NATIONAL INSTITUTE OF GENETICS

JAPAN

ANNUAL REPORT

No. 24 1973

Published by
THE NATIONAL INSTITUTE OF GENETICS
Misima, Sizuoka-ken, Japan
1974

Annual Report of the National Institute of Genetics

No. 24, 1973

Photo by Y. Nakagome

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GENERAL STATEMENT

In August, the XIIIth International Congress of Genetics was held at Berkeley, California. From this Institute thirteen members attended the Congress: Among them, Dr. Y. Tazima participated in it on behalf of the International Genetics Federation as its president; and Dr. M. Kimura and Dr. H. I. Oka were invited to the symposia as speakers. In addition, about ten members of the Institute went abroad to attend other international conferences, to give lectures, and so on.

We also had many visitors from other countries this year. In April a group from the U. S. S. R. Academy of Sciences (ten members headed by Dr. M. A. Markov) visited our institute. Consequent to the establishment of friendly relations between Japan and the People's Republic of China, several Chinese scientists visited Japan. From September to November we received three groups of them: 1) A representative team of botanical workers of Academia Sinica (seven members led by Dr. Chen Tsui), 2) A sericultural mission from the Chinese Association of Agricultural Science Society (four members led by Dr. Yi-Ling Kao); and 3) A representative team of Chinese biologists (ten members led by Dr. Ti-Cho Tung). When we are given the opportunity to welcome scientists from other countries, we ask them to speak at our "Biological Symposium," which met for the 110th time at the end of this year. It was started soon after the establishment of the Institute.

Dr. M. Kimura, the head of the Department of Population Genetics, was elected a foreign associate of the National Academy of Sciences of the United States of America on the 24th of April. As a Japanese geneticist Dr. Kimura is the second who has won the honor, following Dr. H. Kihara, the former director of this Institute.

After Dr. T. Iino was appointed professor of the University of Tokyo, the post of the head of the Department of Microbial Genetics was filled August 1st by Dr. Yukinori Hirota who was the chief of the research unit of cell division, Division of Molecular Biology, Pasteur Institute.

Dr. Kan-ichi Sakai, the head of the Department of Applied Genetics retired under the age limit, and has transferred to Kagoshima University as professor of the Faculty of Agriculture. Dr. Sakai has rendered great services for 24 years to the Institute since he joined in 1949, the year of its establishment. In the scientific field he carried forward his original studies on interspecific competition in plants and genetic variation in forest trees. He has always played a leading part in this field. Dr. Hiko-ichi

Oka, the head of the 3rd Laboratory of the same department succeeds him.

In April Mr. Shin-ichi Tezuka replaced Mr. M. Kudo, the head of the Department of Administration, who was transferred to the Tenth National Youth Home as its director.

STAFF

Director

MORIWAKI, Daigoro, D. Sc., Emeritus Professor of Tokyo Metropolitan University

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* Research members under grant from other organization or visiting researchers.

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FUKUI, Teijiro, Chief of the Finance Section

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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 1973

Department of Morphological Genetics

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Repair processes in radiation mutagenesis (TAZIMA and ONIMARU)
Studies on 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)
Developmental genetic studies on carcinogenesis in tissue culture (KURODA)
Genetics of somatic mammalian cells in culture (KURODA and MINATO)

Department of Cytogenetics

Studies on chromosomal evolution in rodents (YOSIDA, KATO, TSUCHIYA and SAGAI)
Chromosome study on experimental tumors (YOSIDA)
Cytogenetical study on monosomic and trisomic cultured mammalian cells (KATO)
Cytogenetical and immunological studies on mouse plasma cell tumor (MORIWAKI)
Biochemical studies on serum transferrin variations in rodents (MORIWAKI, and TSUCHIYA)
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Cytogenetical study of ants (IMAI)

Department of Physiological Genetics

Population genetics of deleterious genes in natural populations of *Drosophila melanogaster* (OSHIMA, WATANABE and CHOO)
Analysis of inversion chromosome in natural populations of *Drosophila melanogaster* (OSHIMA and WATANABE)
Behavior genetics of phototaxis, diurnal rhythmicity of adult emergence and oviposition in *D. melanogaster* (OSHIMA and CHOO)
Studies on urbanization and noise environments for the habitat and development in *Drosophila* (OSHIMA and CHOO)
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Department of Biochemical Genetics

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Genetical and biochemical studies of human serum proteins (OGAWA and ODAKI)
Genetical and biochemical studies on Japanese middle size dog (OGAWA)
Genetics of isozymes in plants (ENDO)
Effects of exogenous DNA on plant seed formation (ENDO)
Genetics of fresh water hydra (SUGIYAMA and FUJISAWA)

Department of Applied Genetics

Quantitative genetic studies in poultry (KAWAHARA and FUJISHIMA)
Genetic studies in wild populations of Japanese quails (KAWAHARA)
Theoretical studies on breeding techniques (IYAMA)
Behavioral genetic studies in mice (FUJISHIMA)
Studies on competition in plants (IYAMA)
Genetic studies in natural stands of forest tree species (SAKAI, IYAMA and KUDO)
Simulation studies on artificial selection (IYAMA)
Evolutionary studies in wild and cultivated rice species (OKA and MORISHIMA)
Analysis of genetic variations in growth pattern and phenotypic plasticity in rice (MORISHIMA and OKA)
Ecological genetic studies in some grass species (MORISHIMA)
Genetic analysis of isozymes in rice plants (PAI and ENDO)

Department of Induced Mutation

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and KADA)
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Mathematical studies on the genetics of structured populations (MARUYAMA)
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RESEARCHES CARRIED OUT IN 1973

I. MOLECULAR GENETICS

The 5′-Terminal Nucleotide Sequences of the Double-Stranded RNA of Human Reovirus

Kin-ichiro MIURA, Kumiko WATANABE and Masahiro SUGIURA

The 5′-terminal nucleotide sequences of human reovirus double-stranded RNA were determined after labeling the RNA with [^{32}P] phosphate by polynucleotide kinase. Prior to labeling, it was necessary to perform sequential oxidation, β-elimination, and phosphomonoesterase treatment. This operation seems to remove not only a few nucleotides from the 3′ end but also some material attached to the 5′-terminal nucleotide. The 5′-^{32}P-labeled RNA was hydrolyzed by alkali or enzymes, and the resultant mono- and oligo- nucleotides were analyzed chromatographically. The labeled RNA was separated into the ten constituent genome segments by gel electrophoresis, and the 5′ termini of each segment were also analyzed after alkaline digestion. Each genome segment contained the same two 5′-terminal sequences: GpApUp in one chain and G*pCp in the other. G*p is a derivative of guanylic acid which renders the 5′-terminal dinucleotide resistant to hydrolysis by alkali. The results indicate that the transcription of reovirus double-stranded RNA starts from the 3′ end complementary to the G*pCp-terminal resulting in the synthesis of single-stranded mRNA carrying the same 5′ sequence as the G*pCp-chain. The presence of a modified nucleotide at the 5′ terminus of the strand complementary of the mRNA template is a feature common to another double-stranded RNA virus, cytoplasmic polyhedrosis virus.

Identification of the Abnormal Nucleotide Located at the 5′-Terminus of the Genome RNA of Cytoplasmic Polyhedrosis Virus

Kumiko WATANABE, Yasuhiro FURUICHI and Kin-ichiro MIURA

Ten segments of double-stranded RNA of the cytoplasmic polyhedrosis virus (CPV) have the same terminal structure. The 5′-terminal nucleotide in one strand of the double-stranded RNA is abnormal nucleotide, which is resistant to alkaline and ribonuclease digestion. After the 5′-termini of

the RNA was labeled with [^{32}P] phosphate with polynucleotide kinase, the terminal nucleotide was obtained as ^{32}pX by *Penicillium* nuclease, or by venome phospho-diesterase after treatment with *Aspergillus* nuclease. ^{32}pX seemed to be a derivative of adenylic acid from the behavior on chromatography, and to be modified at the 2′-position of ribose moiety, since this nucleotide was resistant to alkali. As a candidate for this abnormal nucleotide, 2′-0-methyladenosine-5′-phosphate (pAm) was synthesized chemically, and it was cochromatographed with ^{32}pX on a filter paper using several kinds of solvent. The radioactive spot of ^{32}pX on a radioautogram was superimposed on the ultraviolet-absorbing spot of pAm in every solvent system. Thus the abnormal nucleotide pX is identified as 2′-0-methyladenylic acid. This is the first report showing the presence of an abnormal nucleotide in a viral RNA molecule.

On transcription of CPV, the same RNA chain as the strand carrying 2′-0-methyladenylic acid at the 5′-end is synthesized. Therefore, this abnormal nucleoside could be a signal which specifies the starting point of transcription.

The 3′-Terminal Structure of the Genome RNA Segments of Rice Dwarf Virus

Yasuhiro FURUICHI and Kin-ichiro MIURA

Rice dwarf virus (RDV) contains twelve genome segments of double-stranded RNA. Total RNA's in RDV particles were extracted by pheonl treatment and oxidized by periodate. The RNA was then reduced by [^{3}H] sodium borohydride to label the 3′-terminal nucleoside. On alkaline hydrolysis the similar amounts of [^{3}H] trialcohols of cytosine and uracil (C′ and U′) were detected. The results of ribonuclease digestion of the [^{3}H] labeled RNA indicate that there are two kinds of the 3′-terminal sequence as follows: -Py-G-A-U and -G-C-C.

Twelve segments of the 3′-terminal [^{3}H] RDV-RNA were separated into eight fractions by gel electrophoresis. Two labeled nucleoside-trialcohols, C′ and U′, were detected equivalently for every fraction. Thus, it is suggested that every RNA segment of RDV has two kinds of 3′-terminal sequences, that is, one chain carries -Py-G-A-U at the 3′-terminus and the other chain -G-C-C.

Methylation Related to the Initiation of Transcription of Cytoplasmic Polyhedrosis Virus Containing Double-stranded RNA

Yasuhiro FURUICHI, Kunitada SHIMOTOHNO and Kin-ichiro MIURA

Cytoplasmic polyhedrosis virus (CPV) contains a virion-associated transcriptase which, in vitro, can copy one of the two chains of the double-stranded RNA segments. It has been noted that the RNA-polymerising activity of CPV preparation was relatively low in comparison with the activity of RNA polymerases obtained from other sources. Since we found that the 5′-terminal nucleotide of one chain of double-stranded RNA of CPV is methylated at the ribose moiety, we thought that methylation may also take place in the *in vivo* transcription system and tried adding a donor of methyl groups for nucleic acid into the reaction mixture for the *in vitro* RNA synthesis of CPV. The addition of S-adenosyl-L-methionine (SAM) to the reaction mixture enhanced RNA synthesis markedly. Synthesis of single-stranded mRNA of CPV proceeded depending on the presence of SAM.

A methyl residue of SAM was incorporated into an RNA molecule. A ribose moiety of adenylic acid in the 5′-terminal region of the nascent RNA was methylated at a very early stage of the trancription. The dependence of the viral transcription on the presence of SAM and the methylation of terminal nucleotide suggests that the transcription of CPV is a "methylation-coupled" reaction.

II. MICROBIAL GENETICS

Studies on Control of Flagellin mRNA Synthesis with *fla*-ts Mutants of *E. coli*

Hideho SUZUKI, Masatoshi ENOMOTO and Yukinori HIROTA

A number of mutants of *E. coli* K12, temperature sensitive with respect to flagella formation, were isolated. They were capable of forming flagella at 30° but not at 41°. The ts-characters were mapped in the *fla* loci. The mutants could be classified into two categories, type I and type II, by the mode of flagella generation on temperature shift during the exponential growth. In the type I mutants a comparatively long lag was required before acquisition of motility on temperature shift from 41° to 30°, and elongation of the flagellar filament stopped immediately upon the temperature shift from 30° to 41°; while in the type II, flagella were generated within a short period on shift from 41° to 30° and filament elongation continued at least for 60 min., when the culture was shifted back to 41°.

m-RNA specific for flagellin in two representative strains belonging to each type was assayed by flagellin synthesizing activity of the extracted RNA in the *in vitro* system. Flagellin synthesizing activity was completely absent in the RNAs derived from 41° cultures of both strains, whereas the RNAs derived from 30° cultures supported synthesis of flagellin equally well. When the cells grown at 30° were transferred to 41°, mRNA activity for flagellin of the type I mutant diminished exponentially with a half-life of 7 min., while that of the type II was maintained at the same level for at least 60 min. at 41°. Administration of 100 γ rifampicin/ml at the time of the temperature shift from 30° to 41° resulted in an exponential diminution of flagellin mRNA activity at a half life of 7 min. In the type I mutant, flagellin mRNA synthesis stopped immediately upon temperature shift to 41°. The inactivation of flagellin mRNA synthesis at the high temperature appears to be irreversible because the type I cells grown at 30° and incubated for 70 min. at 41° revealed a long lag before regaining ability to synthesize flagellin mRNA when shifted back to 30°. When type II cells grown at 41° were fed with 100 γ rifampicin/ml (known as a specific inhibitor for mRNA synthesis) at 10 min. after 30° shift, little mRNA activity for flagellin appeared in the next 10 min. Thus, involvement of a processing step in producing active flagellin mRNA is unlikely, and the absence of flagellin mRNA activity in the mutants at the temperature

non-permissive for flagellation can be ascribed to the failure of flagellin mRNA synthesis, which could be controlled through the *fla* products.

Expression of Salmonella *H2* Genes in *Escherichia coli* K12

Masatoshi ENOMOTO

P1, the general transducing phage of *E. coli*, is active on some rough derivatives of the restriction-negative strain of *S. typhimurium* (e.g., *galE*, *rfaH*, *rfaG* and *galU*) and can be used for transduction between the two genera. *S. typhimurium* has two flagellin gene, *H1* and *H2*, which are separately located on the chromosome and alternately expressed with a constant rate (phase variation); whereas *E. coli* has one flagellin gene, *hag*, corresponding to Salmonella *H1*.

An attempt was made to introduce Salmonella *H2* genes into *E. coli* K12 by P1 transduction, because an *H2* region includes at least a phase determinant and an *H1* repressor gene. A nonflagellate K12 recombinant, with *H1-i ah1*$^-$transduced from *S. typhimurium*, was isolated and used as recipient for *H2* transduction. The three sorts of the Salmonella *H2* region, *H2-1.2 vh2*$^+$, *H2-e, n, x vh2*$^+$ and *H2-e, n, x vh2*$^-$, were tried for transduction into the K12 *H1-i ah1*$^-$ derivative (EJ34). The eighteen transductants were obtained at a frequency 1 to 5×10^{-9}/pfu and all proved to have the phase-2 antigen of the donor. It is expected that Salmonella *H2* incorporated into the EJ34 chromosome will manifest variation between active and inactive states, i.e. flagellate and nonflagellate phases. All transductants except two did not manifest such variation and their *H2* was found to be incorporated into *hag* locus. The *1.2* and *e, n, x* gene at *hag* were unable to repress the *H1* allele and were susceptible to repression by a product of the original *H2* region. The two exceptional transductants, EJ38 and EJ44, showed a high rate of variation between flagellate and nonflagellate phase. EJ38 with antigen *e, n, x* derived from the *H2-e, n, x vh2*$^+$ donor and EJ44 with *e, n, x* derived from the *H2-e, n, x vh2*$^-$ donor were found to receive the *vh2* allele along with *H2-e, n, x* from each donor. The *vh2*$^-$ gene which causes fixation of flagellar expression in either phase-1 or phase-2 in Salmonella was suppressed in an *E. coli* background. The *H2* locus of EJ38 and EJ44 was mapped by Hfr crosses at around 50 minutes, between *lys* and *nalA*, on the standard chromosome map of *E. coli*. *H2-e, n, x* of EJ44 was cotransducible with *tyrA* or *pheA* at a frequency about 3%; whereas with EJ38, contransduction with these two

markers was not observed (<0.3%). This indicates that the integration site of *H2* differs between EJ38 and EJ44.

Isolation of an *H1*-repressor (*rh1*) Mutant from a Salmonella-*E. coli* Hybrid

Masatoshi ENOMOTO

A strain, EJ283, showing an extraordinarily high rate of O-H variation (ca. 1.5×10^{-2}/cell/generation for change from phase-2, *e, n, x* to nonflagellate phase-1) was isolated in the course of P1 transduction of a Salmonella *H2-e, n, x vh2*$^-$ region into the K12 *H1-i ah1*$^-$ derivative (EJ34). EJ287, made by transduction of *H1-i ah1*$^+$ allele into EJ283, was used for isolation of *rh1*$^-$, a mutant of an *H1* repressor gene. It is expected that some cells of the *rh1* mutant will have flagella with both antigens, phape-1 *i* and phase-2 *e, n, x,* because the *i* antigen is always expressed and the *e, n, x* antigen is intermittently expressed with a very high rate. When such mutant cells are present in the parental culture composed by the cells with antigen *i* and those with *e, n, x,* the mutant cells expressing both antigens will agglutinate with the cells expressing either *i* or *e, n, x* by the addition of corresponding anti-flagella serum. The mutant cells will be concentrated in the agglutinated cells by repeating the procedure with alternate use of anti-*i* and anti-*e, n, x* serum.

