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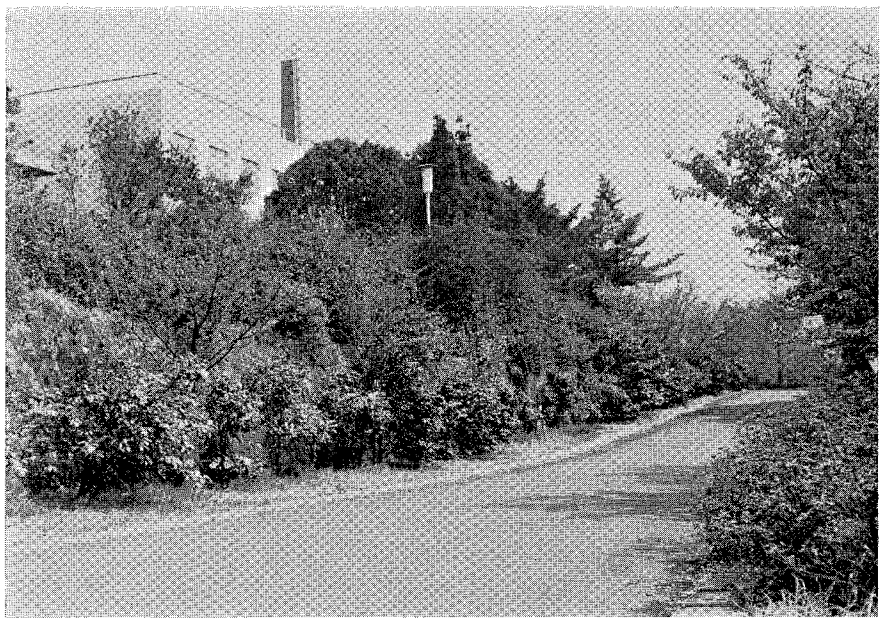

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GENERAL STATEMENT

The National Institute of Genetics attained the twenty-fifth anniversary of its birth. The main commemorative event was the issue of the Commemoration Number of the Twenty-Fifth Anniversary, and we had a small meeting on November 9th, inviting Dr. H. Kihara, the former director, to give us a commemorative lecture. Besides, the annual public lectures were delivered in Tokyo by Dr. Y. Tazima, Head of the Department of Morphological Genetics, and Dr. M. Kimura, Head of the Department of Population Genetics. In the year 1949, when the institute was founded, modern genetics just started to breathe energetically, succeeding "heroic age" of classical genetics. In the past twenty-five years of the institute we can trace a history of the accumulation of endeavor, on the basis of classical genetics, to develop the whole field of genetic studies in the current of modern genetics.

On August 8th we had the honor to have a visit of Prince and Princess Hitachi. Prince Hitachi is majoring in biochemistry and he showed keen interest in the study of cancer cells.

It is worth mentioning that Plant Section of Genetic Stock Center has started. So far important strains of experimental materials such as wheat, rice, morning glory, mouse, silkworm, Drosophila, bacteria, have been maintained in each department, respectively. However, considering increasing burden to the departments to take charge of the increasing strains of genetic importance, we have decided to establish Genetic Stock Center, aiming at "maintenance and storage of the materials necessary for genetic studies and basic investigations in regard to their genetic characters." As a first step the establishment of the plant section of the center has been approved. To this, we intend to add sections for animals and bacteria, so that, while we may be sure to maintain important inherited characters, we will be able to promote studies of higher level by making good use of them.

International exchange of scientists was also active this year. Among many visitors from abroad the following three stayed rather long for cooperative studies: Dr. Montgomery Slatkin of the University of Chicago (June 1–August 31), Professor James F. Crow of the University of Wisconsin (July 16–December 23), and Professor A. Lima-de-Faria of the University of Lund (October 31–December 28). From our side 14 members visited U. S. A., Canada, U. S. S. R., France, Austria, etc., some attending international

conferences, and others for cooperative works.

At the meeting of our Board of Trustees held lately it was approved that I retire in March, 1975, from the director's post. I should like to express hearty thanks to all persons who extended cooperation and encouragement to me during my six year service. I also wish for the great contribution, expansion of research activities, and for further development of the institute.

STAFF

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

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

PROJECTS OF RESEARCH FOR 1974

Department of Morphological Genetics

Genetic studies of the silkworm (TAZIMA and ONIMARU)
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)
Cytogenetics in the silkworm (MURAKAMI and IMAI)
Studies on recombination in the silkworm (MURAKAMI)
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 and KATO)
Chromosome study on experimental tumors (YOSIDA)
Cytogenetical study on sister chromatid exchange in mammalian cells (KATO)
Cytogenetical and immunological studies on mouse plasma cell tumor (MORIWAKI)
Biochemical studies on serum transferrin variations in rodents (MORIWAKI, and TSUCHIYA)
Immunogenetical study on the histocompatibility antigens of wild rodents (MORIWAKI)
Experimental breeding and genetics of mice, rats and other wild rodents (YOSIDA and MORIWAKI)

Department of Physiological Genetics

Studies on effects of noise environment to *Drosophila* (OSHIMA and WATANABE)
Behavior genetics of *Drosophila* (OSHIMA and WATANABE)
Analysis of deleterious and inversion chromosomes in natural populations of *Drosophila melanogaster* (WATANABE)
Studies on fitness of *Drosophila* under controlled environment (OSHIMA and WATANABE)
Ecological genetic studies on the differentiation of *Chrysanthemum* species (NAGAMI)

Department of Biochemical Genetics

Studies on transformation in higher organisms (NAWA and YAMADA)
Genetical and biochemical studies of pteridine metabolisms in insects (NAWA)
Analysis of gene action on cell differentiation in higher organisms (NAWA and YAMADA)
Biochemical studies on the differentiation of muscle proteins in animals (OGAWA)
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)
Genetic analysis of developmental mechanisms in 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)
Genetic studies in natural stands of forest tree species (IYAMA, SAKAI, and KUDO)
Simulation studies on artificial selection (IYAMA)
Evolutionary studies in wild and cultivated rice species (OKA and MORISHIMA)
Ecological genetic studies in some grass species (MORISHIMA)
Genic analysis for isozyme variations in rice (PAI and ENDO)
Genetic effects of environmental pollution on plants (IYAMA, MORISHIMA and OKA)

Department of Induced Mutation

Molecular mechanisms of spontaneous, chemical- and radiation-induced mutations (KADA, SADAIE and NOGUTI)
Environmental mutagens and carcinogens (KADA, SADAIE and TUTIKAWA)
Radiation genetics in mice (TUTIKAWA)
Biochemical factors involved in cellular repair of genetic damage (NOGUTI and KADA)

Mechanisms of recombination repair (SADAIE and KADA)
Mutation and differentiation studies of plant tissue culture (FUJII, AMANO and KADA)
Radiation and chemical interaction in the cells (KADA)
Genetic fine structure analysis in maize (AMANO)

Department of Human Genetics

Genetic studies on common disorders with complex inheritance (MATSUNAGA)
Genetic effects of family planning and population planning (MATSUNAGA)
Clinical cytogenetic studies of congenital abnormalities in man (NAKAGOME and IINUMA)
Molecular cytogenetic studies of human chromosomes (NAKAGOME)
Studies on human chromosome variants (IINUMA, MATSUNAGA and NAKAGOME)
Structural studies on human immunoglobulins (SHINODA)
Isozyme variation in man (SHINODA and MATSUNAGA)

Department of Microbial Genetics

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Genetic regulatory mechanism of cellular division in *E. coli* (HIROTA)
Genetics of bacterial flagella (ENOMOTO and SUZUKI)
Transduction mechanism of phages (ENOMOTO)
Flagellar synthesis and its regulation in a cell-free system (SUZUKI)
Molecular genetics of flagellar synthesis and its regulatory mechanism (SUZUKI, ENOMOTO and HIROTA)

Department of Poputation Genetics

Theoretical studies of population genetics (KIMURA, MARUYAMA and OHTA)
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Mathematical studies on the genetics of structured populations (MARUYAMA)
Linkage disequilibrium in finite populations (OHTA and KIMURA)
Experimental studies on protein polymorphism in *Drosophila* (YAMAZAKI)

Statistical studies on protein polymorphisms in natural populations (MARUYAMA and YAMAZAKI)