The *rh1* mutant, EJ289, could be isolated as expected by this method. The mutant did not form swarms on semisolid medium supplemented with either anti-*i* or anti-*e, n, x* serum, though it did on unsupplemented medium. When P1 grown on EJ289 was applied to an *H1-i* strain on semisolid medium with anti-*i* serum no trails were evoked. This indicates that the transduced *H2-e, n, x* region is unable to repress endogenotic *H1-i*. Further *H1-i* and *H2-e, n, x* of EJ289 were separately transduced into the *H1-i ah1*$^-$ strain.

Expression of the *i* antigen in the *H1-i ah1*$^+$ transductants was repressed by the wild *H2-e, n, x* region. The *H2-e,n, x* transductants, when used as donors in transduction to the *H1-i* strain, had no ability to repress *H1-i* expression. EJ289 was confirmed to carry the *rh1* mutation closely linked to *H2-e, n, x* and the wild *H1-i* allele.

III. BIOCHEMICAL GENETICS

The Genetic Control of Tryptophan Pyrrolase in *Ephestia*

Saburo NAWA and Masa-Aki YAMADA

As reported previously, we obtained individuals with wild-type eyes in experiments where the recessive mutant *a* had been treated with DNA from the wild type. The mutant *a* suffers from the inability to convert tryptophan to formylkynurenine. Although this conversion is catalyzed by the enzyme tryptophan pyrrolase, it is not clear whether the mutant produces none of the enzyme or directs the production of altered polypetides.

Tryptophan pyrrolase is absent also in the mutant of the vermilion cistron of *Drosophila*. It is known that the vermilion mutant produces an altered polypeptide which is in an inactive form in the presence of a tyrosyl t-RNA and that RNase treatment of homogenates of the mutant leads to the activation of the mutant enzyme. In the mutant *a* of *Ephestia*, however, no appearance of tryptophan pyrrolase activity by treatment with ribonuclease T_1 or pancreatic RNase was observed. On the other hand, the activity of tryptophan pyrrolase in the a^+/a heterozygote was super-additive, being greater than half the tryptophan pyrrolase activity in the a^+/a^+. Moreover, when a/a extract was mixed with a^+/a^+ crude extract, the normal a^+ enzyme level was enhanced by 20-40 percent.

The *a*-material responsible for the super-additivity could be purified from a/a extract by the same procedures as used for tryptophan pyrrolase of wild type. The material was shown to be heat labile and precipitable with ammonium sulfate (40-60% saturation), like a protein. The protein was fractionated with Sephadex gel filtration and the degree of super-additivity of each fraction was examined. The material showing the enhancement of tryptophan pyrrolase activity was fractionated in the same place as that for tryptophan pyrrolase of wild type. Thus, if the super-additivity results from a reaction of the *a*-protein with a^+-protein, then the mutant enzyme exists in the a/a extract, in a form with molecular weight indistinguishable from the wild type. We propose that the mutant *a* directs the production of altered polypeptides, suggesting that the *a* cistron is the structural gene for tryptophan pyrrolase.

Structural Study of Fc Disease Protein

Tomotaka SHINODA

Fc disease or heavy chain disease proteins are believed to be identical to heavy chains of normal immunoglobulins, except that the former have internal deletions ranging in size from about 20 to 240 residues. These smaller products seem to have resulted from abnormalities of the mechanism of gene expression rather than from *in vivo* degradation of the intact products. Since the genetic mechanism of these phenomena is not yet well understood and since studies of such proteins are considered to be of great value in connection with the theories concerning the genetic control of antibody synthesis, a sequence study has been carried out using an Fc disease protein designating NIG 43 (γ_1, a^+, x^+, z^-; genetic markers were kindly determined by Dr. H. Matsumoto).

The protein was purified by a variety of methods and finally crystallized. Four fragments, FI, FII, FIII and FIV, were obtained from completely reduced and alkylated protein by CNBr treatment. Of these FII, FIII and FIV were shown to be homogeneous upon amino acid and NH.-terminal analyses, but FI had a heterogeneous NH_2-terminal. Sequence analysis revealed that FI had two NH_2-terminals, serine and threonine, in nearly equal amounts, suggesting that the intact Fc protein was a mixture of at least two molecular species, one beginning with serine and the other with threonine. FIV, a 17-residue fragment, had an identical sequence except one with the COOH-terminal portion of the γ_1 heavy chain; thus it was considered that FIV occupied the COOH-terminal of the protein.

Although there have been several reports on sequences of gamma chains including a partial sequence of heavy chain disease protein, no case has been shown to have amino acid interchange in the COOH-terminal fragment. The present case seems to be the first example which shows that amino acid interchange (i.e. Ala $\rightarrow$ Gly) occurred in the COOH-terminal region. Whether the interchange is associated with some serological type is not known. The phenomenon is provisionally referred to as "Chemotype."

IV. DEVELOPMENTAL GENETICS

In Vitro Cultivation of Developmentally Defect Cells from *Deep Orange* Embryos in *Drosophila melanogaster*

Yukiaki KURODA

In the previous experiment on the cultivation of embryonic cells from the wild-type strain (Oregon-R) of *D. melanogaster*; muscle cells, epithelial cells, fibroblastic cells, small cells and nerve cells were found to maintain their respective characteristics for a prolonged period of cultivation. (For details, see Development, Growth and Differentiation **16**: 55-66.)

In the present experiment cells from genetically lethal embryos, *deep orange* (1-0.3), were cultured to study when and what types of cells were affected by the lethal factors under the same conditions as those used for cultivation of wild-type embryonic cells. Among 463 eggs collected from matings of *dor*/*dor* females and *dor*/Y males, 4.1% died before gastrulation, 38.7% died by abnormal gastrulation, 44.0% died after the stage of sac-like midgut, 13.2% died after segmentation of the body and none were found to hatch.

When cells from *deep orange* embryos after gastrulation were cultured, mesodermal cells such as fibroblastic cells and muscle cells were maintained in an active state for a few weeks beyond the prospective lethal period of the *deep orange* embryos. On the other hand, nerve cells, epithelial cells and small cells (assumed as imaginal disc cells) scarecely grew. No extension or poor extension of nerve fibers, and neither maturation nor differentiation of epithelial cells was found. This suggests that the lethal effects of the *dor* gene may be manifested specifically in the ectodermal or endodermal cells of embryos.

When *deep orange* embryonic cells were cultured in medium containing an unfertilized wild-type egg extract, they were maintained in an active state for a longer period, during which the extension of nerve fibers and the maturation of epithelial cells were observed. The nature and characterization of the effective factors present in the wild-type egg extracts which may repair the *deep orange* defect are now under investigation.

Inhibitory Effect of Cycloheximide on Enhanced Aggregation of Embryonic Quail Liver Cells in Dissociation Medium

Yukiaki KURODA

The effect of cycloheximide on the aggregation of dissociated liver cells from 7-day quail embryos in the presence and absence of the medium in which the cells were dispersed (*dissociation medium*), was examined in rotation-mediated cell cultures.

Cycloheximide had a concentration-dependent inhibitory effect on cell aggregation. With 30 μg/m*l* of cycloheximide the aggregates formed after 24 hours were about one third the size of those in control cultures. Its inhibitory effect appeared after a time lag of about 6 hours and was not completely reversible.

When dissociated cells were centrifuged and resuspended in fresh medium and then rotated without *dissociation medium*, they formed, after 24 and 48 hours, aggregates three fifths and five sixths the size of aggregates formed in *dissociation medium*. Resuspension of the above centrifuged cells in the *dissociation medium* resulted in restoration of aggregate size, and the diameters of the aggregates were comparable to those of the aggregates formed when the original cell suspension was cultured directly.

In the presence of cycloheximide, *dissociation medium* was not effective in enhancing cell aggregation and the size of aggregates formed was comparable to that in cultures of resuspended cells without the *dissociation medium*.

The size of cell aggregates depended on the period of trypsin treatment, with prolonged treatment resulting in smaller aggregates. This decrease in the size of aggregates was more marked in the presence of cycloheximide.

Mutagenesis to 8-Azaguanine Resistance in Embryonic Human Diploid Cells Treated with EMS

Yukiaki KURODA

Human diploid cells derived from a 5-month embryonic lung were treated with ethyl methanesulfonate (EMS) and cultured in selection medium containing 8-azaguanine (8AG) to induce mutation to 8AG-resistance. The effects of factors such as inoculum size and mutation expression time on the induced mutation frequency of cells were examined.

When cells were treated with 10^{-2} M EMS for 2 hours and selected with

10 μg/ml of 8AG, the induced mutation frequency was highest on inoculation of 2.5×10^5 cells and lower with bigger or smaller inocula. When EMS-treated cells were selected with 30 μg/ml of 8AG, the highest induced mutation frequency was obtained on inoculation of 10^5 cells. With similar sizes of inocula, the induced mutation frequencies in cultures selected with 30 μg/ml of 8AG were lower than those in cultures selected with 10 μg/ml of 8AG.

The mutation frequency was higher when 10^5 cells treated with EMS were cultured in normal medium for 48 hours (mutation expression time) before selection with 8AG, than in cultures with longer or shorter mutation expression time. This mutation expression time of 48 hours corresponds to the time during which about half the cell population treated with EMS divides once.

Microscopic examination of the numbers of cells in colonies indicated that with or without EMS treatment, human diploid cells do not all grow exponentially; but that in some cases after each cell division, only one daughter cell continued to divide. These results were reported, in part, in the First International Conference on Environmental Mutagens. (See also Mutation Research **21**: 226.)

Induction of Drug-Resistant Mutations by Furylfuramide in Embryonic Human Diploid Cells

Yukiaki KURODA

Furylfuramide (FF) is now widely used as an antimicrobial food preservative in Japan. Recently this nitrofuran derivative was found to induce chromosome aberrations in human lymphocytes and mutations in bacteria, yeast and silkworms.

In the present experiment the mutagenic effect of FF on 8-azaguanine (8AG) resistant mutations in cultured embryonic human diploid cells was examined. The effects of various concentrations of FF on cell survival were tested and the D_0 values were calculated from the concentration-survival curves. The D_0 values for 2 hour- and 14 day-treatments with FF were 11 μg/ml and 1.9 μg/ml, respectively.

When cells were treated with 1 μg/ml of FF for 2 hours, incubated for 48 hours in normal medium, and selected with 30 μg/ml of 8AG, the induced mutation frequency was 19.2×10^{-5}. When cells were treated with higher concentrations of FF, the induced mutation frequencies increased gradually. Thus cells treated with 30 μg/ml of FF gave the mutation frequency of

73.3×10^{-5}.

When the mutation expression times were varied, the mutation frequency was higher in cultures with 48 hours of mutation expression time than in cultures with longer or shorter mutation expression times. During this mutation expression time about half the cell population treated with FF divided once.

Studies on Serum Factors Supporting Growth of Mammalian Cells in Culture

Kiyoshi MINATO

The nature and functions of serum factors which support growth of cultured mammalian cells were analyzed using HeLa S3 cells in monolayer culture.

HeLa S3 cells were cultured in TD-15 flasks in Eagle's MEM supplemented with calf serum. Cell Growth was determined from the changes in the average numbers of cells per unit area of the culture flasks. In the previous experiments, the growth rate in the logarithmic phase of cell growth was calculated to determine quantitatively the growth-supporting activity of serum or its fractions. This method was based on the fact that at lower concentrations of serum lower growth rates were obtained.

However, in the later experiments, it was found that the relation of serum concentrations to the growth rate of cells varied depending on the lots of serum. Even in this case, the cumulative number of cells which were maintained on each day was found to depend on the concentration of serum supplemented; at low concentrations cells ceased to grow in lower cell density, although the growth rate was not changed.

When dialyzed serum was used, the cumulative number of cells was also dependent on the concentration of dialyzed serum. It was lower than that in control cultures with whole serum, and corresponded to that in cultures supplemented with serum at one fourth concentration, although the growth rate was the same as that in control cultures. Since this dialyzed serum was considered to be deficient in low molecular nutrients such as vitamines and amino acids which were contained in Eagle's MEM, it is likely that the difference in the cumulative number of cells, which was dependent on the concentration of serum, was not due to such vitamins and amino acids. These results indicate that other factors in the serum which were partially lost in the process of dialysis were responsible for the gowth of HeLa S3 cells.

V. CYTOGENETICS

Oceanian Type Black Rats (*Rattus rattus*) with a Subtelocentric M_2 Chromosome obtained from Davis Colony

Tosihide H. YOSIDA, Kazuo MORIWAKI and Tomoko SAGAI

The black rats (*Rattus rattus*) used in the present study were kindly supplied by Dr. Rex Marsh and Mr. Ray Record in the Department of Animal Physiology, University of California at Davis, when we visited there in August, 1973. According to Dr. Marsh's correspondence, three pairs of black rats were collected in San Lorenzo, California in 1970, and since then they have been bred in an outdoor population cage. Ten rats (5 females and 5 males) randomly caught from the breeding colony were given to us. Chromosomes were observed in short-term cultured cells from their tail tips.

All 10 black rats obtained from the Davis colony were characterized as the Oceanian type having 38 chromosomes, except one which had 37 chromosomes. All of them had two large biarmed autosome pairs (M_1 and M_2). Among them the larger one (M_1) was the same metacentric pairs as those found in the other Oceanian type black rats. On the other hand, the smaller one (M_2) in 5 rats was a metacentric homomorphic pair similar to those found in the other Oceanian type black rats, but the remaining 5 rats were remarkable by having a metacentric and a subtelocentric heteromorphic M_2 pair. The morphology of the subtelocentric M_2 chromosome was similar among the 5 rats examined. The metacentric M_2 and the subtelocentric M_2 chromosomes were almost the same size. This suggests that the subtelocentric M_2 was derived from pericentric inversion of the metacentric M_2. The comparison of G-banding patterns between metacentric and subtelocentric M_2 chromosomes strongly suggests that the latter was derived from the pericentric inversion of the former.

Black Rat (*Rattus rattus*) with a Single X-Chromosome

Tosihide H. YOSIDA, Kazuo MORIWAKI and Tomoko SAGAI

Nine of the ten black rats obtained from the Davis colony showed 38 chromosomes as the Oceanian type, but the remaining one showed 37 chromosomes. Chromosome numbers were counted in 6 rats to examine

the distribution. In 5 rats (4 females and one male) the majority of cells showed 38 chromosomes, although a few aneuploid cells were observed. Only one rat among them showed 37 modal chromosome number, although a few aneuploid cells were also observed.

The karyotype of the rat with 37 chromosomes is remarkable by having a single X chromosome. From the G-banding pattern analysis, the single chromosome is clearly shown by its characteristic X band. All autosome pairs are easily distinguished from each other by their characteristic G-bands, but the X chromosome having two dark bands in the middle region of the long arm is unpaired. The external and internal features of the black rat were apparently female. From these observations this rat is characterized by the sex chromosome abnormality with X-O.

Polymorphism of C-bands of Chromosomes in Black Rats, *Rattus rattus*

Tosihide H. YOSIDA and TOMOKO SAGAI

It has already been reported that the Asian type black rats, *Rattus rattus* with $2n=42$ chromosomes, are characterized by chromosome polymorphism with respect to acrocentrics and subtelocentrics. The Giemsa band, however, was quite uniform in all subspecies of the black rats, but also in the related species of genus *Rattus*. The C-band of the animals, however, showed high polymorphism in regard to positive and negative staining, especially in the Japanese black rat, *Rattus rattus tanezumi*. The rats were characterized by 13 autosome pairs (No. 1 to 13) of acrocentrics or subtelocentrics; and 7 autosome pairs (No. 14 to 20) of metacentrics and acrocentric X and Y chromosomes. In Japanese black rats, pairs No. 1, 4, 7, 9, 11 and 13 showed polymorphism in regard to positive and negative C-bands. Pairs No. 2, 5, 6, 8 and 10 have negative bands, while pairs No. 3 and 12 have positive bands. It is interesting that the pair No. 1 chromosomes showing acrocentric and subtelocentric polymorphism were C-band negative in subtelocentrics, but positive in acrocentrics. In pair No. 9 which is also characterized by acrocentric and subtelocentric polymorphism, however, the correlation between the C-band and the chromosome type could not be observed. The above study was carried out in 27 rats obtained from our breeding colony, but in the natural population of Japan the polymorphism was also observed, although the polymorphic chromosome was slightly different from the above rats. The C-band polymorphism was very rare in the Philippine black rat, *R. rattus mindanensis*,

the Hong Kong black rat, *R. r. flavipectus*, and the Malayan black rat, *R. rattus diardii*. In the latter species, however, pair No. 1 was polymorphic similar to Japanese black rats.

In the Oceanian type black rats with $2n=38$ chromosomes, the C-band polymorphism has not been observed. A peculiar feature of the C-band in these animals is that the bands in metacentric pairs No. 11 to 20 were generally smaller than those in Asian type rats. The C-band character in Ceylon type black rat ($2n=40$) was almost similar to that of the Oceanian type.

Induction of Sister Chromatid Exchanges by Chemical Mutagens and Its Possible Relevance to DNA Repair

Hatao KATO

Several chemical mutagens were tested for their ability to induce sister chromatid exchanges in Chinese hamster chromosomes. Among them, the effects of 4-nitroquinoline-1-oxide (4NQO) and mitomycin C (MMC) were very similar to those of ultraviolet light in that the exchange frequency increased with increasing dose of chemicals and decreased markedly in the presence of 1 mM caffeine during a post-treatment period. The frequency of proflavin-induced sister chromatid exchanges was also found to be dose dependent, but it was insensitive to caffeine post-treatment. On the other hand, no appreciable increase was detected in the incidence of sister chromatid exchanges in cells treated with N-methyl-N-nitro-N-nitrosoguanidine (MNNG) over a 100-fold range of variation in chemical dose. Caffeine by itself raised the exchange frequency only slightly over the control level. It was found that 4NQO and MMC exerted remarkable delayed effects on the exchange induction, whereas proflavin did not. This seems to suggest that the lesions caused by the former mutagens would be long-lived and repeatedly provoke sister chromatid exchanges.

These data imply that there are several possible ways in which the initial DNA lesions ultimately lead to the formation of sister chromatid exchanges, and that at least UV-, 4NQO- and MMC-induced sister chromatid exchanges have evolved through a caffeine sensitive repair process, probably related to a post-replication repair of DNA damage. (For details, see Exptl. Cell Res. 85: 239-247, 1974.)

Constitutive Heterochromatin of Indian Muntjac Chromosomes Revealed by DNase Treatment and a C-Banding Technique

H. KATO, K. TSUCHIYA and T. H. YOSIDA

A karyotype of a female Indian muntjac, *Muntiacus muntjak vaginalis*, was studied. The karyotype was unique in that No. 1 and No. 3 homologous pairs were heteromorphic with respect to the size of their secondary constrictions. In these pairs, one of the homologs always had a longer secondary constriction than that of the corresponding homolog. Heterochromatin in the secondary constriction region was visualized with difficulty by a C-banding technique, but was demonstrated clearly by a DNase treatment followed by Giemsa staining, which also revealed the size difference of the secondary constriction. Centromeric constitutive heterochromatin of No. 1 chromosome was also found to differ in size between the homologs.

On the basis of the heteromorphic character of No. 3 chromosome, or an X-autosome complex, it was possible to confirm autoradiographically that X-inactivation had occurred in random fashion in this animal.

The Seventh Cell Population Change Accompanied by a Marker Chromosome E in the Transplantable Mouse Myeloma MSPC-1

Kazuo MORIWAKI

The mouse myeloma MSPC-1 has been serially transplanted in BALB/c mice since 1966. During the eight years, the major cell population has had at least six changes which could be detected either by the successive occurrence of marker chromosomes, A, B, C and D, or by the loss of capability to produce the specific gamma-A globulin. The sixth cell population NP38-ABCD subline, was established in September 1972 at transplant generation 123. It was successfully propagated in the solid form until generation 163 in March 1974, when it was spontaneously converted to the ascites form with tetraploidy. This phenomenon was considered to be certain kind of clonal senescence (Moriwaki and Sadaie, This Annual Report 22: 52, 1972). Our previous data on the successive karyotypic changes in this tumor line (Moriwaki *et al.* J. Nat. Cancer Inst. 47: 623, 1971) suggested that the variant cells with newly emerged marker chromosome can escape from the clonal senescence and actively proliferate to constitute the major cell population in the subsequent transplant generations.

In the case of NP38-ABCD subline, we have maintained two series: One of them changed to the ascties form at generation 163 as stated above, whereas the other one which was once stored in a deep freezer (−80°C) exhibited cell population shift from NP38-ABCD to NP38-ABCDE at generation 140 in March 1974. The new marker chromosome E seems to be produced by a pericentric inversion of the smallest chromosome, No. 19, in the NP38-ABCD subline cell. This was confirmed by either G-band or C-band analysis. The C-banding pattern, especially, has clearly demonstrated that the D-marker chromosome was translocated with the centromeric heterochromatin at the terminal region of the C-marker chromosome and that pericentric inversion near the centromeric region of No. 19 chromosome induced the E marker chromosome. The newly established NP38-ABCDE subline has been propagated until now as a stable solid form.

Synaptonemal Complex and Male Crossing-over in *Drosophila ananassae*

Daigoro MORIWAKI and Mitsuo TSUJITA

The synaptonemal complex has been known to be intimately related to pairing of homologous chromosomes and meiotic exchange, being normally restricted to the nucleus of meiocytes.

Although it has been known that crossing-over does not take place in the male of *Drosophila* species, *D. ananassae* is a notable exception in which spontaneous crossing-over occurs frequently in the males. Individuals from T_{15-4} strain, one of the inbred lines derived from the Tonga strain which had shown the highest recombination value in males, were crossed with double recessives, *b se*, and crossing-over in the F_1 males was counted between *b* and *se*. Crossovers appeared in their progeny with an average frequency of 8.9%, which is 18% of 48.7%, the recombination value between *b* and *se* in females.

Testes were dissected from the third instar larvae of sibs of the F_1 males (*b se*/+T_{15-4}) and examined by electron microscopy to determine whether the synaptonemal complex is formed in pro-spermatocytes. The synaptonemal complex with typical tripartite structure which was seen in pro-oocytes could not be found by examining a number of pro-spermatocytes. According to Grell, Bank and Gassner (1972 Nature 240: 165-167), no structure was found which could be identified as a synaptonemal complex, in spite of their observation of a number of pro-spermatocytes from the testes of *D. ananassae* by electron microscopy. They interpreted this as follows:

"demonstration of high levels of crossing-over in the apparent absence of the complex implies that it is not an inseparable feature of meiotic exchange."

In the present case, however, we were able to find imperfectly developed synaptonemal complexes, though they were not typical. In leptotene and early zygotene cells, the axial filament which makes up the center of each chromosome was clearly observed. Furthermore, in zygotene cells, homologous chromosomes formed incomplete synapses with the aid of an imperfectly developed synaptonemal complex. It is considered, therefore, that male crossing-over takes place between these imperfectly synapsed homologues, although its frequency is much lower than that of females.

Ecological Genetic Studies on the Pollen Germination of *Chrysanthemum* Species

Shuzo NAGAMI[1)]

Pollen grains of 16 *Chrysanthemum* species were cultivated artificially on the medium A and B (NAGAMI, 1972). The results are shown in Table 1. The conclusions are as follows: (1) In general, medium B gives better pollen germination than medium A. (2) There is no, remarkable relation-

Table 1. Species name (H: Hagi strain, Y: Yuhara str., T: Tsubakidomari str., A: Awasima str., M: Manazuru str., K: Kamakura str.), subsection name (SN. D: Dendranthema, L: Leucanthemum, N: Nipponicae), distribution (d. CJ: Central Japan, NJ: North Japan, SJ: South Japan, c: coast species, f: field sp.), chromosome number (cn. $X=9$), and pollen germination rate (grA: rate by medium A, grB: rate by medium B) of chrysanthemums.

species name	SN	d	cn	grA	grB
1. *C. Makinoi*	D	CJ—f	2X	7.5%	6.1%
2. *C. boreale*	D	CJ—f	2X	0	0
3. *C. nipponicum*	N	NJ—c	2X	5.3	15.3
4. *C. indicum*	D	CJ—f	4X	0	5.0
5. *C. indicum* var. *hexaploid* (H)	D	CJ—c	6X	0	6.0
6. *C. indicum* var. *hexaploid* (Y)	D	CJ—c	6X	12.0	22.4
7. *C. japonense*	D	CJ—c	6X	10.3	35.0

1) Professor, Department of Biology, Faculty of Education, Yakohama National University.

(continued from page 28)

	species name		SN	d	cn	grA	grB
8.	*C. Shiwogiku*	(T)	D	CJ—c	8X	1.0	13.5
9.	*C. Shiwogiku*	(A)	D	CJ—c	8X	0	0
10.	*C. Shiwogiku* var. *kinokuniense*		D	CJ—c	8X	0.7	0.6
11.	*C. Weyrichii*		L	NJ—c	8X	6.5	7.7
12.	*C. japonense* var. *octoploid*		D	CJ—c	8X	0	7.1
13.	*C. japonense* var. *crassum*		D	SJ—c	10X	0	5.6
14.	*C. arcticum* ssp. *Maekawanum*		L	NJ—c	10X	6.2	17.5
15.	*C. pacificum*	(M)	D	CJ—c	10X	12.5	32.0
16.	*C. pacificum*	(K)	D	CJ—c	10X	20.0	11.2

ship between taxonomical conditions and pollen germination rate, or between distributional conditions and pollen germination rate. (3) In proportion to the increase of *Chrysanthemum* polyploidy, a gradual increase is found in the size of pollen grains, but no increase is observed in the pollen germination rate. (4) Some species, such as *C. indicum* var. *hexaploid*, *C. Shiwogiku* and *C. pacificum*, show clear intra-specific differences of pollen germination rate.

VI. MUTATION AND MUTAGENESIS IN ANIMALS

Some Aspects of Overdominance Observed in Experiments on Radiosenstitivity in the Silkworm*

Y. TAZIMA

Overdominance is regarded at times as the cause and at other times as the effect of a hybrid vigour. In this paper the relation between ordinary dominance and apparent overdominance is discussed. The argument is based on the results of our experiments with the silkworm in which radiosensitivities at several biological response levels were used as criteria.

By raising two sensitive and one resistant parental strains and their hybrids simultaneously, the radiosensitivity of F_1's was compared with those of parental strains. The modes of dominance contrasted markedly between different reaction levels: Overdominance was always observed with regard to the sensitivity to embryo killing (whole individual level); whereas with regard to mutation induction, sensitivity in F_1 was invariably intermediate between both parental strains (gene level). Between those two reaction levels, with respect to cell killing, either overdominance or the intermediate sensitivity was observed, depending on the combination of the cross (cell level).

These findings indicate that as far as radiosensitivity is concerned, the inherent expression of dominance is of the intermediate type (as in the cases of mutation induction); but may be modified toward the overdominant type because in each strain there is an upper limit for survival of radiation induced lesions, and this limit may be shifted to a higher level in F_1 induviduals than in both parental strains, presumably due to a hybrid vigour. In this regard, the overdominance observed for embryo killing was not a real one, but a modified form of ordinary type of dominance. The overdominance observed for drug resistance, pest resistance, and so on may belong to the latter type.

* Presented at the Second General Congress of SABRAO, held on Feb. 22-28, 1973. A full paper is due to appear in Ind. J. Genet. and Plant Breed.

Results of Mutagenicity Testing for some Nitrofuran Derivatives by Applying a Sensitive Test System with Silkworm Oocytes*

Yataro TAZIMA and Kimiharu ONIMARU

A highly sensitive test system which is applicable for the screening of weakly mutagenic chemicals, has been established in the silkworm using oocytes. It is essentially a kind of specific loci method, using egg color mutants as markers, characterized by an administration of chemicals to wild type females at a mid-pupal stage, so that the chemicals are incorporated into developing oocytes. The method proved to be effective for the detection of a weakly mutagenic compound Panfuran, a derivative of nitrofuran (Tazima, 1973). Using this system we are continuing the mutagenicity testing of other nitrofuran derivatives. The results reported here are for Furylfuramide [2-(2-furyl)-3-(5-nitro-2-furyl) acrylic acid amide], and Furazolidone [3-(5-nitrofurfurylidenamino)-2-oxazolidone].

Both compounds have low solubility in physiological saline solution and injection of 0.025 m*l* of saturated solution, administered doses per head of 3.1 μg for Furylfuramide and 7.2 μg for Furazolidon, did not produce a significant increase in mutation frequency. However, at higher doses, which were prepared by dissolving the chemicals in a mixture of one part acetone and 4 parts of physiological saline solution, Furylfuramide gave clearly positive results. Induced frequencies were 98.0×10^{-5} at $+^{pe}$ locus and 54.5×10^{-5} at $+^{re}$ after injection of 19.2 μg/head and 70.4×10^{-5} at $+^{pe}$ and 41.7×10^{-5} at $+^{re}$ after injection of 9.6 μg/head. In contrast, Furazolidone did not increase the mutation frequency within dose range of 12.5 μg and 25.0 μg per head.

Since Furylfuramide is used widely in Japan as a food preservative, a test for mutagenicity of this compound with a mammalian system is urgently needed.

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* Presented at the Second Annual Meeting of the Environmental Mutagen Society of Japan held at Misima, Sept. 22, 1973.

Production of Mosaic Eggs at Very High Frequency with BUdR Treatment in the Silkworm

Yataro TAZIMA and Kimiharu ONIMARU

It was reported in late 1950 that transition type mutations had been induced in bacteriophage when base analogues of DNA, as 5-bromouracil and/or 2-aminopurine, were incorporated by mistake into DNA during the process of its multiplication. However, our experiments in with silkworm spermatogonia were used as materials, failed to demonstrate a similar mechanism in this insect. Recently, we have been able to establish a very sensitive test system of chemical mutagens using developing oocytes in the silkworm. We again tested the mutagenicity of BUdR to the silkworm using this system. The results were surprising. The incidence of mosaic eggs were observed at strikingly high frequencies.

Wild type female pupae were injected with BUdR at a dose of 50 μg/head in physiological saline solution. Injection was administered 4-5 days before the emergence of the moth. After emergence, injected females were mated with males of a double recessive marker strain, *pe re*. In F_1 the incidence of mosaic eggs having tiny red cell patches among normal (dark brown) serosa cells was observed at a frequency of 1.6×10^{-2}. Mosaic eggs were also observed with regard to pink and normal cells. More frequently observed were mosaics that had either small or large opaque patches on their transparent chorion. Similar treatment with FUdR, BU and/or AP did not produce such aberrations.

Since the chorion is the product of follicle cells of the mother moth, the appearance of opaque patches seems to reflect the abnormal function of those follicle cells, and red or pink patches in serosa cells indicates that the abnormality developed during early segmentation of the zygote. The two phenomena may have some common processes. Presumably, both might have been caused by an error in the process of edcoding genetic information during transcription or translation.

Analysis of a Chromosome-Specific Genetic Instability in the Silkworm

Akio MURAKAMI

In an experiment on radiation-induced mutagenesis carried out in 1971, in which mature larvae of the C108 wild type strain silkworm male were irradiated with 180 kVp X-rays (5 kR: dose-rate of 100 R/min) and crossed

to the marker strain female moths having a recessive visible egg-color mutant gene, *re* (red egg: V-31.7), six mosaic type mutant eggs were found out of 110,000 F_1 eggs. The moths developed from the mosaic mutant eggs were backcrossed again to moths homozygous for *re* gene in order to test their transmissibilities. Among six moths tested, five produced red and black eggs in various ratios as expected, whereas the remaining one produced a large number of unexpected mosaic eggs in addition to expected red and black eggs. Furthermore, when all these mosaic eggs which appeared in the BF_1 were crossed to moths homozygous for *re* gene, they again produced a number of unexpected mosaic eggs (BF_2) besides expected red and black eggs as observed in the BF_1 eggs. By repeating procedure, a similar tendency has been observed in the production of BF_3 eggs.

From the finding in the single *re* locus tested so far, it seems likely that the above instable (or mutable) line, tentatively MV^{INSTA}, may be classified as the line having locus-specific genetic instability. Also, it can be said that this MV^{INSTA} line behaves as a semidominant one.

In the foregoing description, the relevant event occurred at the *re* locus on chromosome 5. It is of interest to analyze whether or not the MV^{INSTA} line has an effect upon another gene located on chromosome 5, *pe* (pink egg: V-0.0). It was observed that the chromosome in the MV^{INSTA} line was affected at the *pe* locus as well as at the *re* locus. This was also confirmed in studies on the other two gene loci locating on the chromosome 5, *ok* (*kinshiryu* translucent, V-4.5) and *oc* (chinese translucent, V-40.8). Both genes control a larval oily skin character which is due to lack of uric acid in epidermal tissues.