Department of Molecular Genetics

Studies on the chemical structure of genome of viruses containing double-stranded RNA (MIURA, SUGIURA, FURUICHI, SHIMOTOHNO, WATANABE, URUSHIBARA, YAZAKI, MUROFUSHI and KIMURA)

Studies on the interaction between RNA polymerase and template nucleic acid (MIURA, SUGIURA, SHIMOTOHNO, FURUICHI and HORI)

Genetics Stock Center

Studies and conservation of germplasm resources in rice and wheat species (FUJII and OKA)

On the sex expression in monoecious plants (FUJII)

Specificity of mutagen tolerance in higher plants (FUJII)

RESEARCHES CARRIED OUT IN 1974

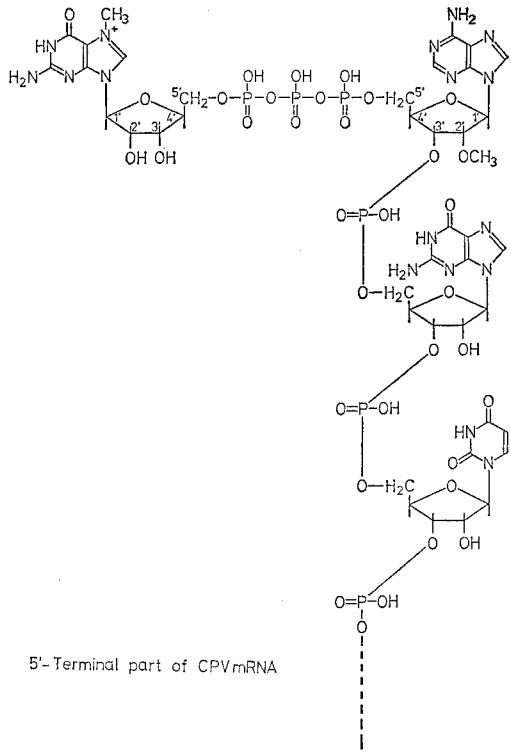
I. MOLECULAR GENETICS

A Novel Modified Structure at the 5′-terminus of Messenger RNA of Cytoplasmic Polyhedrosis Virus

Yasuhiro FURUICHI and Kin-ichiro MIURA

The messenger RNA of cytoplasmic polyhedrosis virus (CPV) containing double-stranded RNA can be synthesized *in vitro* with the use of the substrates XTP and the virion itself. The addition of a methyl donor S-adenosylmethionine (SAM) to the reaction mixture enhances this RNA synthesis and causes methylation of the product mRNA. The structure of the methylated mRNA was analysed.

The RNA was synthesized in the presence of [^{3}H methyl] SAM and β-[^{32}P] ATP. The product RNA was digested with ribonucleases T_1 and T_2, pancreatic ribonuclease IA, alkali, and *Penicillium* nuclease P_1. The digests were analysed on DEAE-urea column using salt concentration gradient in 7M urea solution. Both the radioactivities of [^{3}H] and [^{32}P] were detected in the same peak of an oligonucleotide in any case of the digestion samples. The smallest labeled oligonucleotide component was obtained by *Penicillium* nuclease P_1. Since the [β-^{32}P phosphate] attached to adenylic acid should locate at the 5′-terminus of the synthesized RNA, the methylation by [^{3}H methyl] must occur only at the 5′-terminal part of the RNA. The [^{32}P] phosphate in the oligonucleotide component was not released by phosphomonoesterase treatment. Therefore, the β-phosphate of the 5′-terminal adenylic acid should be blocked by some material as X^{32}ppA. Then the smallest oligonucleotide component obtained by the *Penicillium* nuclease P_1 digestion was further treated with venom phosphodiesterase. Three radioactive components were detected by paper electrophoresis at pH 3.5: [^{32}P]-inorganic phosphate, [^{3}H]-2′-O-methyl-adenylic acid (pAm), and a [^{3}H]-containing neutral component (X) which did not move from the starting point. Upon digestion of the last component with phosphomonoesterase, [^{3}H methyl] was found in a component carrying a plus charge. This was



identified as 7-methylguanosine by paper-chromatographic analysis using several kinds of solvent. The previous neutral component (X) was then identified as 7-methylguanosine-5′-phosphate (pm^{7}G) by comparison with authentic material on chromatography and by proof of presence of a cis-diol (free OH groups in the adjacent positions in 2′ and 3′). As the phosphates in the original material (XppA) were not released by phosphomonoesterase, the original terminal component should be in the structure of m^{7}G$^{5'}$ppp$^{5'}$Am. 7-methylguanosine-5′-phosphate is linked to the β-phosphate of ppAm through a pyrophosphate linkage. The terminal nucleotide sequence of CPV mRNA is concluded as m^{7}G$^{5'}$ppp$^{5'}$Am-G-U- (see illustration), judging from the above mentioned ribonuclease digestion experiment and the former experiments with non-methylated CPV mRNA.

The blocked and methylated structure at the 5′-terminus of CPV mRNA

is the first indication as a modified structure in the messenger RNA molecule. This structure could play an important role in the performance of mRNA synthesis and/or the function of mRNA. This modified structure would be resistant to exonucleolytic attack, so that it may be able to control a life of mRNA.

This work was published in Nature 253: 374–375 (1975).

A Modified 5′-terminal Structure of Messenger RNA of Vaccinia Virus

Toshiyuki URUSHIBARA, Yasuhiro FURUICHI, Kunitada SHIMOTOHNO, and Kin-ichiro MIURA

It is known for vaccinia virus carrying double-stranded DNA genome to contain various kinds of enzymes in a coat. When the virion is treated with trypsin, it is able to synthesize mRNA *in vitro*. If SAM was added to this system, mRNA was methylated. The methylation was restricted to the 5′-terminal part of the mRNA. Two 5′-terminal nucleotide components were isolated by chromatography or paper electrophoresis after digestion with *Penicillium* nuclease P_1. The [^{3}H] methyl-labeled mRNA were digested with venom phosphodiesterase into pm^{7}G, pGm and pAm in the ratio of 5: 3: 2. When [β-^{32}P] ATP or [β-^{32}P] GTP was used for mRNA synthesis, [^{32}P] inorganic phosphate was obtained by the same digestion methods. Finally two kinds of the 5′-terminal components were identified as m^{7}G$^{5'}$ppp$^{5'}$Gm and m^{7}G$^{5'}$ppp$^{5'}$Am (in the ratio of 3: 2). Vaccinia virus genome contains several or more cistrons, so that some mRNAs carry m^{7}GpppGm while other mRNAs carry m^{7}GpppAm at the 5′-termini. These unusual modified structures at the 5′-terminal of vaccinia mRNAs are the same to the the mRNA of CPV. The commonness of these abnormal structures suggests that the modifications at the 5′-terminus are necessary for the function and/or the control of metabolism of mRNA.

The preliminary report was published in FEBS Letters 49: 385–389 (1975).

Nucleotide Sequence at the 5′-terminus of Reovirus Messenger RNA

Kunitada SHIMOTOHNO and Kin-ichiro MIURA

The 5′-terminal nucleotide sequence of mRNA synthesized *in vitro* by reovirus was determined. The fact that the 5′-terminal nucleotide is ppG

was known by the incorporation of [β-^{32}P] GTP into the RNA. From the digestion of the labeled material with pancreatic ribonuclease, the second nucleotide was analyzed as a pyrimidine nucleotide. [α-^{32}P] CTP and [α-^{32}P] UTP were prepared as the substrates of this part. The labeled material was digested with pancreatic ribonuclease and then digested with spleen phosphodiesterase. These treatments yield mononucleotides from inner part of RNA and a terminal oligonucleotide, because only the nucleotide carrying the 5′-phosphate resists to the attack of spleen phosphodiesterase. Only when [α-^{32}P] CTP was used, the terminal oligonucleotide was labeled. [α-^{32}P] of UTP was not incorporated in the terminal oligonucleotide. The labeled terminal oligonucleotide was isolated by chromatography and analyzed. Its structure was determined as ppG^{32}pC^{32}p. As only [α-^{32}P] CTP was used as a source of ^{32}P in this experiment, the neighbor of the 3′-side should be C. Therefore, the 5′-terminal three nucleotide sequence is ppGpCpC------. Thus the 5′-terminal sequence of reovirus mRNA is the same to the 5′-terminal modified strand in the genome double-stranded RNA as the case of another double-stranded RNA containing virus: CPV.

Phosphohydrolase Activity in the Virion of Cytoplasmic Polyhedrosis Virus

Kunitada SHIMOTOHNO and Kin-ichiro MIURA

Messenger RNA synthesized *in vitro* by CPV particle carries the following 5′-terminal structure: ppA–G–Y-------. There is no γ-phosphate at the 5′-terminus. An enzyme activity which deletes γ-phosphate was found in the CPV particle.

The optimum conditions for this enzyme activity were pH 9.5, 45°C, and 4~8 mM Mg^{++}. The activity was inhibited to 50% by 2.5 mM inorganic phosphate, 5 mM pyrophosphate, 0.1 M KCl, or 0.15 M NaCl. Only γ-phosphate of nucleoside triphosphate (NTP) was released by the enzyme, and nucleoside diphosphate (NDP) was left. Among the four kinds of NTP, ATP was readily hydrolysed in a few times higher rate than other NTPs. The RNA sample, which contains pppA– as a 5′-terminus and has 4 S size, was hydrolysed only 1/30 in rate to the case of ATP. The rate of hydrolysis was further lower for the 30 S RNA sample (below 10% of NTP). Therefore, this enzyme acts on the γ-phosphate of small nucleotide, which can enter into the virus particle, but not on the terminal γ-phosphate in a long RNA chain. When the relationship between deletion of protein

subunits from a virion by pronase treatment and loss of enzyme activity was studied, it was suggested that the phosphohydrolase does not face to the outside of a virion.

Modified Structure in the 5′-terminus of Genome Double-stranded RNA of Reovirus

Kin-ichiro MIURA and Kumiko WATANABE

When the 5′-terminal nucleotide sequence of human reovirus double-stranded RNA was determined, removement of a few nucleotides from the 3′-terminal was required prior to [^{32}P] labeling of the 5′-termini by polynucleotide kinase. If the 5′-terminal phosphates of the double-stranded RNA is blocked by 7-methyl-guanylic acid as shown in mRNAs of CPV and reovirus, the 2′- and 3′- positions in the ribose moiety of this 7-methylguanosine should form cis-diol and should be oxidized by periodate and labeled with [^{3}H] by the borohydride reduction. This method is the same to the 3′-terminal labeling. When the 3′-terminal nucleosides of CPV and reovirus were analyzed, an unknown [^{3}H]-labeled material was detected in about half amount of the sum of the [^{3}H] trialcohols from the 3′-termini. Now we have determined this material as the trialcohol of 7-methylguanosine.

Reovirus RNA was labeled by [^{3}H] using borohydride as mentioned above, and digested with *Penicillium* nuclease P_1 and phosphomonoesterase. Besides [^{3}H] nucleoside trialcohols from the 3′-termini, a [^{3}H]-labeled material similar to the modified structure obtained from the 5′-end of mRNA was detected. On hydrolysis of this material with phosphodiesterase, [^{3}H] trialcohol of 7-methylguanylic acid was obtained. These results led us to the conclusion that the 5′-terminus of one RNA chain in a double-stranded RNA involves a modified structure same to the 5′-terminus of mRNA as follows: $m^7G^{5'}ppp^{5'}Gm$–C-------.

Thus we can write a structural relationship between genome double-stranded RNA of reovirus and its mRNA as follows:

$m^7G^{5'}ppp^{5'}Gm$–C ——————→—————— A–U–$C^{3'}$

$_{3'}$C – G ——————←—————— U–A–$G_{5'}$

↓ transcription

↓ modification

$m^7G^{5'}ppp^{5'}Gm$–C ——————————————$_{3'}$

Reovirus synthesizes single-stranded mRNA transcribing one strand of

genome and releases only the completed mRNA outside of the virion. After the mRNA has finished a role for protein synthesis, it functions as a template for synthesis of the counterpart strand. Here, the latter (minus strand) would combine with mRNA to make a double-stranded state and enter into a progeny virion. This idea for the multiplication process of reovirus is suggested from the fact that both the plus strand in genome and the mRNA contains the same modified structure at the 5′-terminus. This supports the asynchronous replication mechanism proposed by Acs *et al.* (Proc. Nat. Acad. Sci. U. S. A.: 68, 505 (1971)), which is different from the replication mechanism of DNA.

Transcription of Double-stranded RNA by the DNA-dependent RNA Polymerase from E. coli

Masahiro SUGIURA and Kin-ichiro MIURA

In order to test the possibility to analyze the signals for transcription —operator, promotor, terminator and so on—the *in vitro* transcription of double- stranded RNA by *E. coli* RNA polymerase was studied, as the nucleotide sequence is readily analyzed for RNA. Formerly RNA synthesis was not detected in this system (Virus, 18: 555 (1968)). For the present experiments, highly purified DNA-dependent RNA polymerase from *E. coli* was prepared according to the Burgess' method followed by two-cycle chromatography on Agarose and DNA-cellulose chromatography. When the *in vitro* RNA polymerizing system was added with Mn^{++} ion instead of Mg^{++}, a little but definite amount of RNA was synthesized, depending on the addition of double-stranded RNA of CPV as a template. This RNA synthesis required addition of four kinds of NTP. It was inhibited by rifampicin, streptoligidin, and ethidium bromide, but not by deoxyribonuclease and actinomycin D, which does not bind with double-stranded RNA. The synthesized RNA was 13 S in size and was able to hybridize with double-stranded RNA of CPV specifically. Since this RNA synthesis depended completely on the sigma factor, the *E. coli* RNA polymerase would recognize a specific part, which would correspond to the promotor site in DNA, to start RNA synthesis.

II. MICROBIAL GENETICS

A Mutation which Causes a Change in the Structure of a Lipoprotein Bound to Peptidoglycan

Hideho SUZUKI, Yukinobu NISHIMURA, Hiroko IKETANI and Yukinori HIROTA

The lipoprotein bound covalently to peptidoglycan of *Escherichia coli* was found by Braun and his co-workers (1970, Eur. J. Biochem. 13: 336).

A mutant of *E. coli* K12 which produced an altered lipoprotein was found. The mutation appeared to result in the failure of a transfer reaction of fatty acid diglyceride to the SH group of N-terminal cystein in the lipoprotein. From the analyses by crosses and transduction, it was concluded that the location of the gene responsible for this reaction was around 31.5 min, between *gurA* and *aroD*, on the chromosome map of *E. coli* (A. Taylor 1972, Bacteriol. Rev. 36: 504).

When the peptidoglycan-lipoprotein complex of the mutant was digested with egg white lysozyme and subjected to the SDS-gel electrophoresis followed by staining with Coomassie Blue, there appeared no band which represented a lipoprotein with a minimal unit of peptidoglycan resulted from lysozyme action, but instead, two new bands were observed at positions of higher molecular weights. These bands, which were absent in the electrophoretic pattern of the preparation from the wild type cells, disappeared upon reduction of the lysozyme digest with mercaptoethanol to give rise to two bands corresponding to a wild type lipoprotein with a peptidoglycan unit and its free form. The lipoprotein of the mutant was reactive with monoiodoacetate when exposed for an hour at pH 8.5, but the authentic lipoprotein was not under the same condition. In the former nearly 1 mole of carboxymethyl group was incorporated into 1 mole of lipoprotein. The presence of one cysteinyl residue per lipoprotein molecule of the mutant was detectable by a common procedure for an amino acid analysis. In the authentic lipoprotein, the sulfhydryl group of the N-terminal cystein is known to be occupied by diglyceride of fatty acids through thioether bond resistant to acid hydrolysis. In order to examine if the mutant-lipoprotein lacked this diglyceride leaving an SH group free, the cellular lipid was labelled by addition of 2-(^{3}H)-glycerol to the growth medium. The incor-

poration of the glycerol into the lipoprotein of the mutant was absent when compared with that into the lipoprotein of the wild type cells.

Possible phenotypic changes resulted from this mutation were examined with respect to the following points: sensitivities to bacteriophages, T1, T5, T4, T6, λ, P1, P2; to growth inhibitors, penicillin, methylene blue; to detergents, deoxycholate, SDS; to a chelating agent, EDTA; and to compounds of mercury, mercuric chloride, p-chloromercuribenzoate, phenylmercuric acetate, including seven additional compounds, and permeation for sugar such as lactose. The physiological characters related to the outer membrane as represented by the passive transport, receptors for bacteriophages, etc. were not changed by this mutation, but the mutant exhibited the pronounced sensitivity for the compounds of mercury.