It is of further interest to know whether the MV^{INSTA} line has an effect upon chromosomes other than chromosome 5. In this connection, experiments were carried out using several gene loci, *os* (sex-linked translucent, chromosome 1-0.0), *lem* (lemon, chromosome 3-0.0), w_2 (white egg-2, chromosome 10-3.4) and *ch* (chocolate, chromosome 12-9.6). No genetic effect of the MV^{INSTA} line on these loci and/or chromosomes was found. These findings may lead to a conclusion that the MV^{INSTA} line shows a chromosome (5)-specific instability.

Stage Sensitivity and Dose-Response of X-ray-Induced Recombinations in Oogenesis of the Silkworm

Akio MURAKAMI

The object of the present study was to investigate the stage sensitivity and the dose-response relation in X-ray-induced recombinations in silkworm female gametogenesis as well as in *Drosophila* male gametogenesis, in which the crossing-over does not occur normally.

Silkworm females from early instar larval stage to the late stage pupal stage, having *pe* (V-0.0) and *re* (V-31.7) mutations in trans type heterozygotes were exposed to 1000 R of X-rays and mated to males homozygous for *pe* and *re*. In this test system, recombinants occur as exceptional black $\left(\frac{+\ +}{pe\ re}\right)$ and pink $\left(\frac{pe\ re}{pe\ re}\right)$ eggs in addition to normal pink $\left(\frac{pe\ +}{pe\ re}\right)$ and red $\left(\frac{+\ re}{pe\ re}\right)$ eggs. Since the recombinant pink eggs can not be distinguished from the normal ones, a progeny test has been done only for the black eggs. In view of the mating scheme, however, the recombinant black eggs cannot be distinguished from other exceptional types, such as polyploid eggs, which may come from apparent non-disjunctional events. Thus, by backcrossing the exceptional black eggs with marker strain moths homozygous for *pe* and *re*, they can be identified as recombinant eggs and further classified into either homologous exchange (crossover) or non-homologous interchange type.

The results of the experiments indicated that some recombinants were induced by X-rays at any stage in female germ cells. A considerable number of recombinants was induced by X-rays in 4th or 5th instar larvae, suggesting that a peak of the sensitivity to X-ray-induced recombinations occurs at the early stage of prophase I oocytes, which in general, is the most likely stage for the crossing-over to take place. At other developmental stages, however, the incidence of recombinations was almost equal to or somewhat less than that of the untreated control series. It is of interest to note that no cluster effect of induced recombinants was observed even at the early larval stage of 1st to 2nd inster larvae, suggesting that gonial cells do not respond to the induction of recombinations by treatment with X-rays. Among 122 recombinants recovered in X-irradiated groups in the present experimental series, the frequency of homologous exchange type recombinants was about three times as high as that of non-homologous interchange types.

When recombinants induced by different doses of X-rays were analyzed, it was found that the yield in the mature larval oocytes which showed a peak sensitivity to induction of recombinations increased curvelinearly up to a dose of 2500 R. The yield of recombinants in oocytes of 4th instar larvae increased also curvilinearly up to 1000 R and then decreased, probably due to cell-killing. In pupal oocytes, no increase in recombinants was observed up to 2500 R, while a markedly large number of polyploid type exceptionals were found.

Ultraviolet-Light Sensitivity of an X-ray Sensitive Silkworm Mutant *rb*

Akio MURAKAMI and Yataro TAZIMA

A silkworm mutant *rb* (red blood) is one of X-ray sensitive stocks for either mutation induction or killing effect (Tazima, 1956). In order to investigate the mechanism underlying the biological effects of ionizing-radiations, the UV sensitivity of *rb* mutant was examined and compared with that of some other UV sensitive stocks. The stocks *Kansen* and *Kojiki* are resistant to UV light; whereas the stock *Aojuku* is sensitive. Also, as reported already, the stocks *Kansen* and *Aojuku* is of the X-ray resistant class; whereas the stock *Kojiki* is of the class relatively sensitive to X-rays (Tazima and Murakami, 1971). After mature oocytes and/or eggs at early stages of cleavage from the above stocks were irradiated with UV, their sensitivities to killing effects and to mutation induction were determined by measuring embryonic mortality and by scoring *pe* : *re* egg-color specific locus mutatins, respectively.

As to the killing effect of UV-light on mature oocytes and/or eggs of the mutant *rb*, a large shoulder was found for dose-embryonic hatchability curve in semilogarithmic plot with UV resistant stocks, whereas a slight shoulder and an almost linear dose-response relation were found for UV sensitive stocks. This finding suggests that the *rb* stock may be one of the UV resistant stocks.

As to the mutagenic effect, the mutant *rb* showed almost the same rate of induced-mutation as those of UV resistant stocks (*Kansen* and *Kojiki*). As has been reported elsewhere, the stock *Aojuku* was UV sensitive for mutation production. Thus, it was suggested that stocks which are UV sensitive for killing effect are also UV sensitive for mutation-production and *vice versa*.

These experiment indicated that an X-ray sensitive stock *rb* was not

UV sensitive, but UV resistant. Furthermore, there may be no positive correlation between UV and X-ray sensitivity of oocytes and/or eggs to both biological effects; and the mechanism(s) involved in killing and mutagenic effects of UV-light may be, at least in part, different from those of ionizing-radiations.

Induction of Recombinants by Mitomycin C in Oogenesis of the Silkworm

Akio MURAKAMI and Isao ARIGA[1)]

Although no crossing-over occurs normally in silkworm females, it has been reported that a significant number of recombinants were clearly detected in prophase I oocytes by treatment with X-rays. The present communication reports our findings on the induction of recombinations in oogenesis of the silkworm by treatment with mitomycin C (MC) which is known to effectively break DNA strands.

For detection of recombinants, the egg-color method was employed. The detailed principle for the method was described in the preceding report. Females from the first instar larval stage to the middle pupal stage having two recessive egg-color mutant genes, *pe* (pink egg: V-0.0) and *re* (V-31.7) in the state of trans type heterozygotes, were treated with either feeding or injection of MC dissolved in 0.85% NaCl solution, and crossed to male moths homozygous for both mutant genes.

The results indicated that the presence of recombinants as well as polyploid type eggs was detected in any stage of germ cells after treatment with MC. The stage showing a peak sensitivity to MC-induced recombinations was the last (mature) larval stage, probably corresponding to the nuclear phases from zygotene to diplotene which is the most likely stage for crossing-over to take place. At the peak sensitive stage the frequency of homologous exchange (crossover) type recombinantions was 4.12×10^{-5} (8/193,984) and that of non-homologous interchange types was 6.70×10^{-5} (13/193,984). This ratio of the frequencies of homologous exchange to non-homologous interchange type recombinations is contary to that of experimental results with X-rays, suggesting that mechanisms related to the induction of recombinations by treatment with MC may be different from those with X-rays. It should be noted, however, that the present finding in the silkworm appears to agree with the result of experiments on *Drosophila* treated with MC (Schewe *et al.*, 1971).

1) Kanagawa-Ken Sericultural Research Center, Ebina-Shi, Kanagawa-Ken.

In the control series, seven eggs out of 189,007 F_1 eggs so far tested were products of recombinations. Six of them were classified as the homologous exchange (crossover) type and the remaining one was an interchange type recombinant. Thus, the spontaneous frequency of homologous exchange type recombinations was calculated to be 3.17×10^{-5}. This finding and others suggested that crossing-over is not completely absent in the silkworm female, but it may be suppressed in some ways.

Radio-Sensitization of Cultured Mammalian Cells by 5-Iodouridine

Yukiaki KURODA, Akiko YOKOIYAMA and Tsuneo KADA

It is well known that cells are more sensitive to ionizing radiation or ultraviolet light when some chemical agents are present at non-toxic low concentrations during irradiation. Such enhancement of the radiosensitivity of cells by halogenated thymidines such as 5-bromodeoxyuridine or 5-iododeoxyuridine was thought to be due to the replacement of thymidine in DNA with thymidine analogues (Zamenhof *et al.*, Nature **181**: 827, 1961; Kaplan *et al.*, Radiat. Res. **16**: 98, 1962; Erikson and Szybalski, Cancer Res. **23**: 122, 1963). On the other hand, our previous studies have shown that radiosensitization with some iodine compounds such as 5-iodouracil, 5-iododeoxyuridine or 6-chloropurine took place by γ-irradiation at 0°C without drug incorporation into bacterial cells, but may involve radiation-provoked radicals containing iodine atoms which might attack certain cellular sites (Kada *et al.*, Int. J. Radiat. Biol. **18**: 281, 1970).

When Chinese hamster cells at acidic pH's were exposed to γ-rays and the number of colonies was scored after 6 to 11 days of incubation, many more cells were inactivated in the presence of the drug than in its absence. 5-iodouridine was markedly effective at concentrations more than 1.0 mM, whereas 6-chloropurine had no such radio-sensitizing effect even at a concentration of 3.0 mM.

This radio-sensitizing effect of 5-iodouridine may be due to radiation-induced toxic products from the reagent, because the cells were inactivated efficiently only by contact with the previously irradiated drug solution. The toxicity of the irradiated drug solution increased markedly when the pH was shifted to the acidic side. Such a toxicity was not detected with 6-chloropurine. It is concluded that the radio-sensitization with 5-iodouridine does not require its incorporation into cellular DNA, at least under the conditions employed in the present studies.

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN MICROORGANISMS AND PLANTS

An Early Step Enzyme Participating in the Excission Type Repair of γ-ray Damaged DNA in *Bacillus subtilis*

Takehiko NOGUTI and Tsuneo KADA

Ionizing radiation produces complex DNA damage including lesions in nucleoside moieties as well as simple chain scission in phosphodiester bonds. Most complex lesions are assumed to be modified and converted to single strand interruptions with 3'OH termini prior to subsequent excision-type repair process, because this terminus structure is essential for the initiation of the DNA polymerase I action involved in it (this Annual Report, No. 23: 72-73, 1972). In this connection we have shown that a protein factor exists and enhances the priming activity of γ-ray impaired DNA. Because

Table 1. Effect of the primer activating enzyme (Phosphocellulose fraction) on DNA's subjected to different treatments

Treatment	PA enzyme treatment*	Priming activity for DNA polymerase I (%)
Control	−	100
	+	105
γ-ray (0.5 kR)	−	42
	+	167
Control	−	100
	+	100
Heat (70°C, pH 5.0, 30 min)	−	111
	+	196
Control	−	100
	+	116
MMS (25 mM, 37°C, 30 min)	−	82
	+	344
UV (15 erg/mm^2, 1 min)	−	78
	+	93
UV (15 erg/mm^2, 4 min)	−	63
	+	78

* 0.64 μg enzyme protein was mixed with 60 μg T7 DNA and incubated for 30 minutes at 37°C.

this enzyme was expected to play a role in an early step of the excision repair, we purified the factor from *Bacillus subtilis* cells by means of DEAE cellulose and phosphocellulose chromatography. One of the two peaks which appeared in phosphocellulose fractions was first examined as to its specificity. As shown in Table 1, this enzyme enhanced the priming activity of heat or MMS treated DNA like γ-ray irradiated DNA. On the other hand, the enzyme had little effect on the priming activity of intact DNA or UV irradiated DNA. The exonuclease activity of the enzyme was very low. Endonuclease activity was examined by sedimentation analysis in sucrose density gradient. When T7 DNA samples which had been previously denatured with alkali or formamide were analysed in parallel in neutral sucrose density gradient containing formaline, a significant number of nicks were introduced by subsequent action of the enzyme at alkali labile lesions produced by ionizing radiation. This nicking action on the alkali labile region of DNA was more clearly demonstrated if DNA was heated to 70°C for 30 minutes at pH 5.0. It is very probable that the DNA lesion susceptible to the above enzyme may be apurinic or apyrimidinic sites because it is known that such lesions are alkali labile and considered to be formed in general by heating, MMS treatment and γ-ray irradiation of DNA. Our primer activating enzyme may be analogous to the apurinic site-specific enzyme reported by Verly *et al.* (Nature New Biology 244: 67, 1973).

Metabolic Activation and *Escherichia coli* Mutagenesis of Furylfuramide, a Nitrofuran Food Additive

Tsuneo KADA

The author has formerly reported that 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (furylfuramide) shows a potent DNA-damaging capacity as shown by the rec-assay utilizing *Bacillus subtilis* strains, and very efficiently induces reversions in *Escherichia coli* strains B/r WP2 *try* and WP2 *try hcr* (Jap. J. Genetics 48: 301, 1973). Similar observations were also made by S. Kondo and H. Ichikawa-Ryo (Jap. J. Genetics 48: 295, 1973). Our tentative experiments have shown recently that the genetic capacity of transforming DNA extracted from *Bacillus subtilis* strain 168T was not inactivated by treatment with furylfuramide in phosphate buffer (pH 7.0). This result in addition to the positive *in vivo* activity of furylfuramide would indicate that the chemical may be metabolically converted to an active form in the cell and react with cellular DNA to produce mutations.

Similar activations must take place also in cultured human cells to induce chromosome aberrations and DNA repair synthesis (Tonomura and Sasaki, Jap. J. Genetics 48: 291, 1973); and in silkworms to induce visible colour mutations (Tazima, Y., 1973).

The above metabolic activation hypothesis of mutation inductions by furylfuramide indicates that mutations would not be induced in cells lacking this activation capacity. It is also supposed that these cells may be resistant to the lethal action of the drug because the supposed activated form should be involved in the cell killing through production of DNA damage. We therefore tried to isolate such strains from *E. coli* WP2 *try*. In a typical one isolated, reverse-mutations were hardly induced by furylfuramide and supplementation of rat liver extract was required for mutation induction.

Cells of WP2 *try* were grown overnight in broth and spread densely on solid broth agar containing furylfuramide (10 μg/m*l*). A number of substrains were selected and purified from drug-resistant colonies which appeared. A typical strain called C801 was used in the following experiments: Cells in broth were collected during exponential growth and washed once by 15/M phosphate buffer (pH 7.0); they were resuspended in the same buffer at a concentration of about 2×10^8 cells per m*l*. Furylfuramide was dissolved in distilled water at a concentration 100 μg/m*l*. Liver homogenate was prepared as described by B. N. Ames *et al.* (Proc. Nat. Acad. Sci. 70: 782, 1973). This fraction contained NADP and glucose-6-phosphate as supplements and was sterilized by filtration with a Millipore membrane. For experiments of mutation induction, two m*l* of bacterial suspension, one m*l* of furylfuramide solution and one m*l* of S9 were mixed at 0°C and kept at 37°C for 10 minutes; the cells were then collected and washed by centrifugation with 15/M phosphate buffer in cold (0°C) and finally resuspended in 0.5 m*l* of the same buffer. The number of viable cells and the frequency of revertants were determined by spreading appropriately diluted cellular suspensions on broth-supplemented minimal agar (MB agar; T. Kada *et al.* Mutation Res. **16**: 165, 1972). For the controls without drug and/or S9, corresponding volumes of buffer were supplemented and incubated similarly.

Results obtained are shown in Table 1. The following points are clear:
1) Reversions were hardly induced by furylfuramide (25 μg/m*l*, at 37°C for 10 minutes) in the strain C801 derived from WP2 *try*. In the case of the original drug-sensitive strain, it was found that a similar treatment yielded mutations that were more than 200 Try^+ cells per 10^7 survivals.
2) When supplemented with S9 fraction the drug produced a significant

Table 1. Activation by rat liver homogenate of furylfuramide-induced reversions in a drug-resistant derivative C801 of *E. coli* WP2 *try*. Treatment of the cells was done at 37°C for 10 min. (See the text for details of experiments.)

Supplements for treatment of bacteria	Number of viable cells per m*l* in suspensions of treated and washed cells	Number of revertant Try^+ colonies found on MB agar; 0.1 m*l* portions were spread and a mean of triplicated plates was obtained	Frequency of induced Try^+ revertants per 10^7 viable cells
Furylfuramide and S9	1.3×10^9	337	24 ± 0.5
Furylfuramide only	1.5×10^9	24	less than 1.0
S9 only	1.6×10^9	32	less than 1.0
None	1.6×10^9	20*	(0)

* These colonies appeared during residual growth of auxotrophic cells in minimal agar supplemented with a small amount of broth.

induction of reversions, 24 times more than that found without S9. Such a mutation induction was not observed with supplementation of S9 without drug.

The above results indicate very clearly that mutation induction by furylfuramide requires a metabolic activation by liver homogenate. It is supposed that a similar metabolism may take place in the cells of *E. coli*. Further studies are required to determine whether our drug-resistant cells are really lacking the supposed activation enzymatic capacity.