III. BIOCHEMICAL GENETICS

Detection of Hybrid Enzymes Specified by Acpt Alleles in Wild and Cultivated Rice Strains

Chiang PAI, Toru ENDO and Hiko-Ichi OKA

It was concluded from genetic experiments that leaf acid phosphatase zymograms observed among the rice strains belonging to *Oryza perennis* Moench (wild) and *O. sativa* L. (cultivated) were specified by alleles at three loci, Acp_1, Acp_2 and Acp_3. At Acp_1 were detected seven codominant alleles, Acp_1^{-17}, Acp_1^{-9}, Acp_1^{-4}, Acp_1^{4}, Acp_1^{9}, Acp_1^{12} and Acp_1^{24}, each producing a group of three major (A, M and C) and three minor (a, m and c) bands on the starch gel. The alleles determined mobility of the band group, in which the relative position of the six bands was almost constant. In addition, a null form lacking all these bands was found in two cultivated strains which had a recessive allele, Acp_1^{nul}.

We once considered that hybrids between strains with different mobilities produced three major hybrid bands, so that they had nine major bands in total though some of them overlapped one another (Pai and Endo 1972; Ann. Rep. 22). This year we examined four hypothesis, *i. e.*, 1) no hybrid bands occur in heterozygotes, 2) three major and three minor hybrid bands are produced each by a set of corresponding parental bands, 3) corresponding parental major bands produce three major hybrid bands (our previous assumption), and 4) corresponding minor band produce three minor hybrid bands. Statistical analysis of the data taken from densitometric tracings of zymograms indicated that the second hypothesis was most plausible *i. e.*, the F_1 hybrids produce three major (A^H, M^H and C^H) and three minor (a^H, m^H and c^H) hybrid bands.

IV. DEVELOPMENTAL AND SOMATIC CELL GENETICS

Polymorphic Characters of the External Orifice of Uterus in Inbred Strains of Rats

Tosihide H. YOSIDA and Kunitoshi SANO

It has been known as an established fact that the uterus of the rat has two external orifices; namely the cervice of the uterus opens directly to the vagina by two orifices. Morphology of the external orifice of the rats, however, differs makedly from strain to strain. In some strains of rat the two external orifices are observed as shown in the textbook, but in some other only one orifice is observable. In the latter case, two cervices of the uterus join together to constitute one external orifice that opens to the vagina. To know the strain differences of the morphology of the external orifice, nine inbred strains of rats, ACI, Albany Buffalo, Fisher, Long Evans, NIG, Wistar-M (WM), Wistar-King-A (WKA) and Wistar-King-S (WKS) were examined. Among them only two strains (WKA and WM) showed two external orifices, but the other 6 strains (ACI, Albany, Buffalo, Long Evans, Fisher and NIG) had one external orifice. Only one strain (WKS) showed an intermediate type of the above two forms. Morphology of the external orifice does not change through the sexual cycle or the other physiological condition of the host. It seems to be a rigid genetical characteristic of each strain of the rat.

Cross experiments between one- orificed and two-orificed rats and between two orificed and intermediate type rats were carried out to know their genetical characters. All F_1 hybrids between them showed two orifices. Thus, two-orificed type of the rat seems to be inherited as a dominant character to the one-orificed and intermediate types.

Isolation of Nematocyte Deficient Mutants of Hydra

Toshitaka FUJISAWA and Tsutomu SUGIYAMA

Nematocytes are hydra's stinging cells and they are located mainly on the tentacles where they are used primarily to capture and paralyse prey. *Hydra magnipapillata*, a common species in Japan, has four types of nematocytes (type A, B, C and D) which together account for one-third to one-fourth of

the total body cells.

The nematocyte deficient mutants of *H. magnipapillata* were isolated by crossing male and female strains collected from the same ponds and examining their F1 and F2 progenies for the food capturing ability and the nematocyte compositions.

Nem-4, one of the mutants found by this procedure, was able to capture the laboratory food, *Artemia salinas* nauplii, but was unable to paralyse them. Miscroscopic examination revealed that the type A nematocyte (stenotele) which contained neurotoxin to paralyse prey was absent from the tentacles of this strain, but that the same nematocyte was present in its body region. Since it is known that the differentiation of the nematocytes from the interstitial cells takes place in the body region and that only matured nematocytes migrate to the tentacles, the defect of nem-4 seemed to be in the mechanism for the migration of the newly-differentiated type A nematocyte from the body region to the tentacles.

Other nematocyte mutants isolated included a strain which lacked type B nematocyte and a strain which lacked all four types of nematocytes in the tentacles, and characterization of these mutants are currently in progress.

Regeneration Mutants of Hydra

Tsutomu SUGIYAMA and Toshitaka FUJISAWA

The regeneration deficient strains of *Hydra magnipapillata* were isolated by the inbreeding of field strains as described in the preceeding paper.

After amputation of its head and foot, a wild type hydra regenerated a new head and a new foot in their original places within a few days. In contrast, the mutant strains exhibited greatly different patterns of regeneration under the same coneditions. For example, one of the mutant, reg-1, regenerated the foot normally but it frequently failed to regenerate the head. Another mutant, reg-8, regenerated the head normally but it frequently produced an extra head in place of the foot, thereby producing a double-headed, or bipolar, animal. The third type of mutant was unable to regenerate any visible structures at all.

It is generally believed that hydra has polarity gradient (s) along its body column that specifies the distal to proximal nature of its body. The mutants are being examined with the view that they may have abnormal polarity gradient (s).

Analysis of Time Specificity of *dor*$^+$ Gene Action in Embryonic Development of *Drosophila melanogaster*

Yukiaki KURODA

In a previous work, it has been found that embryonic cells homozygous or hemizygous for an X-linked (1–0.3) recessive lethal gene, *deep orange* (*dor*) were maintained in a chemically defined medium with some macromolecular supplements for a relatively extended period over the prospective lethal phase of the embryos, although some defects in properties such as syncytium formation of muscle cells, formation of cellular spheres, proliferation of small cells, and droplet formation on nerve fibers were found. It has been also found that the addition of unfertilized wild-type egg extract to the culture medium of *dor* embryonic cells resulted in repair of their defective properties (for details, see Nature 252: 40–41, 1974).

In the present experiment *dor* embryonic cells were cultured to determine the specific time of *dor*$^+$ gene action introduced by the wild-type sperm in embryonic development. Embryos obtained from matings of *dor*/*dor* females and *dor*$^+$/Y males were allowed to develop to the stage of blastoderm formation (3 hours after fertilization), gastrulation (5 hours after fertilization), head and trunk segmentation (8 hours after fertilization), and muscular movement (14 hours after fertilization), and used as donor embryos from which extracts were prepared.

The all defective behavior and functions of *dor* embryonic cells were not repaired by the extract from embryos at the stage of blastoderm formation. The extracts from embryos at the stages after gastrulation were effective in repairing the defective properties of *dor* embryonic cells. This suggests that some effective substances having a repair effect may be produced by paternal *dor*$^+$ gene between 3 hours and 5 hours after fertilization.

Effective Substances Having a Repair Action of *dor* Embryonic Defects in *Drosophila melanogaster*

Yukiaki KURODA

It has been previously found that *dor* embryonic cells had some defects in properties such as syncytium formation of muscle cells, formation of cellular spheres and droplet formation on nerve fibers when they were cultured *in vitro* and that these defects were repaired by the addition of unfertilized wild-

type egg extract to the culture medium of *dor* embryonic cells.

The effective substances having this repair effect were contained only in the wild-type egg extract, and not in unfertilized *dor* egg extract. The behavior and functions of embryoic wild-type cells were not inhibited by the addition of *dor* embryonic egg extract. These results suggest that the repair action of the wild-type egg extract may be due to the action of some effective substances contained in the extract and not due to the removal of inhibitory substances in embryonic *dor* cells.

When the wild-type egg extract was heated at 80°C for 10 minutes, its activity was lost. This indicates that the effective substances in the wild-type egg extract may be some enzymes or heat-labile substances.

Isoxanthopterine and xanthopterine added to culture medium had no effects on repair of the defective properties of embryonic *dor* cells, although 2-amino-4-hydroxypteridine had a slight effect on stimulation of droplet formation on nerve fibers of *dor* cells. Further investigation is required to identify the chemical nature of the effective substances.