Host-Mediated Assay of Mutagenicity of Furylfuramide

Kiyosi TUTIKAWA and Tsuneo KADA

Host-mediated assays are usually devised to detect the mutagenic activity of metabolites of chemical agents in experimental animals (M. C. Cabridge and M. Legator, Proc. Soc. Exptl. Biol. Med. 130: 831, 1969). In the present example, studies were carried out to evaluate the genetic toxicity in the peritoneum of mice to which furylfuramide had been administered orally.

The experimental procedures are based on the host-mediated rec-assay formerly described (T. Kada, K. Tutikawa and Y. Sadaie, Mutation Res. 16: 165, 1972). Seven to nine weeks female or male of closed colony ddY-

SLC were used in the experiments. The chemical substances furylfuramide (FF, 2-(2-Furyl)-3-(5-nitro-2-furyl)-acrylamide), was suspended in 0.5% sodium carboxymethyl cellulose solution and administered orally with doses ranging from 190.6 to 4.7 mg per Kg of body weight. One hour after administration of the drug, a 0.5 m*l* portion of bacterial suspension consisting of 17A (Rec$^+$ *arg*$^+$ *try*$^-$ *str-r*) and 45T (Rec$^-$ *arg*$^-$ *try*$^+$ *str-r*) cells of *Bacillus subtilis* in a modified Vogel Bonner medium without glucose (MM) was injected into the peritoneal cavity of each mouse. One hour after injection of the cellular suspension, the mouse was sacrificed by cervical dislocation and was injected with 1 m*l* of MM into the peritoneum. Immediately the abdomen was kneaded gently in order to promote suspen-

Table 1. Determination of the DNA-damaging capacity in the peritoneal cavity of mice (ddY-SLC) orally administered FF by means of the host-mediated rec-assay

Experimental number (sex of mice used)	FF (mg/Kg) given to each mous		Number of viable cells of *B. subtilis* strains per m*l* of bacterial suspension. Means of 3 mice were obtained	
			17A Rec$^+$	45T Rec$^-$
I (males)	—	Administrated to mice before drug treatment	7.9×10^6	2.1×10^6
	0.0	Recovered from mice	2.3×10^6 (100%)	9.2×10^5 (100%)
	47.7	Ibid.	2.4×10^6 (104%)	2.5×10^4 (2.7%)
II (females)	—	Administrated to mice before drug treatment	8.0×10^5	7.5×10^4
	0.0	Recovered from mice	1.6×10^5 (100%)	6.5×10^4 (100%)
	47.7	Ibid.	2.6×10^4 (16.3%)	0.5×10^2 (0.08%)
III (males)	—	Administrated to mice before drug treatment	2.7×10^6	2.9×10^5
	0.0	Recovered from mice	7.6×10^5 (100%)	2.8×10^5 (100%)
	9.0	Ibid.	6.5×10^5 (85.5%)	9.7×10^4 (34.6%)

sion of the bacterial cells in the injected fluid. Then the fluid was withdrawn directly with an injection syringe. About 1 m*l* of fluid could thus be recovered from an animal and the exudate was filled up to 10 m*l* with MM. The recovered bacterial suspension was diluted appropriately with MM and plated on two kinds of agar media: *MM+Arg−Try* and *MM−Arg+Try* to count the number of viable cells of the original and recovered suspensions.

The results obtained are summarized in Table 1. With an administration of 190.6 mg/Kg, the toxic activity found in the peritonial cavity of female mice was very high and both the *rec*$^+$ and *rec*$^-$ cells were killed completely. Similar activity, though less enhanced, was observed with 95.3 mg/Kg. With a dose of 47.7 mg/Kg (about 1.5 mg per mouse), some DNA damaging activity appeared in the peritoneal cavity 60 minutes after administration, as suggested by the fact that many more cells of 45T Rec$^-$ were killed than 17A Rec$^+$. In one series of experiments, male animals were administered a dose of 47.7 mg/Kg. The genetic toxicity found seemed to be more than that observed in female mice. However, we avoid concluding anything about the sex difference because these experiments are not yet repeated enough. With a dose of 9 mg/kg, the toxic activity decreased remarkably compared to that observed with higher doses, but some activity was still detected.

In order to estimate quantitatively the level of DNA damaging activity *in vivo*, model experiments were carried out *in vitro*. Mixture of Rec$^+$ and Rec$^-$ bacteria were kept at 37°C in MM containing different amounts of furylfuramide, and survival curves were obtained for two kinds of cells. The toxicity level obtained in one series of experiments (Table 1, b) was thus found to be between 0.015 ppm and 0.15 ppm of furylfuramide tested *in vitro*. The activities found may be due to either FF which was not metabolized or certain metabolized compounds which permeated into the peritoneal cavity through the intestinal wall or veins (presented at the 1973 Technicon Symposium, Tokyo).

DNA-damaging Products from Reaction between Sodium Nitrite and Sorbic Acid

Tsuneo KADA

Compared to toxicological studies of single substances, little attention has ever been given to products derived through reactions taking place among chemicals in our environment. Sodium nitrite and sorbic acid often

co-exist in food materials as food additives. We found that they produce "rec-assay positive" substances under moderate or natural conditions. The rec-assay procedures were useful for easy detection of DNA-damaging chemicals or potential mutagens by comparing growth sensitivities of wild and recombination repair-deficient strains of *Bacillus subtilis* against test chemicals (Mutation Res. *16*: 165, 1972; Jap. J. Genetics *48*: 301, 1973; Mutation Res. *26*: 243, 1974).

Sodium nitrite was weakly positive in the rec-assay at high concentrations but the effect was not detectable at low concentrations. Sorbic acid was negative in the assay. When sodium salt sorbic acid and sodium nitrite were dissolved in distilled water to obtain one molar concentration for each chemical, the solution slightly coloured at 0°C or at room temperature indicating some reactions taking place. The reaction seemed to be further enhanced by heating at 100°C. When the reaction mixture was rec-assayed, some positive results were observed compared to those obtained in the control experiments in which each single chemical was assayed. The positive effect showed its maximum value at fifth minuts in the course of heating at 100°C, then decreased.

Dr. H. Hayatsu and his collaborators (University of Tokyo) have carried out chromatography analysis of the reaction products and obtained several fractions possessing rec-assay positive characters. Their preliminary chemical analysis suggested formation of some nitroso-compounds. Studies are under way to find mutation specificity of the above DNA-damaging products from reaction between sodium nitrite and sorbic acid.

Dose Rate Effect of Gamma-rays in Maize Pollen

Taro FUJII

Recovery of premutational damage or mutation frequency decrement in maize pollen grains treated with ionizing radiations has been studied with fractionation dose treatment with gamma-rays or fast neutrons, and we concluded that the premutational damage could be reparable in the case of gamma-rays if a 2-hr interval period was adopted (Ann. Rep. 23: 78-79). In order to get further information about such a reparability, the dose rate effect of gamma-rays was studied using 1 or 40 kR/h intensities of 3 different doses. Two different dose rates were used in these experiments at different distances from a 6 kCi ^{137}Cs-source. A 40 kR/h intensity was most commonly used with our facility. Materials and method used in the

mutation detection were similar to those used in our previous experiments.

In the 40 kR/h series, 1, 2 and 3 kR lots showed mutation frequencies of 2.18, 4.60 and 5.92% in "total" (sum of whole and partial type) mutations. In the 1 kR/h series, however, a clear decrease in mutation frequency was observed, showing 1.30, 3.07 and 3.90% in the 3 dose lots. The mutation frequency decrement in the low dose rate series was about 30% of the high dose rate series. From these results, we can say that some efficient recovery of premutational damage might have occurred with the 1 kR/h intensity. The frequency of whole type mutations was markedly lower in low dose rate lots than in the high dose rate lots, while the frequency of partial type mutations did not show any significant difference in the low and high dose rate treatments.

Though the actual nature of whole and partial mutations is not clear, the above results are in accord with those of our previous experiments in which single and fractionation dose were done. Thus, recovery of about 30% of premutational damage induced by gamma-ray occurred with fractionation dose treatment over a 2-hr interval or by low dose rate treatment with 1 kR/h. However, such a recovery seems to be concerned only with the whole type mutation.

Effects of Gamma-Rays on Sex Expression in Gynoecious Cucumber

Taro FUJII

In monoecious plants, the sex expression is influenced by genetic, environmental and hormonal factors. Reduction in the number of pistillate (female) flowers in monoecious cucumber by gamma-ray treatment has already been reported (Ann. Rep. 23: 76-77). Gynoecious strain (MSU) in cucumber normally produces only female flowers under genetic control, and in the gibberellin treatment at an early seedling stage is required to develop staminate (male) flowers or to obtain homozygous seeds. Dry seeds of MSU-713-5, kindly supplied by Dr. S. Komochi of Hokkaido Agric. Exp. Station, were exposed to 10, 20 and 40 kR of gamma-rays, and the growth habit and flower development examined up to the 50 th nodal stage. Twenty seeds were used in each lot. All plants in 10 and 20 kR lots showed no difference in growth with good germination percentages like in the non-irradiated control lot. One among 18 plants of 40 kR lot had developed 88 male flowers in total over 18 nodes between 4th to 35th nodes, in addition

to female flowers. In this monoecious-like plant (MSU-713-5 M), two fruits were obtained by selfing, but no ripened seed was observed. Other male flowers were crossed to the original MSU-strain and 15 fruits with ripe seeds were obtained. Fertility and germination percentages in the F_1 seeds examined so far was about 50%. Most of the F_1 plants showed normal gynoecious type, but 4, 1 and 2 plants among 33, 5 and 14 plants from 3 examined fruits again developed male flowers. The number of male flowers in these plants was merely 2 or 3 on one or two basal nodes in each plant, while the male parent had developed many male flowers. Although we can not say at present that the male flower development in the gynoecious strain might be due to mutation, it may be possible that some transmissible alteration of the sex expression might have been induced by gamma-ray treatment. Selfing and back- or test-crosses using male flowers developed in F_1 plants are now underway to check whether or not the altered sex expression is introduced by heritable change. Modification of the sex expression, either genetic or non-genetic, might be useful in studies of the sex differentiation in plants.

Germination of *Podostemaceae* Seeds

Etsuo AMANO

Podostemaceae plants are found mostly in tropical streams or rapids and have very strange flowering habits. When the water level of the river decreases and the plants are exposed, they produce flowers and seeds for a short period. There have been some reports on the taxonomy and evolution of this family, as well as some notes on their strange physiology of self maceration when the tissues are taken out of water. However, there is no detailed report on the life cycle or on the genetic relationships among species. Even the chromosome number is not yet known. In cooperation with the Kihara Institute for Biological Research, the author succeeded in germinating the seeds which had been collected by a member of the Kihara Institute from the tropical rivers in Surinam, South America.

Among the media tried, the best result was obtained with 1/10 diluted salts mixture of Murashige and Skoog's medium. Other conditions adopted were constant temperature (25°C) and continuous illumination with Biolux fluorescent lamps. Of the four kinds of seed material tested, *Mourera fluviatilis*, *Apinagia longifolia* and *Apinagia* sp. successfully germinated. However, *Apinagia* sp. having the smallest capsules and seeds failed to germinate. The germination took place on the surface of agar medium,

but the seeds had to be submerged in liquid medium for further growth. The seeds were generally very small. The length of the largest seeds (*M. fluviatilis*) was about 1/4 mm, and that of the smallest (*A.* sp.) was about 1/6 mm. The seeds seemed to have some gluing materials which were released by wetting. Thus they could be attached to slide glass or ground glass surfaces by wetting the seeds with distilled water and redrying them. These slides with adhering seeds could then be surface-disinfected by dipping into equal mixture of 3% H_2O_2 and ethanol for two to five minutes. After rinsing twice with sterilized distilled water, they were put into a deep Petri dish containing liquid medium.

Most seeds germinated on the third day after sowing. The fertility of the seeds was usually more than 80% and the germination rate was over 80% of the fertile seeds. The seedling that emerged from a seed coat with the root part first was green and had two cotyledon-like leaves, which may really be cotyledons. There was no significant root organization except fine root hairs that grow directly from the round bottom of the seedling. The cotyledons opened flat, and had a thick disk-shaped meristematic tissue between them. Two long, narrow, flat leaves emerged from the meristematic disk at right angles to the cotyledons, forming a flat "cross". The third and fourth leaves came out in a similar manner, but, instead of forming a radial rosette, they came out on one side of the previous leaves, suggesting shift of the apical meristem toward one of the cotyledons. After the fourth or fifth leaf appeared from the shifted meristem, the leaves had branches, one on each side of the leaf. The leaves had several remarkable spots which glistened in incident light. These spots looked like some kind of condensed crystal. Since the seedlings are easily damaged by algae, they have been kept under aseptic culture conditions.

Callus Culture of *Haplopappus gracilis*

Etsuo AMANO

Cultured cells of a *Compositae* plant, *Haplopappus gracilis* (Nutt) Gray, have a low chromosome number ($2n=4$) and grow relatively fast on modified Murashige and Skoog's agar medium containing 2, 4 D (2 ppm), yeast extract (0.3%), vitamins and sucrose (2%). Even after two years' culture with about 50 transfers, the chromosome number has remained stable at $2n=4$.

To obtain haploid cell lines, flower bud cultures were tried. Young flower buds were taken from plants grown in the green house about two to four days prior to opening of the ligulate flower. Florets of the central

part of the flower were picked out and placed on the same agar slant as used for the callus culture (2 ppm of 2, 4 D). The anthers were too small to inoculate separately from another part of the floret. No special disinfection procedure was used for the materials, but nearly half of the slants were free of contaminating microorganisms. The floret grew on the agar medium and bloomed some days later. About a month later, callus tissues were found on the floret. They were then transferred to new medium and roughly grouped according to the part of the floret where the callus originated. After five transfers on the agar medium, chromosome numbers of the cells were examined. Of 15 lines examined, all carried the two pairs of chromosomes that are expected for normal diploid somatic cells.

There were some morphological differences among these callus lines, but there seems no relation to the original tissue. A very soft callus has been selected and maintained as HB line for future experiments.

The *Haplopappus* callus cells grew so fast at 25°C and under dark conditions, that transfers were necessary every ten days. However, we had some difficulties with experiments in liquid culture. Conditions for the stable suspension culture were examined and the following points suggested: (1) Sufficient shaking is necessary during incubation to disperse cells in the culture medium. (2) Better results were obtained with 1/2 concentration of the medium than full strength or 1/10 dilution with the same 2, 4 D concentration. (3) Fast and good growth was observed at the initial concentration of 2 mg wet weight per m*l*. Condition (3) seems to be important because, if the initial concentration was too low, growth is very slow until the cells reach this concentration. Under favorable conditions, the maximum rate of the growth was about 7 mg wet weight/m*l*/day.

Experiments on the Grafting of *Capsicum* Mutants

Etsuo AMANO

Graftings of leaf color mutants onto the normal plants of parental line were done to test modification of the genetic traits by grafting. The phenomenon has been reported in *Solanaceae* plants, but it is necessary to test it in a genetically purified experimental system in order to study the genetic aspects or even to confirm the phenomenon itself. For this purpose, some usable leaf color mutants have been induced in *Capsicum annuum* L. var. Sapporo Wase, by ethyl methanesulfonate (EMS). The characters lgA3 and yg^2 could be grafted on normal green homozygotes of parental line above the 2nd leaf. Both mutants segregated in a ratio of one mutant

to three normal plants, suggesting that they are simple recessive mutants. If a normal dominant factor (gene?) can be transferred to the scion or to the seed, normal green tissues or seedlings may be expected.

The grafts were cultured in the greenhouse. The lgA3 character can be observed best in very young leaves; they recover normal green color as they mature. A yellow green or light green mutant, yg2, is a stable and distinct mutant of the leaf color. Leaves of some 17 yg2 scions were examined but no significant color patch of normal green color was found in these leaves. To test the progeny of the grafts, each flower but of the scions was covered by a small paper bag to ensure self pollination.

Seeds thus obtained were sown onto steam-sterilized soil in wooden flats in the green house. The color character lgA3 can be examined on the cotyledon, but better on the first and second young leaves. Out of 34 selfed fruits, 780 seedlings were examined and all of them were mutants. The yg2 mutant can be examined on the cotyledon. Out of 50 selfed fruits, 1271 seedlings were examined and all of them showed *yg*2 phenotype. Sincc leaves of the scions were not removed during the culture of the grafted plants, nutritional dependence of the scion on the stock might be small. Another leaf color mutant, yl, may be a good material for such a nutritional dependence on the stock. The character yl is a recessive yellow color mutant at the seedling stage. It is very weak or lethal unless grafted on the normal green stock. When it was grafted on the parental normal green plant, growth was nearly normal. The tissues near nodes had some chlorophyll but leaves were pale yellow or creamy white. The leaves had a relatively short life and were replaced by new leaves. On these pale yellow leaves, a few small green patches could be observed. The shape of the green patches was such that the mutational events might have taken place in the leaf primodia. The frequency of the green patches, however, was not high, and only three patches were found among 216 yl leaves. Furthermore, the patches were about 1 mm wide and 4 mm long at the largest. Rough calculation using the number and the area of leaves indicated that the frequency of mutational events might be as low as 1.4×10^{-4}. This frequency is much lower than that reported as around 10^{-2}. In the case of yg2 scions, no normal green patches were found in 374 leaves from 17 graftings.