Induction of 8-Azaguanine Resistant Mutations by Phloxine in Embryonic Human Diploid Cells

Yukiaki KURODA

The red dye, phloxine, is widely used as an additive in cakes and fish pastes in Japan. It has been reported that in the "rec-assay" phloxine showed a greater lethal effect on Rec^- than on Rec^+ strain of *Bacillus subtilis* and it had a mutagenic action on *Escherichia coli* B/r WP_2 try^+.

In the present experiment the mutagenic effect of phloxine on 8-azaguanine (8AG) resistant mutations in cultured embryonic human diploid cells was examined. Phloxine had a severe cytotoxic effect on the cells at concentrations of 1 to 10 μg/ml. At concentrations of more than 30 μg/ml of phloxine no further decrease in cell survival was found. This cytotoxic effect of phloxine was not dependent on the duration of treatment. After treatment with phloxine for 2 hours division of cells in normal medium was inhibited for 120 hours.

When cells were treated with phloxine at various concentrations for 2 hours, cultured in normal medium for 48 hours, and then selected with 30 μg/ml of 8AG, an increase in the induced mutation frequency was found. This increase in mutation frequency was dependent on the concentration of

phloxine used as a mutagen and treatment with 100 μg/ml of phloxine increased the frequency to six times that in untreated cultures. For details, see Mutation Research 30: 239–248.

Protection by Vitamin E Against Bisulfite-Caused Defect in Growth of Cultured Human Cells

Yukiaki KURODA

It is known that bisulfite induces gene mutations in bacteria, yeast and bacteriophages. The induction of these gene mutations is produced by a transition process from cytosine to uracil in DNA molecules. At low concentrations bisulfite was found to inactivate the transforming activity of bacterial DNA by free radical-mediated reactions in the co-existence with oxygen. On the other hand, it is generally accepted that vitamin E has an antioxidant activity in various metabolic processes in higher animals.

The present experiment reveals that vitamin E protects against a bisulfite-caused defect in growth of cultured human cells. The cells used were the S–3 subline of HeLa cells, human cervical carcinoma cells. Triplicate inocula of 10^3 cells were incubated for 2 hours at 37°C in 60-mm petri dishes, and then bisulfite and/or vitamin E at various concentrations were added to the medium. After incubation for 10 days at 37°C, the colony-forming activity of cells was determined.

Bisulfite had a severe cytotoxic effect on HeLa S–3 cells at a concentration of 10^{-4} M, causing complete inhibition of colony formation. A concentration of 10^{-5} M bisulfite had a moderate cytotoxic effect on the cells, reducing their colony-forming activity to one third of that of untreated control cultures. Addition of vitamin E at a concentration of 10^{-7} M restored the colony-forming activity of 10^{-5} M bisulfite-treated cells to 71% of that of control cultures. Addition of 10^{-6} M vitamin E resulted in almost complete recovery of colony-forming activity of bisulfite-treated cells to that of control cultures. The counteraction of vitamin E to the cytotoxic effect of bisulfite on human cells was suggested. For details, see Experimental Cell Research 94: 442–445.

Isolation and Characterization of Variant Clones of Chinese Hamster Cells after Treatment with Irradiated 5-Iodouridine

Yukiaki KURODA, Akiko YOKOIYAMA and Tsuneo KADA

Previously we reported that the colony-forming ability of Chinese hamster cells decreased on treatment of the cells with γ-irradiated 5-iodouridine at acidic pH's. We suggested that this might be due to radiation-induced cytotoxic iodine radicals.

In the present work some variant clones were isolated from Chinese hamster cells treated with irradiated 5-iodouridine. The following characters of a primary variant clone, C–11 and a secondary variant clone, C–24 were compared with those of the original clone C–1: colony-forming activity, growth rate in the presence of irradiated and unirradiated 5-iodouridine, distribution of chromosome numbers and cell cohesion.

The variant clone C–11 and C–24 were partially resistant to unirradiated 5-iodouridine at lower concentration and C–24 cells were slightly resistant to short-term treatment with irradiated 5-iodouridine. Unlike clones C–1 and C–11 the variant clone C–24 showed no lag phase on growth in 5-iodouridine medium. The modal numbers of the chromosomes of all three clones were 22, like that of normal Chinese hamster diploid cells.

Of the three clones, the variant C–24 cells showed the least mutual cohesion and the original C–1 cells showed the most. The possibility that an alteration in cellular membrane might be related to an increase in the resistance to radiosensitizing agents was suggested. For details, see Mutation Research 33: 285–298.

Effect of Serum on Proliferation of Mammalian Cells in Culture

Kiyoshi MINATO

It has been previously found that the cumulative number of cells which were maintained without medium change was dependent on the concentration of serum supplemented initially. In further studies on this phenomenon, a stable proliferation of cells was not obtained very frequently by unknown reason.

In the present experiment, the reason why the stable proliferation of cells

had not been obtained was investigated. This unstable cell growth was related to a low quality of serum which was dependent on its lots. Considerable differences were found in population-dependent requirement (PDR) for cell growth, doubling time, saturation density of cells, when cells were cultured in different lots of serum; serum with low quality had bad effects on these properties of cells.

It was also found that serum with low quality brought about an incomplete repair of mechanical damages produced in the process of dissociation of cells, and that this resulted in unstable proliferation of cells in further cultivation.

V. CYTOGENETICS

Frequency of A/A, A/S and S/S Chromosomes in Pairs No. 1, 9 and 13 in 133 Black Rats Collected in Various Localities

Tosihide H. YOSIDA

Chromosome polymorphism of the largest pair no. 1 with respect to acrocentrics (telocentrics) and subtelocentrics has been previously reported in a small population of Japanese black rats (Yosida *et al.* 1965, Chromosoma 16: 70–78). Thereafter, the frequency of A/A, A/S and S/S pairs no. 1 in 453 rats corrected in 19 localities in Japan has been published (Yosida *et al.* 1971, Chromosoma 33: 30–40). Appearance of chromosome polymorphism in pairs no. 9 and 13 in regard to the acrocentrics and subtelocentrics has also been reported by Yosida *et al.* (1969, this report 19: 12; 1970 this report 20: 10; 1971, this report 21: 55–56) in Japanese population, and Gropp *et al.* (1970, Z.f. Säugetier. 35: 363–371) in Thailand rats. Statistical survey of the frequency of chromosome polymorphism of the pairs no. 9 and 13 has not been carried out yet, except a study of 16 rats collected in Misima, Japan (Yosida *et al.* 1971, this report 21: 55–56). To know the frequency of the polymorphism of pairs no. 1, 9 and 13, 133 rats were examined. Among them 95 rats were *R. rattus tanezumi* collected in Japan, 6 rats were *R. rattus diardii* from Malaysia, 19 rats were *R. rattus mindanensis* from the Philippines, and the remaining 13 rats were *R. rattus rattus* from Oceania. In Japan rats were collected in four localities; Hokkaido (22), Niigata (15), Misima (35) and Nagasaki (23). In Hokkaido population nos. 1 and 9 were all acrocentrics, but no. 13 were polymorphic. In Niigata, no. 1 were all acrocentrics but nos. 9 and 13 were polymorphic. In Misima and Nagasaki populations pairs no. 1, 9 and 13 were all polymorphic. In *R. rattus diardii*, pairs no. 1 and 13 were polymorphic, but no. 9 was all S/S type. In *R. rattus mindanensis* pair no. 1 was S/S type, except one rat with A/S, while pairs no. 9 and 13 were polymorphic. Pairs no. 1 and 9 of *R. rattus rattus* were S/S, except one with A/A pair no. 9. Most of pair no. 13 were acrocentrics, but 2 were heteromorphic A/S type. Besed on the above investigation, it can be said that the frequency of polymorphism in the smallest pair no. 13 is higher than those of the pairs no. 1 and 9, and inversion of the no. 13 chromosome could occur considerably earlier than the others.