In the all present experiments, no distinct graft mutation phenomenon was observed. The genetic markers induced by EMS were not significantly modified by the stock as reported by different authors. It is likely that the so-called graft mutations might involve physiological modifications or cytoplasmic influences, or some effects of infection with the stock used.

Relationship between Damage Caused by Ageing and by Irradiation in Rice Seeds

Sujit BAGCHI

In order to look into the relationship between the damages caused by ageing and irradiation in rice plants, seeds of fifty-one rice varieties which were kept in a desiccator at a room temperature for five years and then in a 0-1°C room for seven years were tested for their germinabilities and seedling heights. Among them, two varieties with high (451, Surjamkhi from India and 647, Padi Ase Banda from Indonesia) and two with low (545, Shinriki and 564, Nakamura both from Japan) germination percentages were selected. The fresh seeds of those selected varieties were irradiated by gamma-rays with doses of 10 kR, 30 kR and 50 kR. The germination and seedling height of the treated as well as unirradiated control plots were measured.

It was found that varieties with high and low germinabilities in aged seeds also showed high and low resistances to irradiation for both germination and seedling height, when their freshly harvested seeds were irradiated by gamma-rays. It was suggested from the result that there may be similar biological effects between damages due to ageing and irradiation.

VIII. POPULATION GENETICS (THEORETICAL)

The Age of a Neutral Mutant Persisting in a Finite Population

Motoo KIMURA and Tomoko OHTA

Formulae for the mean and the mean square age of a neutral allele which is segregating with frequency x in a population of effective size N_e have been obtained using the diffusion equation method, for the case of $4N_e v<1$ where v is the mutation rate. It has been shown that the average ages of neutral alleles, even if their frequencies are relatively low, are quite high. For example, a neutral mutant whose current frequency is 10% has an expected age roughly equal to the effective population size N_e and a standard deviation $1.4\,N_e$ (in generations), assuming that this mutant has increased by random drift from a very low frequency. Also, formulae for the mean "first arrival time" of a neutral mutant to a certain frequency x have been presented. In addition, a new, approximate method has been developed which enables us to obtain the condition under which frequencies of "rare" polymorphic alleles among local populations are expected to be uniform if the alleles are selectively neutral. It was concluded that exchange of only a few individuals on the average between adjacent colonies per generation is enough to bring about such a uniformity of frequencies. For details see Genetics 75: 199-212.

A Model of Mutation Appropriate to Estimate the Number of Electrophoretically Detectable Alleles in a Finite Population

Tomoko OHTA and Motoo KIMURA

A new model of mutational production of alleles was proposed which may be appropriate to estimate the number of electrophoretically detectable alleles maintained in a finite population. The model assumes that the entire allelic states are expressed by integers $(\cdots, A_{-1}, A_0, A_1, \cdots)$ and that if an allele changes state by mutation the change occurs in such a way that it moves either one step in the positive direction or one step in the negative direction. It was shown that for this model the 'effective' number of selectively neutral alleles maintained in a population of the effective size

N_e under mutation rate v per generation is given by

$$n_e = \sqrt{1+8N_e v}\,.$$

When $4N_e v$ is small, this differs little from the conventional formula by Kimura and Crow, *i. e.* $n_e = 1+4N_e v$, but it gives a much smaller estimate than this when $4N_e v$ is large. For details see Genet. Res. Camb. 22: 201-204.

Effect of Linkage on the Behavior of Mutant Genes in finite Populations

Tomoko OHTA

The effectiveness of the apparent or "associated" overdominance caused by linked selected loci for maintaining genetic variability was investigated using diffusion models. It was shown that when a population is subdivided, the amount of linkage disequilibrium is determined by the local population size if the recombination fraction is much larger than the migration rates. Also, it was shown that, although the associative overdominance temporarily creates a sort of inertia against perturbation in gene frequencies, it cannot determine any definite equilibrium values. Hence, it has no long-term effect on the rate of mutant substitution in evolution. For details, see Theoret. Pop. Biol. 4: 145-162.

IX. POPULATION GENETICS (EXPERIMENTAL)

Analysis of Heterozygosity in Regard to the Neutral Theory of Protein Polymorphism

Takeo MARUYAMA and Tsuneyuki YAMAZAKI

In conjunction with a theoretical calculation, a distribution of heterozygosity (more precisely, $2x(1-x)$, where x is the allele frequency) was examined with special reference to the neutral hypothesis of protein polymorphisms. Data of some 400 polymorphic proteins collectively are consistent with the theoretical expectation based on the neutral hypothesis, although other possibilities are not ruled out.

The distribution of neutral alleles for randomly-mating populations is known (Kimura and Crow, 1964), and from it we can calculate the distribution of the amount of heterozygosity. If we let N be the population size and u be the mutation rate, the number of alleles whose frequency is in the interval $x\pm\delta x/2$ is proportional to $x^{-1}(1-x)^{4Nu-1}$. Therefore, if we multiply this formula by $2x(1-x)$, we get the amount of heterozygosity. Namely,

$$2(1-x)^{4Nu}\,, \qquad (1)$$

which is proportional to the sum of the heterozygotes whose gene frequency is in the interval $x\pm\delta x/2$.

From the same literature used in our previous paper (Science 183: 1091, 1974), we have collected 1053 alleles (over 400 loci), involving many genera and species. All alleles were classified according to their frequency into twenty equally spaced classes: 0—0.05, 0.05—0.1, ···, 0.95—1. Then the heterozygosity was calculated from the allele frequency, and the values were summed. Each sum represents the value of the heterozygosity of that class.

To determine the theoretical expectation, we need to know the value of $4Nu$ in formula (1). It can be shown that if f is the probability that two randomly chosen genes are identical by descent, $1/f=1+4Nu$. The value of f is rather invariant over a wide range of organisms, but it varies among different loci. The estimated values of $4Nu$ for some loci are close to zero, while those for other loci are as high as 0.5, which is about the highest we found. Therefore, we present in Figure 1 the curves given by (1) for two extreme values of the parameter, $4Nu=0.5$ and 0 together with observations. The data appear to be consistent with the theoretical

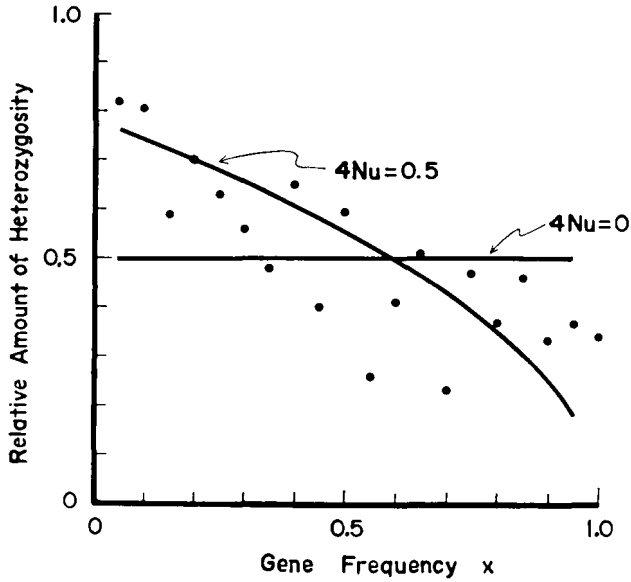

Fig. 1. The curves indicate the theoretical expectations based on the neutral hypothesis. The dots indicate the observed results. The amount of heterozygosity is normalized so that the total area under each curve and the dots is one.

expectation. Expectations based on other assumptions have different patterns. For example, that based on the assumption that the majority of polymorphisms are maintained by some sort of balancing selection with equilibrium frequency uniformly distributed has a high peak at the neighborhood of $x=1/n$, where n is the number of alleles maintained in the population. Therefore, this result supports the neutral hypothesis that genetic drift plays the main role in the maintenance of protein polymorphisms in natural populations.

Genes Affecting Productivity in Natural Populations of *Drosophila melanogaster*

Takao K. WATANABE

Two hundred second chromosomes were extracted from the Katsunuma population in October of 1972, and the viabilities and productivities of homozygotes and heterozrgotes were examined. Viability was measured by

the *Cy*-method and the productivity by the number of progeny produced per female. The frequency of lethal-carrying chromosomes was 0.315. When the average heterozygote viability was standardized as 1.000, the average homozygote viability was 0.595 including the lethal lines, and 0.866 excluding them. The frequency of recessive sterile chromosomes among 131 non-lethal lines was 0.092 in females and 0.183 in males. There were two instances in which homozygosis for the second chromosome caused sterility in both sexes, which was close to the number expected (2.2) from a random basis of $0.092 \times 0.183 \times 131$. When the average heterozygote productivity of 200 lines was standardized as 1.000, the average homozygote productivity was 0.532 including femals steriles, and 0.584 excluding them. The ratio of detrimental load to lethal load was 0.383, while the ratio of partial sterile load to complete sterile load was 5.767. The average viability of lethal heterozygotes was slightly, but not significantly, lower than that of lethal-free heterozygotes; while the average productivity of lethal heterozygotes was significantly lower than that of lethal-free heterozygotes. There were no statistically significant differences in viability or productivity between sterile heterozygotes and non-sterile heterozygotes. The heterozygous effects of viability and productivity polygenes were examined by regressions of the heterozygotes on the sum of corresponding homozygotes. The regression coefficients were consistently positive for both viability and productivity if lethal and sterile chromosomes were excluded. The correlation between viability and longevity was significantly positive in homozygotes; the correlation between viability and productivity in homozygotes was significantly positive when sterile chromosomes were included, but the significance disappeared when the sterile chromosomes were excluded. In the heterozygotes there were no detectable correlations among viability, longevity and productivity. The homozygous sterile chromosomes in both sexes had significantly lower viability in homozygotes.

Tabel 1. Average viability and productivity of lethal heterozygotes.

Genotype	No. of crosses	Viability±s. e.	Productivity±s. e.
Non-lethal/Non-lethal'	90	1.0044±.0048	1.0302±.0129
Lethal/Non-lethal	94	.9972±.0055	.9804±.0147*
Lethal/Lethal'	16	.9873±.0113	.9462±.0210**

* Statistical significance at the 5% level.
** Statistical significance at the 1% level.

Genetic Changes in Natural Populations of *Drosophila melanogaster*

Takao K. WATANABE, Taishu WATANABE and Chozo OSHIMA

Annual censuses of genetic variation on the second chromosome have been taken in a Japanese natural population for more than ten years; the frequencies of both recessive lethals and polymorphic inversions have been examined every fall in the population at Katsunuma. The lethal ferquency was about 15% in the 1964-1968 period; it increased from 1969 to 1970 to 30%. The frequency doubled in the two years, and the population has maintained this new lethal frequency in recent years (1970-1973). The allelism frequency of the lethals did not change so much, showing no significant correlation with the increased frequency of lethals. The accumulation of mutations other than lethals was also measured by the homozygous genetic load, *i. e.* the detrimental load (non-lethals) and the lethal load (lethals). The ratio of detrimental load to lethal load (D/L) was slightly but not significantly decreased as the lethal frequency increased.

Another sudden change observed in the population was in the frequencies of chromosomal arrangements. Two polymorphic inversions, *In*(2*L*)*B* and *In*(2*R*)*C*, had been maintained at high levels of about 35% and 25% during the early sixties. In 1969 and 1970 their frequencies began to decrease, until they were reduced by half in the early seventies. Thus, the frequency

Table 1. Frequencies of second chromosome lethals, their allelism and inversions, and the ratio of detrimental load to lethal load (D/L).

Year	No. of chr. tested	% of le.	No. of crosses	% of allelism	% of			D	L	D/L
					In B	*In C*	*ST*			
1963	668	20.2	6441	2.64	32.0	21.0	53.7			
1964	826	15.7	3403	4.20	27.0	25.5	54.4			
1965	905	15.5	8646	1.95	35.0	25.5	48.4	.128	.167	.767
1966	323	12.4	1830	2.02	40.5	30.0	41.7			
1967	974	15.0	6216	6.44	—	—	—			
1968	571	15.2	3741	3.80	29.0	20.5	56.4	.121	.166	.729
1969	201	23.1	—	—	26.5	14.5	62.1			
1970	412	30.8	6085	4.35	15.5	14.0	71.4	.232	.366	.634
1971	410	29.5	3160	1.46	17.0	9.0	75.1	.256	.349	.734
1972	351	34.8	1994	2.96	16.5	9.5	75.1	.221	.428	.516
1973	204	31.9	666	5.71	13.0	12.5	74.0	.213	.385	.553

of inversion-free chromosomes (*ST*: standard arrangements) increased in the population as the homozygous load increased. Why this should happen is unknown. Additional data collected from other Japanese populations (Yamagata, Okinawa) and U. S. populations (Raleigh, Texas) fit very well the linear regression of lethal frequency on standard arrangement frequency which was obtained for the Katsunuma population.

X. EVOLUTIONARY GENETICS

Mutation and Evolution at the Molecular Level

Motoo KIMURA and Tomoko OHTA

Some consequences of the neutral mutation-random drift hypothesis of molecular evolution and polymorphism were worked out with special reference to the relationships among evolutionary rates, mutation rates, protein electrophoretic mobilities, functional constraints and molecular structure. A graphical method was devised to show the pattern of extinction, multiplication and mutational steps involved for selectively neutral mutants in a finite population in the course of evolution. Using the observed mammalian evolutionary rate of protein-encoding cistrons and average heterozygosity for human isozyme polymorphisms, and assuming that the latter are transient states of neutral evolution, it is inferred that, very roughly, a new allele is substituted in each cistron every 10 million years, each individual substitution taking an average of 2 million years. The upper limit of the evolutionary rate is the mutation rate. Using data on the incidence of rare hemoglobin variants in Japanese populations, it was estimated that the mutation rate per amino acid site is about 9×10^{-8} per generation. It was shown that in hemoglobins, the rate of amino acid substitutions at the surface parts of the molecules is about 10 times that in the heme pocket in the course of mammalian evolution, although there is no indication that mutation rates differ at different parts of the molecule. For details see Genetics (Supplement) 73: 19-35.

Eukaryote-Prokaryote Divergence Estimated by 5S Ribosomal RNA Sequences

Motoo KIMURA and Tomoko OHTA

In order to estimate the evolutionary distances among 5S ribosomal RNA sequences, we made the alignment using published data on human, yeast and bacterial (*Escherichia coli* and *Pseudomonas fluorescens*) sequences, and counted the numbers of base differences by comparing them to each other. From the fraction of different sites between sequences, the average number of nucleotide substitutions per site that separate the sequences was estimated. The number turned out to be 0.817 between the eukaryotes and prokaryotes, whereas it was 0.561 between the human and yeast. The

remoteness of the eukaryotes-prokaryote divergence relative to the human-yeast divergence can be estimated by the ratio 0.817/0.561, which is approximately 1.5. From comparative studies of cytochrome *c* sequences, the time of the human-yeast divergence was estimated to be about 1.2×10^9 years. Therefore the divergence between eukaryotes and prokaryotes is estimated to go back to some 1.8×10^9 years. For details see Nature New Biology 243: 199-200.

Slightly Deleterious Mutant Substitutions in Evolution

Tomoko OHTA

The concept of neutral mutant substitution in a population by random genetic drift can be extended to include random fixation of very slightly deleterious mutations. In fact, there are a few observed facts which suggest that this process is important in molecular evolution. In particular, Fitch's concept of covarion (concomitantly variable codons) can be understood readily by considering the secondary or tertiary structure of the molecule: If the first mutant disturbs very slightly the normal functioning of the molecule, there are probably many ways of compensating for it and hence the first mutant provides a new possibility for evolutionary change. A simple example is coupled base substitutions in the helical region of transfer RNA. It was pointed out that many facts can be explained by assuming that the class of mutations with very slight negative effects are important. For details, see Nature 246: 96-98.