Segregation of Chromosomes in the Second Spermatocytes in F_1 Hybrids between Asian and Oceanian Type Black Rats

Tosihide H. YOSIDA

F_1 hybrids between Asian ($2n=42$) and Oceanian type black rats ($2n=38$) are characterized by having 40 chromosomes in diploid number which consists of each haploid set from Asian and Oceanian rats. Therefore, the F_1 rats have one large metacentric (M_1) which is derived from fusion of acrocentric pairs no. 4 and 7, and another large metacentric (M_2) arising from fusion of acrocentric pairs no. 11 and 12. The rat also had unpaired acrocentrics no. 4, 7, 11 and 12 (Yosida *et al.* 1969, Jap. J. Genet. 44: 84–91; Yosida *et al.* 1971, Chromosoma 34: 40–50). Idiogram of the F_1 hybrid in respect of pairs no. 4, 7, 11 and 12 is formulated as $M_1(A_4+A_7)/A_4$, A_7, $M_2(A_{11}+A_{12})/A_{11}$, A_{12} (M and A mean large metacentrics and acrocentrics, respectively). In meiosis of the hybrid, synapsis between $M_2(A_4+A_7)/A_4$ and A_7, and between $M_2(A_{11}+A_{12})/A_{11}$ and A_{12} can be performed, and in the first spermatocytes 17 bivalents and 2 trivalents are produced. At the first maturation division the following four second spermatocytes are expected to be produced by segregation of the metacentrics M_1 and M_2 and acrocentrics A_4, A_7, A_{11} and A_{12}.

(A) $M_1(A_4+A_7)$ and $M_2(A_{11}+A_{12})$.
(B) $M_1(A_4+A_7)$, A_{11} and A_{12}.
(C) A_4, A_7 and $M_2(A_{11}+A_{12})$.
(D) A_4, A_7, A_{11} and A_{12}.

Owing to the distribution of the large metacentrics (M_1 and M_2), the haploid chromosome number in the second spermatocytes is expected to

Table 1. Frequencies of the second spermatocytes with four different chromosome types in the F_1 hybrids between Asian and Oceanian type black rats

Chromosome types (pair no.)				n	No. of cells observed
No. 4	No. 7	No. 11	No. 12		
$M_1(A_4+A_7)$		$M_2(A_{11}+A_{12})$		19	13
$M_1(A_4+A_7)$		A_{11}	A_{12}	20	17
A_4	A_7	$M_2(A_{11}+A_{12})$		20	14
A_4	A_7	A_{11}	A_{12}	21	21
		Total			65

vary from 19 to 21. Microscopically, the second spermatocytes with 19 chromosomes having two metacentrics, with 20 chromosomes posessing one metacentric and with 21 chromosomes having no metacentrics (Fig. 3) were really observed. The second spermatocytes with the above four types are expected to be equal in their frequencies. In 65 spermatocytes analysed the four types were observed in almost equal number, although the frequency of cells with 21 chromosomes was slightly higher than that of the others (Table 1).

Relation between the Decrease of C-band Heterochromatin and Differentiation of *Rattus* species

Tosihide H. YOSIDA

In the previous report the author (Yosida and Sagai 1974, this report 24: 24–25; 1975, Chromosoma 50: 283–300) described that the black rat (*Rattus rattus*) showed clear-cut and large-sized C-bands in the centromeric region of all chromosome pairs, but polymorphism of the bands involving the presence and absence or their size was observed in some subspcies of the animals. From a view point of karyotype evolution of the animals the author suggested that the C-bands show a tendency toward the decrease, so far as the closely related subspecies of the black rat are concerned. In order to know whether the tendency found in subspecies of the black rats occurs in the case of differentiation of species in genus *Rattus*, C-bands in 8 species, *R. r. argentiventer*, *R. r. jalorensis*, *R. norvegicus*, *R. annandalei*, *R. exulans*, *R. muerelli*, *R. fuscipes* and *R. conatus*; were investigated by way of comparison with that of *R. rattus*.

Results of observations showed that all 8 species examined here presented small sized or vaguely stained C-bands in contrast to those of *R. rattus*. From view points of karyotype evolution in genus *Rattus* the author (Yosida 1973, Chromosoma 40: 285–297) suggested that the karyotype of *R. rattus* with $2n=42$ is an ancestral form, from which the karyotypes of the other 8 *Rattus* species investigated here have differentiated by pericentric inversion and Robertsonian fusion. Based on these evidences a following suggestion presents that the size of heterochromatic C-bands has also a tendency to decrease in the closely related species of the genus *Rattus*.

Effects of 2-(2-furyl)-3-(5-nitro-2-furyl)-Acrylamide (AF-2) on Bone Marrow Chromosomes of the Rat at Different Ages

Tosihide H. YOSIDA and Hideo TSUJI

Cytogenetical effect of 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF–2, a food preservative) on rat bone marrow cells has been reported by Sugiyama (1975, Mut. Res. 31: 241–246). The present authors reinvestigated the effect of the chemical on bone marrow cells of the rat and found that the effect was considerably different depending on the age (body weight) of the rat used. Two rat strains (Wistar King-S and Long Evans) weighing 60 to 420 gr were used in the present study.

The chemical (AF–2) was used at a concentration of 300 mg/k dissolved in physiological saline. According to Sugiyama, the effect of the chemical to bone marrow chromosomes is most prominant at 6 hours after injection. As we also obtained the same result in a preliminary study, chromosome observations were done exclusively on the 6 hour sample. The experiment was carried out with 6 young rats weighing 60 to 190 gr and 3 old rats weighing 380 to 390 gr.

Results of experiment are as follows: (1) Chromosomal abnormalities caused by the chemical were all chromatid breaks and gaps, and no other abnormalities were detected. The chemical might affect cells at the G_2 stage of the cell cycle because the abnormalities was observed 6 hours after treatment. (2) The effect was not different in two rat strains used. (3) In young rats (60–190 gr) chromosomes of 600 cells were examined. Among them 42 cells (7%) showed chromatid aberrations such as chromatid breaks (5 cells) and chromatid gaps (42 cells). In old rats (280–390 gr) 300 cells were counted, and only 5 cells (1.6%) had chromatid aberrations. The difference between these aberration frequencies is statistically significant, but the latter value is not significantly different from that obtained in the control. From the above experiments it can be said that effect of AF–2 differs markedly depending on the age of rats used.

Evidence against that the Sister Chromatid Exchange is Single-Strand Exchange

Hatao KATO

Phenotypic similarities between the induction of sister chromatid exchanges

(SCEs) in mammalian chromosomes by ultraviolet (UV) irradiation and recombinational repair of UV-induced DNA damage in *E. coli* led previous workers (Bender, M. A. *et al.* 1973 Mutation Res. 20: 387) to postulate that chromosomal aberrations are outcome of incomplete SCEs arising through molecular processes akin to those involved in recombinational repair. However, the fact that the recombinational repair mechanism involves exchange of single polynucleotide chains of DNA seems to offer various difficulties in the explanation for the SCE induction based on the context mentioned above (Kato, H. 1974 Exptl. Cell Res. 85: 239; 1974 Nature 249: 552). Firstly, in recombinational repair the size of a gap to be filled in by a segment of single strand transferred from the sister molecule is believed to be about 1000 nucleotide long. This size seems to be too small to be detected as SCEs in mammalian chromosomes. Secondly, single-strand exchange requires denaturation of very long segments of DNA and their eventual annealing with the complementary strands in the sister chromatid. These processes may be achieved without unwinding difficulty by crossed strand migration (Lee, C. S. *et al.* 1970 J. Mol. Biol. 48: 1). However, the distance for the crossed strand to migrate is considered to be unrealistically long in such a giant molecule as the mammalian chromosome: Suppose if one single strand exchange is initiated at the midpoint of a Chinese hamster No. 1 chromosome which is about 6×10^8 nucleotide pair long, the crossed strand has to migrate through 3×10^8 nucleotide pairs to accomplish the SCE process. Meselson (1972 J. Mol. Biol. 71: 795) calculated that at 37°C about 20 seconds would be needed for such migration through 1000 nucleotide pairs. This means that the completion of the given SCE needs 6×10^6 seconds, *i. e.* 167 hours.

Recently a new method was developed to distinguish sister chromatids without the usage of tritium label. The method involves labeling of cells with 5-bromo-2′-deoxyuridine (BUdR) and staining of fixed chromosomes with acridine orange (Kato, H. 1974 Nature 251: 70), and can demarcate unequivocally chromatids substituted unifilarly with BUdR from unsubstituted as well as bifilarly-substituted chromatids. By using this technique, the question as to whether the SCE is single-strand exchange or not was re-examined. If cells are labeled with BUdR for one round of cell cycle, a BUdR-labeled metaphase chromosome would be composed of sister chromatids each containing unifilarly-substituted DNA. Exchange of any single strands of the same polarity would result in the appearance of chromosomal

segments which would be now composed of an unsubstituted DNA duplex in one chromatid and a bifilarly-substituted one in its sister chromatid at corresponding regions. These segments would be distinguished clearly from each other as well as from the rest of chromosome regions by acridine orange staining. On the other hand, if exchange occurs at a double strand level, recombined chromatids would be still composed of unifilarly-substituted DNA along their entire length and the chromosome would be stained uniformly.