XI. HUMAN GENETICS

Fingerprints in Ainu

Ei MATSUNAGA and Ei IKEDA

As part of anthropological studies of the Ainu in Hokkaido, fingerprints were taken from pupils of three junior high schools located in Niikappu, Urakawa and Piratori townships, in the District of Hidaka. The material yielded a sample of 923 unrelated subjects, of which 173 were indentified as Ainu in origin through genealogical investigations carried out by other members of the team. The rate of admixture with the surrounding Wajin, *i.e.* the ordinary Japanese in the same environment as the Ainu, was estimated to be about 30-40%.

It was found that the Ainu had more ulnar loops and less whorls than the Wajin, and that they had a significantly larger pattern size of ulnar loops. In both Wajin and Ainu groups, however, the mean ridge-count of whorls per digit was larger in boys than in girls, while no such difference was found for loops; the result has confirmed the one previously obtained by us for Honshu Japanese. The distribution of total finger ridge-count in the Ainu showed a marked variation from locality to locality, probably because of random drift. Details of the studies will be published elsewhere.

Sequence Analysis of Human IgA Immunoglubulin

Tomotaka SHINODA

Amino acid sequence analyses were performed using fragments obtained by CNBr cleavage at methionyl bond from a completely reduced and alkylated heavy chain of human IgA myeloma globulin. Following the cleavage, four fragments, FI, FII, FIII and FIV, were separated by column chromatography in a urea-propionate system. Of these, FII and FIV were further purified by ion exchange column chromatography in phosphate-urea-EDTA buffer. FIV had an identical sequence with that of the COOH-terminal portion of the alpha chain. FII was further digested with proteolytic enzyme, and the smaller peptides were separated with a column of AG 50−×2, AG 1−×2 and by paper electrophoresis at pH 3.7. FI was a large fragment and contained the total carbohyrates of the intact alpha heavy chain; therefore it was concluded that FI covered almost the entire

Fdc-Fc region. Several CMC-containing peptides were isolated from FI after tryptic digestion. Among them was a proline-rich peptide, which was later identified to be the hinge peptide. Several other CMC-peptides were also completely or partially sequenced but the order of each peptide in FI has not been determined. The results are summarized as follows:

FII, Glu-Val-Gln-Leu-Val-Glu-Ser-Gly-Gly-Gly-Leu-Val-Lys-Pro-Gly-Gly-Ser-Leu-Arg-Leu-Ser-Cys-Ala-Ala-Ser-Gly-Phe-Thr-Phe-Thr-Arg-Gly-Gly-Leu-Glu-Trp-Val-Arg-Gln-Ala-Pro-Gly-Lys-Ala-Leu-Glx-Trp-Val-Leu-Val-Phe-Ser-Val-Thr-Gly-Asx-Asx-Lys-Phe-Tyr-Thr-Glu-Ser-Leu-Asn-Gly-Arg-Phe-Thr-Ile-Ser-Arg-Asx-Asx-Ser-Lys-Asn-Thr-Leu-Tyr-Leu-Glx-Met; FIV, Ala-Glx-Val-Asx-Gly-Thr-Cys-Tyr; hinge, His-Pro-Thr-Asn-Pro-Ser-Glu-Asp-Val (Thr, Val, Pro, Cys, Pro, Val, Pro, Ser, Thr, Pro, Pro, Thr, Pro, Ser, Pro, Ser, Thr, Pro, Pro, Thr, Pro, Ser, Pro, Ser, Cys, Cys, His, Pro, CHO)-Arg; FIT1, Leu-Ser-Leu (His, Arg. Pro, Ala, Leu, Glx, Asx, Leu, Leu, Leu, Gly, Ser, Glx, Ala, Asx, Leu, Thr, Cys, Thr, Leu, Thr, Gly, CHO) Leu-Arg; FIT2, Asx-Phe (Pro, Pro, Ser, Glx, Asx, Ala, Ser, Gly, Asx, Leu, Tyr, Thr, Thr, Ser, Ser, Glx, Leu, Thr, Leu, Pro, Ala, Thr, Glx, Cys, Leu, Ala, Gly, Ser) Lys; FIT3, Asx-Leu-Cys-Gly-Cys (Tyr, Ser, Val, Ser, Ser, Val, Leu, Pro, Gly, Cys, Ala, Glx, Pro, His, Gly, Asx) Lys; FIT4, Gly-Asx-Thr-Phe-Ser-Cys-Met; FIT5, Ser-Val-Thr (Cys, His, Val) Lys.

In addition to these peptides there were a few CMC-peptides, but they were not pure enough for sequence study.

Isozyme Variations in Man

Tomotaka SHINODA, Ei MATSUNAGA and Jushiro KOSHINAGA*

Recent developments in biochemical analyses have made it possible to assess the degree of genetic variation of individuals to a much more precise level than a decade ago. Using tissue extracts, we have undertaken a survey on a variety of enzymes by means of gel electrophoresis and specific staining. Last year we analyzed 16 different enzyme systems on 64 samples, and found that 6 loci were polymorphic. This time we have tested 25 different loci on a total of 56 tissue samples collected in Tokyo. Samples were relatively fresh and there existed hardly any problems of secondary isozymes due to *in vivo* modifications.

Of 25 different loci tested, 7 were found to be polymorphic with practically the same frequencies as in the previous study. They are: adenosine

* Medical Examiner's Office, Tokyo.

deaminase (ADA 1, 54; ADA 2-1, 2; total 56), alcohol dehydrogenase (ADH_3 1, 20; ADH_3 2-1, 27; ADH_3 2, 9; total 56), 6-phosphogluconate dehydrogenase (PGD A, 49; PGD AC, 7; total 56), phosphoglucomutase-1 (PGM_1 1, 35; PGM_1 2-1, 19; PGM_1 2, 2; total 56), phosphoglucomutase-3 (PGM_3 1, 36; PGM 2-1, 18; PGM_3 2, 2; total 56), s-glutamate-pyruvate transaminase (GPT 1, 22; GPT 2-1, 25; GPT 2, 9; total 56), m-glutamate-oxaloacetate transminase (GOT 1, 49; GOT 2-1, 7; total 56).

In addition to these enzyme systems, a mitochondrial tetrazolium oxidase was found to exist in multiple forms (m-TOX 1, 55; m-TOX 2-1, 1; total 56). NADP-dependent isocitrate dehydrogenase, aldehyde dehydrogenase and xanthine dehydrogenase were also found to be in multiple forms. These phenomena, however, are not likely to exist in genetic origin but in secondary modifications caused by, for example, allosteric effect due to unbinding/binding with coenzyme.

Amplicon Model for Antibody Variability

Tomotaka SHINODA

Among the most fascinating and challenging problems in mammalian genetics, the nature of the genetic control of antibody variability is yet to be solved. Vertebrate organisms are believed to be endowed with the ability to synthesize thousands of different species of antibodies, each presumably encoded by a different antibody gene. So far two major lines of hypotheses have been proposed concerning the mechanism of antibody variability: the germ line and somatic theories. The somatic theory postulates that antibody genes arise by appropriate mutational events during somatic differentation, whereas the germ line theory claims that each organism has a separate germ line for each antibody polypeptide chain. Both theories appear to have paradoxes within themselves from the standpoint of basic biological economics.

To escape the paradox, an alternate model referred to as "Amplicon model" is proposed for antibody variability, based on our own and other's sequence data on immunoglobulins. The basic idea of the model is as follows:

Since the total number of germ line genes in haploid mammalian species may not, on the average, exceed $\sim 10^6$, and since the organisms can hardly spare genes only to code antibody molecules, the maximum number of the genes which code for antibodies should be far less than the above figure. If we follow the assumption that each antibody polypeptide chain, both

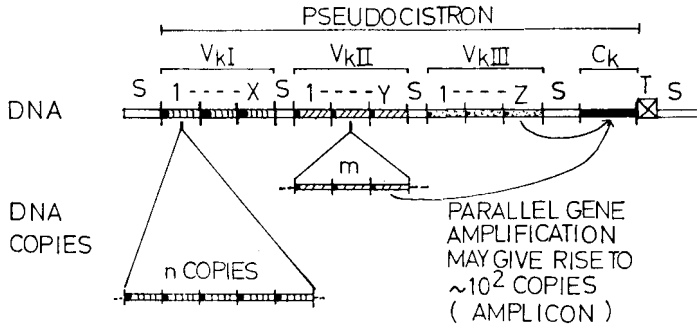

Fig. 1. Amplicon model for antibody variability.

light and heavy, is encoded by a set of separate genes–V and C genes–then at least 14 different genes may be assumed to be in the constant region of antibodies. As for the variable region, it has become increasingly clear that the first 110 residues of both light and heavy chains can be classified into subgroups according to their sequence similarity. If it is assumed that each V-region subgroup is encoded by a certain number of germ line genes, ~10 for example, then the total number of the germ line genes for antibodies may be on the order of $\sim 10^2$. This figure corresponds to no more than 1/1000 of the total informational part of the genetic material of mammalian genome, if we follow an assumption that only a few per cent of the genetic material is informationally active in mammalian organisms. The idea is illustrated in Fig. 1, where stacks of a certain number of repeated DNA segments are oriented in the order within each subcistron which constitutes a "pseudocistron" together with other subcistrons of different subgroups. Each subcistron consists of a set of different but similar DNA segments of about 400 nucleotide pairs. As for light chains, the model postulates that each subcistron can produce a number of copies called "amplicon" which later undergoes genetic fusion with the C gene(s) within the same pseudocistron. In the case of the heavy chains, the model requires at least 5 sets of the C genes, and these genes can fuse randomly with any one of the genes of any subgroups to form a complete stretch of antibody gene.

Frequencies of *C* and *Q* Variants in Human Chromosomes

Kazuso IINUMA and Ei MATSUNAGA

Unrelated individuals with normal karyotypes, a total of 27 males and 28 females, have been studied with *C* and *Q* banding methods. Their karyotypes have further been characterized by distinctive patterns of *C* and *Q* polymorphisms in those regions defined as variable in the Paris Conference (1972). After *C* banding treatments, two sizes of heterochromatin blocks in secondary constrictions were observed. The large one, appearing as the most frequent type, was designated as '*C*', and the other infrequent one as '*c*'. Frequencies of '*c*' of #1, 9 and 16 chromosomes were 0.18, 0.19 and 0.07, respectively. Fluorescence intensity of the paracentric regions was determined of #3, 4, 13, 14, 15, 21 and 22 chromosomes. Strongly fluorescing ones were designated as '*F*', and weakly fluorescing ones as '*f*'. Frequencies of '*F*' were 0.58, 0.31, 0.48, 0.14, 0.02, 0.12 and 0.20, in orders of the chromosomes mentioned above. For each of these chromosomes, the expected incidences of '*CC*' or '*FF*', '*Cc*' or '*Ff*' and '*cc*' or '*ff*', were calculated from the frequencies, and the values obtained were compared with those observed in our samples. No significant discrepancy ($p<0.05$) was seen in any chromosome except #4 and 13. *Q* variants of #4 are in fact difficult to distinguish from each other because both of them have a strongly fluorescing band adjacent to the very variable region near the centromere. However, *Q* variants of #13 are much more easily identified, because of the absence of any confusing segment around the region, and of a clear-cut appearance of '*F*' in contrast to 'f'. The implication of this discrepancy observed in #13 is not clear.

In addition, 5 patients with regular 21 trisomy and their normal parents were studied of their *Q* variants of #21 chromosomes. In one family, it could be safely denied that the non-disjunction had occured in the second stage of maternal meiosis.

A part of this study was reported in the 18th annual meeting of the Japan Society of Human Genetics (1973).

XII. BEHAVIORAL GENETICS

Short-Term and Long-Term Memories in Different Strains of Inbred Mice

Tohru FUJISHIMA

This experiment was designed to obtain basic information for a selection experiment on the learning ability of mice. Adult mice of 15 inbred strains, each comprising 10 males and 10 females, were measured for right-left discrimination ability, conditioned avoidance and escape responses, and activity using a Y-maze apparatus in which lamp and buzzer were used as conditioned stimulus and electric shock as unconditioned stimulus. Two sessions, each consisting of 50 trials, were carried out at 24 hour intervals, and the short-term and long-term memories were measured in percentage. The long-term memory was calculated as the difference between the performance in the first session (denoting short-term memory within the same day) and that in the second session.

The results indicated that there were significant differences in all characters among the strains. As to the short-term and long-term memories in discrimination ability, respectively, C3H/He and C57BL/6 was high and high, D103 low and low, C57L, C57BR, DBA/2 and DBAf/Lw low and high, and SWM and RF high and low. In general, related strains showed similar performances in learning ability.

XIII. APPLIED GENETICS

Analysis of Genes Controlling F_1 Sterility in Rice by the Use of Isogenic Lines

Hiko-Ichi OKA

In order to look into the genetic basis of intervarietal F_1 sterility in rice (*Oryza sativa* L.), a series of backcrosses (up to B_{13}) was carried out using Taichung 65 (Japonica type) as the recurrent parent and several Indica varieties as donor parents. A number of "isogenic F_1-sterile lines" were isolated by test-crossing fertile F_2 plants obtained from the selfing of partly pollen-sterile backcross segregants. Crossing experiments with the isogenic lines confirmed the author's previous hypothesis that there are sets of duplicate gametic lethals (*s* genes) and gametes carrying a double recessive combination (s_1 s_2) of them deteriorate during development: though in the present hypothesis the genes are considered to affect the development of microspores only. Assuming that Taichung 65 has s_1/s_1 $+_2/+_2$ and a donor parent as well as an isogenic F_1-sterile line derived from it have $+_1/+_1 s_2/s_2$, pollen grains with $+_1 s_2$ have shown in the genetic background of Taichung 65 a higher fertilizing capacity than those with $s_1 +_2$, while those with $+_1$ $+_2$ have a lower fertilizing capacity. This certational advantage of alien genes was considered to be an internal mechanism that helped the development of F_1 sterility relationships among rice varieties. The isogenic F_1-sterile lines derived from different donor parents each had a set of *s* genes at different loci. Linkage relations were detected between the *s* loci and three gene markers. (Genetics, in press)

Breeding of Isogenic Lines of Rice Carrying Various Gene Markers and Reciprocal Translocations

Hiko-Ichi OKA and Hiroko MORISHIMA

This work has been continued for 10 years though not reported in previous issuse of the Annual Report. Isogenic marker lines were isolated from the selfing of heterozygotes backcrossed to the recurrent parent 7 to 16 times. Donor parents carrying different markers were obtained through the kindness of Dr. M. Takahashi of the Hokkaido University, and Taichung 65 (T65, a Japonica type from Taiwan) was used as the recurrent parent throughout

the experiments. The marker genes so far isolated in isogenic lines are: *wx* (glutinous endosperm, 1st chromosome), d_2 (Ebisu dwarf, 2nd), *lg* (liguleless, 2nd), *Ph* (phenol reaction, 2nd), *Rd* (red pericarp, 3rd), *Rc* (brown pericarp, 4th), d_1 (Daikoku dwarf, 6th), *la* (lazy habit, 8th), *ne* (bract development, 9th), *bc* (brittle culm, 11th), *gb* (glabrous leaf, 12th), etc. The pleiotropic effects of these genes are under observation.

Further, 30 isogenic lines of T65 carrying different reciprocal translocations were obtained from the progeny of radiation-induced semi-sterile plants after recurrent back-crossing (3 times) and test-crossing. Which chromosomes are involved in each of them is still under observation. As these lines will be of some use for rice geneticists, their seeds are offered to the workers who need them.

Character Variation and Inheritance of Sterility in the Backcross Progenies of a Species Hybrid in Rice

Hiko-Ichi OKA and Hiroko MORISHIMA

The F_1 hybrid between two cultivated rice species, *Oryza sativa* L. and *O. glaberrima* Steud. (highly pollen sterile though meiotic chromosome pairing is normal), was backcrossed by the parental strains, and the characters distinguishing between the parental species and pollen fertility were recorded for B_nF_1 and B_nF_2 plants. This experiment has been continued since 1966, and the B_8F_2 plants were observed this year. The pattern of character variations as shown by correlation coefficients differed according to the genetic background of the plants estimated by the coefficient of relationship to one of the parents. As to the genetic basis of sterility, it was suggested that some unfavorable interaction between the sporophytic and gametophytic effects of particular genes could be the major factor causing the deterioration of microspores. Further experiments on the genetic basis of this hybrid sterility are under way.

Phenotypic Plasticity and Yield Stability in Rice Cultivars

Hiroko MORISHIMA and Hiko-Ichi OKA

Our part as coworkers of the International Rice Adaptation Experiment (IBP/UM) was to look into varietal variations in the plasticities of grain yield and yield-component characters. The data studied came from tests of 10 rice varieties of different origins at seven experimental stations

distributed in Japan, Taiwan, India, and U.S.A. which were continued for two years (1971 and 1972; Phase B). Though the details will be published elsewhere, one point suggesting the nature of genotypic difference in yield stability is presented here. An examination of interrelationships between the standard deviations of various agronomic characters indicated that plasticity in a developmental stage could be associated with stability in the subsequent stage. Thus, yield stability appeared to depend on a certain degree of plasticity in the tillering stage and non-plasticity in later stages. It was also pointed out that successive selection for better yield of hybrid materials raised in different cropping seasons, called "disruptive seasonal selection", was an effective method for improving yield stability.