Chinese hamster cells, D–6, were exposed to either 5 j/m^2 UV or 10^{-6}M mitomycin C (MMC) and then grown in culture medium containing 0.5μg/ml BUdR for 8 h before harvest. It was confirmed previously that UV and MMC at these doses induced SCEs at frequencies of 2.5 and 3.1 per No. 1 chromosome, respectively, when they were applied during the 2nd postlabeling S. Over a hundred metaphases were examined carefully for the presence or absence of differentially stained segments but all the chromosomes were found to be stained uniformly as expected if the SCE had occurred at double-strand level. It is thus very likely that even if the initial step of the SCE formation can be exchange of single strands arising from each sister chromatid, breakage of the remaining strands would soon follow enzymatically at or near the exchange site, resulting in double strand exchange as postulated by previous workers (Whitehouse, H. L. K. 1963 Nature 199: 1034; Holliday, R. 1964 Genet. Res. 5: 282). Obviously these processes are different from the mechanism proposed for recombinational repair of DNA damage in UV-irradiated *E. coli* (Rupp, W. D. *et al.* 1971 J. Mol. Biol. 61: 25).

Chromosome Analysis of Sublines of a Mouse Hepatoma (MH134) with a Ribosomal RNA Variation

Kazuo MORIWAKI, Hirotami IMAI, Ryo KOMINAMI* and Masami MURAMATSU*

It has already been reported that two sublines (C and Os) of mouse hepatoma MH134 are lacking in a spot-20 (CUAACAϕCUG) on the fingerprint pattern of 28 S RNA, though either the other subline (Y) of this tumor and normal liver have it (Kominami, Hashimoto and Muramatsu, Proc. Jap. Cancer Assoc., 33: 37, 1974). In order to trace the possible position during serial propagation where the ribosomal RNA variation may have occurred,

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the pedigree of eight sublines of this tumor was surveyed. Since 1955, this tumor line has been maintained by Prof. H. Sato in the Tohoku University from where four sublines, Ms, Se, Y and Ib, originated after 1967. In 1959, a part of this tumor was transferred to the Tokyo University Institute of Medical Science (Prof. T. Yamamoto) from where the other four sublines, I-65, Os, C and If, started after 1965. The cytogenetical survey by G-band analysis on the karyotypes of those subline cells well approved this pedigree. As summarized in Table 1, M4 and M5 marker chromosomes are common to all the sublines, whereas M1 and M2 markers are observed only in the four sublines directly originated from the Tohoku University-line. The occurrence of the ribosomal RNA variation seems to be limited to the sublines started from the Tokyo University-line. Furthermore, in sublines Os and C the extreme eleongation of the secondary constriction in a No. 15 chromosome was observed. Considering the repetitive nature of ribosomal genes in the higher organisms, one may not explain simply the cause of the variation in ribosomal RNA.

Table 1. Occurrence of Specific Marker Chromosomes in MH134 Sublines

Sublines	Frequencies of the marker chromosomes (% of 50 cells)							
	Ms	Y	Se	Ib	Os	If	C	I-65
Modal chromosome No.	39	38	44	43	43	41/42	42	42
Marker chromosomes								
M1 (6–2 TRL.)	100	100	100	98	0	0	0	0
M2 (7–8 TRL.)	100	100	100	98	0	0	0	0
M3 (15 ISO.)	0	0	94	92	0	0	0	0
M4 (A)	94	100	98	100	100	100	94	100
M5 (B)	96	100	0	0	20	72	94	65
M6 (4 ISO.)	0	0	0	96	0	0	0	0
M7 (C)	18	0	0	0	0	0	48	0
M8 (D)	58	40	0	0	0	0	58	0
M9 (15 2nd Const. Elg.)	82	82	0	0	100*	90	100*	90
No. 16 Chromosome	1	1	2	3	1	1	1	1
Ribosome 28S-RNA-Spot 20**	+	+	+	+	−	−	−	

* Extreme elongation ** Kominami, Hashimoto and Muramatsu, 1974

Genetical Analysis of a Newly Gamma-ray Induced Translocation between W and V Chromosomes in Pupal Oocytes of the Silkworm

Akio MURAKAMI and Akio OHNUMA

In the course of progeny tests for ^{137}Cs gamma-ray induced egg-color specific locus mutations in pupal oocytes of the silkworm, a red egg (*re*, V–31.7) locus mutation was detected. Genetical analysis suggests that this mutant resulted from a break in the vicinity of *re* locus on chromosome-5 and that one part of the chromosome including *pe* (pink egg, V–0.0) was translocated on to the W (or Y) chromosome. In the silkworm, the male is homogametic ZZ or XX and the female is heterogametic ZW or XY depending on the nomenclature used for the sex chromosome.. This stock also has a section of chromosome-5 bearing the marker *oc* (chinese oily, V–40.8) gene locus. This translocation line therefore has the chromosome constitution $Z/\widehat{W \cdot V^{pe^+}_{\cdot} V^{oc^+}}/V$ (Figure 1).

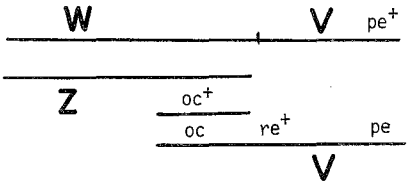

Fig. 1. Chromosome constitution of a newly gamma-ray induced translocation between W and V chromosomes.

This translocation line also produces a number of non-disjunctional individuals. In the female progeny of a cross between a female of this line and a marker strain male, individuals of three different chromosome types occurred ($Z/\widehat{W \cdot V^{pe^+}_{\cdot} V^{oc^+}}/V$, $Z/\widehat{W \cdot V^{pe^+}}/V$ and $Z/\widehat{W \cdot V^{pe^+}_{,} V}/V$) while in the male progeny, there were also three types of individuals (Z/Z V/V, $Z/Z\ V^{oc^+}_{,} V/V$ and $Z/Z\ V^{oc^+}$ /V). Some embryos of male progeny with $Z/Z\ V^{oc^+}/V$ in a cross between the translocation line female and the marker male died, probably due to the loss of a large part (ca. 60%) of chromosome-5. Similarly, in the progeny of a cross of the $Z/\widehat{W \cdot V^{pe^+}_{,} V}/V♀ \times Z/Z\ V/V♂$, neither disjunctional males from normally colored eggs (+) nor females from yellowish-white egg (*pe*) were observed, while in a cross between $Z/\widehat{W \cdot V^{pe^+}_{\cdot} V^{oc^+}}/V$ and Z/Z V/V a small

number of disjunctional products segregated. These observations suggest to be nondisjunctional events in meiotic divisions because of the complex chromosome constitution of this translocation line.

VI. MUTATION AND MUTAGENESIS IN ANIMALS

Attempts to Induce Non-disjunction by Means of Irradiation and Chemical Treatment in the Silkworm*

Yataro TAZIMA and Akio OHNUMA

Since the induction of aneuploidy by radiation attracted our great concern from the view point of genetic hazard to man, experiments have been carried out in our laboratory with silkworm. The results obtained hitherto indicate that non-disjunction can hardly be induced by direct means, although innumerable trisomic strains have been known in this organism. The mutagenic agents used so far are ionizing radiations, high temperature, CO_2 gas, organic mercury compounds and MMC. Both sex-chromosome and autosome systems have been employed. The latter system, in which egg color genes were used as markers, permitted to deal with a large number of experimental individuals with relative ease.

Females heterozygous for *pe*+/+*re* were subjected to mutagenic treatment and crossed to non-treated *pere* males. Individuals due to non-disjunction, if occurred, could be recovered as a wild type among F_1 eggs which comprise 1 *pe*: 1 *re*. The incidence of wild type was fairly high both in the control and treated groups: for instance, 13/59120 in the control, 25/99765 in X-irradiated groups and 41/123413 in methyl mercuric chloride groups. Wild type individuals analysed so far were 70 females and 1 male. 70 females comprised 1 4*n*, 63 3*n* and 6 2*n* and 2*n* females were confirmed as the products of recombination. One male was 2*n* but its genotype was not determined. Thus, even a case of non-disjunction of marked chromosomes has not been recovered yet, contrasting sharply to the findings in *Drosophila*. Almost all trisomies thus far obtained in the silkworm occurred as a consequence of chromosomal unbalance due to deficiencies, inversions and/or translocations. Why non-disjunction can hardly be induced by direct means may be ascribed to the holokinetic nature of the chromosome. Further analyses are in progress.