Intervarietal Variations in Growth Pattern Obtained from the International Rice Adaptation Experiment

Hiroko MORISHIMA and Hiko-Ichi OKA

Variations in logistic growth curve, among rice varieties were investigated using dry-matter weight data from the International Rice Adaptation Experiment (IBP/UM). Two major trends of variation were detected, one represented by the developmental stage at which growth rate becomes maximum (variation between "early- and late-vigor" types), and the other represented by the durability of a high growth rate (between "sustained- and dash-vigor" types). These variations were found to be controlled by varietal genotypes. Early vigor contributes to the production of many spikelets, and late vigor to filling the spikelets with grain. Sustained-vigor types gave more grain yield than dash-vigor types. The magnitude of change in these vigor types due to fertilizer application also appeared to be a varietal characteristic.

Variation in Weediness Among Strains of Dallisgrass

Hiroko MORISHIMA

Seeds were collected from several natural populations of dallisgrass (*Pasupalum dilatatum*) at different sites in Kyushu and in the Misima area, and the plants from the seeds were examined with the hope of demonstrating ecological characters supporting the weediness of this species. Observations were made in an experimental field for variations in metric characters, the response to walking-over by man, density response, and

competition with a closely related native species, *P. thunghergii*. Dallisgrass produces its seeds mainly by apomixis. As expected from this breeding behavior, strains from different populations did not differ much in characters though the differences were partly significant statistically. The response to walking-over by man significantly differed among strains. When mix-grown with *P. thunghergii*, dallisgrass was advantageous at a low planting density but was disadvantageous at a higher density. The weediness of dallisgrass as compared with other weed species is still under observation.

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

Chiang PAI, Toru ENDO and Hiko-Ichi OKA

Peroxidase zymograms of the leaf blade, leaf sheath and lemma-palea were observed in progeny of crosses between strains of *Oryza perennis* Moench and *O. sativa* L. Bands OC, 2A, and 4A were found to be specified by codominant alleles, Px_1^{OC}, Px_1^{2A}, and Px_1^{4A}, respectively. These alleles produced hybrid bands 1A and 3A in their heterozygotes. Regarding band 4C specified by Px_2^{4C} (independent of Px_1), it was found that a *perennis* strain (W120-4, derived from an Indian natural population) had the band in the leaf sheath only, while another (W120-5, from the same population) showed it in the leaf blade only. Observations of their selfed progenies and hybrids suggested that there were two independent dominant genes repressing band 4C, one ($R_{LB}{}^{4C}$) acting in leaf blade and the other ($R_{LS}{}^{4C}$) acting in leaf sheath. On this basis, the organ-specific intensity variations of the 4C band observed in four different crosses could also be explained. These genes were considered to be "regulatory genes" repressing the activity of Px_2^{4C}.

(Published in Can. J. Genet. Cytol. 15: 845-853, 1973)

Intergenotypic Competition and Cooperation in Mix-Planting of Rice Strains

Tsuguhiro HOSHINO and Hiroko MORISHIMA

A series of mix-planting experiments was conducted with various rice varieties and hybrid-derived lines to study intergenotypic competition and cooperation in mix-planting of rice strains. Three different models given

by P. Jacquard and J. Caputa (1970, Ann. Amèlior. Plantes 20: 115-158) were employed. They were: 1) evaluation of aggressiveness and resistance to aggression, 2) analysis of "domination matrix", and 3) estimation of regression coefficient on mean values for various combinations of mix-planting. In addition to the strong effect of competition, cooperation effect as shown by a gain or a loss in both mix-planted genotypes was demonstrated in certain combinations of strains. The detail will be published elsewhere.

Variations in Isozymes and Other Characters Among F_8 Lines Derived from *Oryza perennis* × *O. sativa* Hybrids

Chiang PAI and Hiko-Ichi OKA

The Asian strains of *Oryza perennis* Moench, the wild progenitor of *O. sativa* L., carry a number of different alleles for peroxidase and acid phosphatase variations. However, only a small part of them is found among cultivars of *O. sativa*. Why were many isozyme alleles lost when the wild plants were domesticated? In order to look into this problem, 142 F_3 lines from 6 *perennis-sativa* crosses which had different isozyme alleles were observed for three characters distinguishing between wild and cultivated forms of rice, *i. e.*, seed shedding, rachis angle, and awn length. No particular linkage relations were found, however, between genes for those characters and isozymes. The problem should now be considered from a different angle.

Further Investigations of Geographical Variation in Isoperoxidase in Populations of *Pinus Thunbergii*

Shin-ya IYAMA

Variation in isoperoxidase of *Pinus Thunbergii* populations was investigated in the area along the Japan Sea coast. Needle leaves were collected from about 40 trees randomly selected from each of six local populations in Toyama, Ishikawa and Fukui prefectures. Sap squeezed from the needle leaf was used for electrophoretic study of isoperoxidase variation. Incidences of several bands of peroxidase isozyme were investigated. Comparing the result with those obtained previously for the populations in Eastern and Western Japan, the populations used for the present study were similar to

those from Eastern Japan and had lower frequencies of the bands than those in Western Japan. For example, the average frequency of band *Q* in the present populations was 8.8%, while those in Shizuoka and Niigata prefectures (Eastern Japan) were 24% and 21%, and those in Hiroshima and Tottori prefectures (Western Japan) were 89% and 62%, respectively.

Gamma-ray Induced Increase in Intra-Line Variability in *Arabidopsis thaliana*

Sujit BAGCHI and Shin-ya IYAMA

We reported before (Ann. Rep. N. I. G. No. 23) an increase in intra-line variability in M_4 generation due to gamma-ray irradiation. To clarify if this increase was due to developmental instability or merely genetic segregation within line, further investigations were conducted in the M_7 and M_8 generations which were obtained by the "one plant one offspring" scheme.

For the present investigation, about 100 plants were selected at random from each of the M_6 populations (Control, 20 kR and 80 kR; each consisting of about 1000 plants), and M_7 lines each consisting of approximately 20 plants were observed. Measurements were taken on days to first flowering and plant height at 40 th day, on a single plant basis. The results of analysis of variance showed that (1) radiation induced little change in population means except for days to first flowering in 20 kR populations; (2) inter-line variation was increased by irradiation, and the irradiated populations had larger genetic variances than the control; (3) intra-line variability in both characters increased in the irradiated populations, particularly in the 80 kR population; (4) no significant correlation was found between the line mean and within-line variability in 80 kR population. Since the parental M_6 plants were assumed to be almost homozygous on account of self-pollination, increased intra-line variability was considered to be due to induced mutations that increased developmental instability. To reconfirm

Table 1. Estimated mean, genetic and environmental variances for two quantitative characters of *Arabidopsis thaliana*

Population	Days to flowering			Plant height (cm)		
	Mean	σ_g^2	σ_e^2	Mean	σ_g^2	σ_e^2
Control	28.53	0.0196	1.1285	24.00	0.0713	1.6193
20 kR	24.02	0.0714	1.5093	26.82	0.0739	1.5366
80 kR	29.89	0.0370	2.3501	22.33	2.3661	2.8499

this, a selection experiment was conducted. M_7 lines showing high and low intra-line variability for plant height were selected from 80 kR population, and the intra-line standard deviation of their progeny lines was investigated. Selection was effective and estimated heritability (0.34) was found to be significant. It may be concluded that irradiation has induced genetic changes not only in quantitative characters but also in their developmental stability.

Genetic Changes of Japanese Wild Quails Under Domestic Conditions

Takatada KAWAHARA, Akihiko MITA, Masami SAITO and Norio SUGIMOTO

This experiment was carried out to find the genetic changes in Japanese wild quails reared for several generations under domestic conditions. Examinations were made on several characters of the quails which had been reared without any artificial selection for five generations since they were captured in the field.

The results indicated that the most striking changes of the birds toward the domestic types occurred in time of sexual maturity (which was earlier), and in fertility, hatchability, survival rate, egg production rate, body weight and fitness index (which all increased). A decrease in the variability of characters was also observed in progressive generations.

Interstrain Variation in Gamma-ray Induction of Hindlimb Deformities in Chick Embryos

Takatada KAWAHARA

Gamma irradiation of eggs at a very early stage of embryonic development produced facial anomalies, and at 48 to 60 hr of incubation induced deformities in the hind- and forelimbs. Strains differed in the frequency and degree of deformities in hindlimbs. Barred Plymouth Rock showed the lowest frequency, and White Leghorn the highest of the three breeds tested. The reciprocal F_1 hybrids between the two breeds, when irradiated, showed that the incidence of deformities could be maternally determined. In the backcrosses, however, the paternal genotype had a strong influence on the incidence. The Nagoya breed showed an incidence intermediate between the above two breeds. Its reciprocal F_1 hybrids with White Leghorn

showed transgressive matrocliny, and a maternal effect was apparent in the backcrosses. The heritability estimate for the frequency of hindlimb deformities was 0.303 on the average. The incidence and the magnitude of hindlimb deformities were highly correlated. The incidence was negatively correlated with the number of somites at 48 hr of incubation, but was uncorrelated with shell thickness.

(Published in Radiation Research 57, 332-341, 1974)

Serum Amylase Isozymes in the Japanese Quail

Takatada KAWAHARA, Seiki WATANABE and Haruhiro YOSHIDA

The sera from a domestic and a wild strain of Japanese quails, and their F_1 hybrids, were examined for amylase isozymes by the use of polyacrylamide gel electrophoresis.

Seven anodal bands were detected and were classified into three groups: Regions I, II and III. Observing 641 birds of the three strains to determine the gene frequencies for two bands appearing in Region II, there were three phenotypes, AA (a fast band), AB and BB (a slow band) which were controlled by autosomal codominant alleles called *Amy*-1^A and *Amy*-1^B. The frequency of *Amy*-1^B was generally higher than that of *Amy*-1^A. The frequencies of *Amy*-1^A were 7.7% in the wild and 21.7% in the domestic strain.

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ABSTRACTS OF DIARY FOR 1973

January	26	204 th Meeting of Misima Geneticists' Club
February	23	205 th Meeting of Misima Geneticists' Club
	26	101 st Biological Symposium
March	9	206 th Meeting of Misima Geneticists' Club
	13	102 nd Biological Symposium
	22	207 th Meeting of Misima Geneticists' Club
April	27	208 th Meeting of Misima Geneticists' Club
May	1	103 rd Biological Symposium
	10	104 th Biological Symposium
	14	209 th Meeting of Misima Geneticists' Club
June	15	210 th Meeting of Misima Geneticists' Club
	23	211 th Meeting of Misima Geneticists' Club
	23	105 th Biological Symposium
August	8	106 th Biological Symposium
September	10	107 th Biological Symposium
October	1	212 th Meeting of Misima Geneticists' Club
	26	213 th Meeting of Misima Geneticists' Club
November	2	214 th Meeting of Misima Geneticists' Club
	16	108 th Biological Symposium
	22	109 th Biological Symposium
	22	215 th Meeting of Misima Geneticists' Club
December	11	216 th Meeting of Misima Geneticists' Club
	22	217 th Meeting of Misima Geneticists' Club

FOREIGN VISITORS IN 1973

February	26-27	HAMKALO, B. A., Oak Ridge Natl. Lab., U. S. A.
March	16-17	ADLER, H. I., Oak Ridge Natl. Lab., U. S. A.
April	6	MARKOV, M. A. & others, Academy of Sciences, U. S. S. R.
May	1	BEALE, G. H., University of Edinburgh, Great Britain
July	22-24	SUZUKI, D. T., University of British Columbia, Canada
November	9	TUNG, T. C., & others, A representative team of Chinese biologists, People's Republic of China
	16	EIGEN, M., Max-Planck-Institut, Germany
	22	IRVING, C. C., Veterans Administration Hospital, U. S. A.

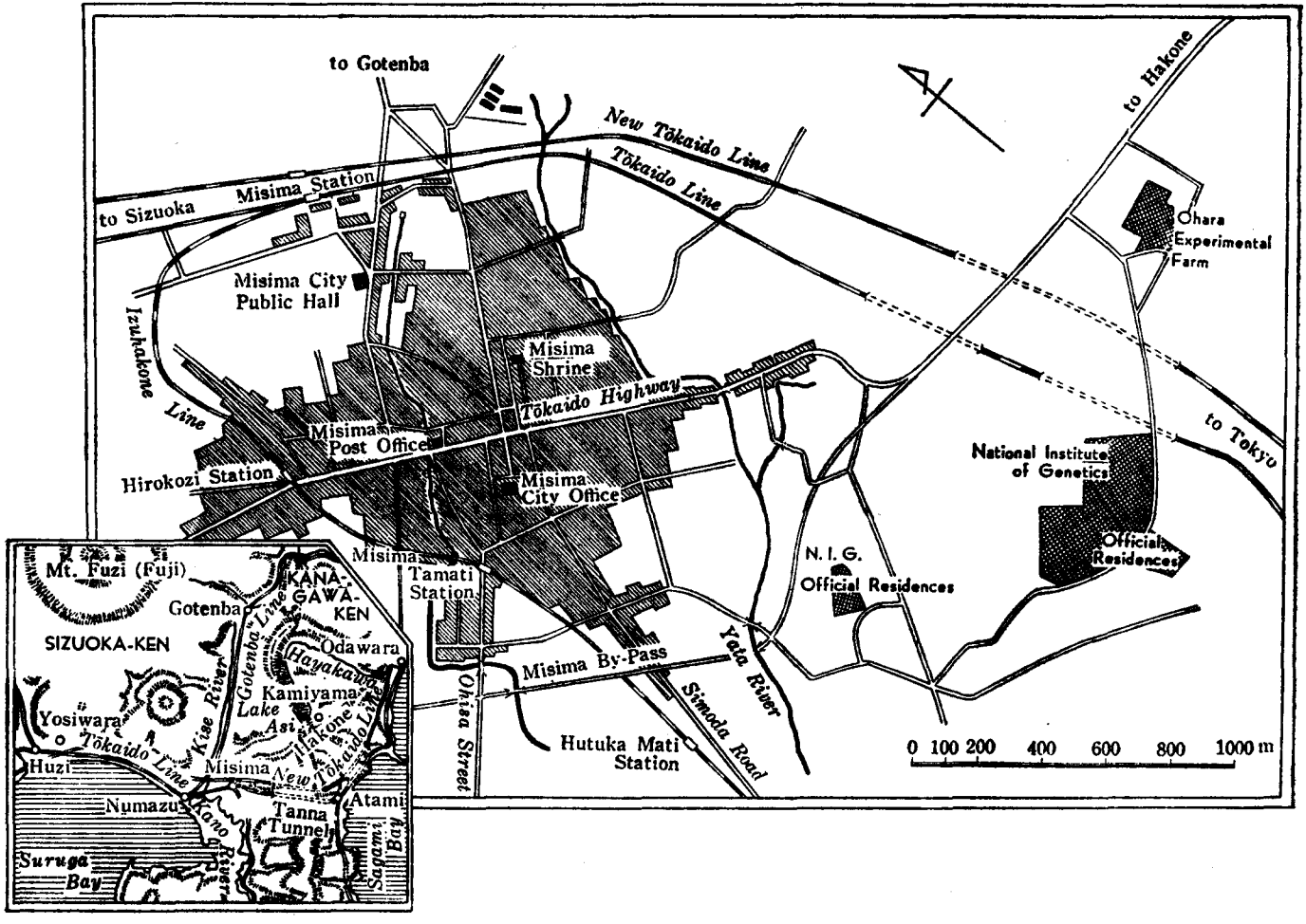
ACKNOWLEDGEMENTS

The editor wishes to express his sincere gratitude to Dr. M. Slakin, Mrs. S. Slatkin and Dr. J. F. Crow for their kindness in reading the original manuscripts. (Y. Nakagome)

AUTHOR INDEX

国立遺伝学研究所欧文年報　第24号
昭和49年12月20日 印刷
昭和49年12月25日 発行

発行者　森脇大五郎
国立遺伝学研究所内
編集者　中込弥男
国立遺伝学研究所内
印刷者　笠井康頼
東京都新宿区戸塚町3-270
印刷所　株式会社 国際文献印刷社
東京都新宿区戸塚町3-270
発行所　国立遺伝学研究所
〒411　静岡県三島市谷田1111
電話・(三島0559)(75)0771, 0772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