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* Presented at the fifth International Congress of Radiation Research held at Seattle, July 19, 1974.

Mutagenic Effectiveness of an Internal β Emitter, Tritium. (1) A Preliminary Experiment on Somatic Mutation in the Silkworm*

Yataro TAZIMA, Kimiharu ONIMARU, and Yosoji FUKASE

With the purpose of developing a new experimental system suitable for the assessment of mutagenicity of internal emitters, studies have been carried out with silkworm, in which duration of egg stage, hence the duration of exposure of embryo, can be controlled by experimental conditions. In this preliminary experiment ^{3}H–TdR was injected into the body cavity of wild type females of a strain C108 at a mid-pupal stage, so as to allow the incorporation of the ^{3}H–TdR efficiently into the egg. Injected dose was 50 μCi per individual pupa in 0.05 ml of physiological saline. The average radioactivity transmitted to the deposited egg was 0.002 μCi/egg.

Injected females were crossed, after emergence, to *pe ok* males for the detection of induced somatic mutations. Mutations were expected to appear as tiny dots either of *pe*-cells among wild type serosa cells or of translucent cells among opaque larval integument cells. There were 103 days from deposition to hatching, during which the embryos were exposed to β-rays. The results of the experiment are shown in Table 1.

Table 1. Somatic mutations induced by ^{3}H-TdR injection
++♀×*pe*, *ok* ♂ (740)

Treatment	Total No. obsvd.	No. of *ok* mutants			Total No. of dots	Dots per mut. bearing larva
		dot	mosaic	whole		
Control	7161	55	0	0	83	1.51
Frequency		0.77 (0.59–0.99)*				
^{3}H-TdR	6381	407	1	1	515	1.27
Frequency		6.38 (5.80–7.05)*				

C108 ♀, average body wt.; 1.33 g
Date of oviposition; Dec. 11, 1974: Date of hatching; March 16, 1975
* 95% confidence interval

No significant increase was observed in the incidence of eggs with *pe* dots in the injected group but the injection of ^{3}H–TdR was distinctly effective in the production of *ok* dots in larval integuments.

* Presented at the 17th meeting of the Japan Radiation Research Society, held at Tokushima, Oct. 7, 1974.

These results clearly show that the method of administration of radioisotope could be used effectively for the assessment of the genetic effects of internal emitters.

Effects of Low-temperature Post-treatment on the Repair of Premutational Damage in Spermatogonia of the Silkworm

Yataro TAZIMA and Yosoji FUKASE

As a part of serial work to elucidate the nature of radiation-induced damages on DNA, an experiment has been carried out by applying low-temperature treatment after X-irradiation of the silkworm spermatogonia.

It has been known that enzymes that concerned with repair of DNA damages are suppressed of their activity at cold temperature as 0°C. It is interesting to know whether the suppression of repair may result in the failure of rejoining of broken ends of DNA strand or lead to successful repair via slow acting but exact error correction process. If the former holds true, it can be expressed as an increase in mutation frequency. While in the latter case we may observe the decrease in mutation frequency.

Wild type larvae of strain C108 were used as materials. The newly hatched larvae were divided into three groups: non-irradiated control, irradiated and post-treated at 27°C, and irradiated and post-treated at 5°C. For irradiation larvae were contained in petri dishes, one with ice and the other with solid paraffine, both rotating simultaneously under X-ray (300 R/min) beam. The radiation dose was 1000 R. Immediately after irradiation irradiated group on ice were kept at 5°C in ice jar for five hours and those irradiated on paraffine were kept in 27°C room for the same duration. After treatment

Table 1. Results of low-temperature post-treatment of silkworm spermatogonia (742)

Treatment	Total No. observed	No. of mutants				Mutation frequency ($\times 10^{-5}$)			
		pe	pe mos	re	re mos	pe	pe mos	re	re mos
Control	74,988	7	3	0	2	9.3	4.0	0	2.7
27°C	83,516	16	1	1	2	19.1	1.2	1.2	2.4
5°C	86,292	35	7	24	4	40.6	8.1	27.8	4.6

irradiated and control groups were raised in rearing room at 25°C throughout their life cycle. For the measurement of mutation frequency specific loci method using *pe* and *re* was employed. The results are given in Table 1.

The results clearly show that low-temperature post-treatment increased the radiation induced mutation frequency at both loci. An average effectiveness was -1×10^{-5}/locus per 1°C. Analysis of induced mutants is in progress.

Silkworm Oocyte System for Testing Mutagenicity*

Yataro TAZIMA and Kimiharu ONIMARU

As reported previously, a highly sensitive system has been developed for the detection of mutation in the silkworm in which oocytes are treated with chemicals. The system proved itself to be very effective for the demonstration of mutagenicity of several nitrofuran derivatives including furylfuramide. In these experiments injection of the test chemical was performed five days before emergence, because at this stage maximum mutation yield had been revealed by our previous experiments with mitomycin C.

It was also known that silkworm oocytes are extremely sensitive to ionizing radiation as to mutation induction around meiotic metaphase, which takes place immediately after egg deposition. If the same is true for chemical mutagens, the chemical must remain active in silkworm hemolymph for more than five days until deposition. This is quite dubious for furylfuramide, because the compound is known to be easily metabolized in bacterial cells or mammalian metabolic systems. The evidence that furylfuramide exhibited clearly mutagenicity even when injected five days before emergence could be ascribed to two alternative mechanisms: either the injected chemical reacts immediately upon genetic materials or the chemical remains unchanged until it reacts upon genetic materials at their surmised most sensitive stage, perhaps around meiotic metaphase. To determine which of the mechanisms holds true, an experiment was carried out using EMS, of which metabolic half-life has been reported to be fairly short. EMS was injected in saline into the female pupae of a wild-type strain C108 at different developmental stages, every 24 or 48 h. The administered dose was 20 μg/pupa. Mutation frequency was estimated by the specific loci method using *pe* and *re* as

* Presented at the Third Annual Meeting of The Japanese Environmental Mutagen Society, held September 28, 1974.

markers. Induced frequencies varied with two peaks, major and minor. Mutation response was lowest three or four days before emergence and then it rose slightly showing a minor peak one–three days before emergence. This finding seemed to support the first hypothesis, *i. e.*, reaction takes place around meiotic metaphase. A major peak of one or two orders of magnitude higher was found at the earlier mid-pupal stage, which seemed to support the second hypothesis, *i. e.*, immediate reaction. Perhaps the latter may represent the main response.

Thus the system appears to be applicable also to the detection of mutagens of short-life activity.

Embryonic Lethality in Progeny of X-Irradiated Silkworm Moths: Inherited Lethality and Sex-Ratio Distortion

Akio MURAKAMI

Germ-cells of the silkworm moths were irradiated with X-rays at different doses ranging from 0.5 to 21 kR and then were crossed with unirradiated moths in the opposite sex. The deposited fertilized eggs were examined for either hatched or non-hatched eggs. The latter incidence was regarded as embryonic lethal mutations. The larvae were further examined for their mortality and sex-ratio. The surviving P_1 progeny was again crossed with normal moths of the opposite sex. The embryonic lethality and other biologival incidences of F_1 progeny were also investigated in the same manner as did in the P_1 progeny.

The results obtained so far clearly indicated that dose-response curves in the P_1 progeny as well as in the F_1 progeny were linear at lower doses and non-linear at higher doses. The embryonic lethality of the F_1 progeny from the treated male (XX) moths (or mature sperm) was markedly increased as compared with that of the P_1 progeny at corresponding dose, regardless of mating types. This inherited lethality was clearly dose-dependent. This phenomenon was interpreted as a delayed lethal effect. It should be noted, however, that the lethality of the BF_1 progeny showed a decrease of about 50 percent as compared with the preceeding progeny. In contrast to these findings, when the female (XY) moths or prophase I oocytes have been irradiated with X-rays no remarkable such the inherited lethality was observed in F_1 and BF_1 progeny. The cause of these embryonic lethalities observed in these F_1 and BF_1 progeny from the treated male moths would be due to

translocations which may be able to pass through meiotic divisions.

Treated male moths produced much more male than female progeny (or lost much more female than male progeny in the course of embryogenesis). This sex-ratio distortion was depended on the dose. However, irradiated female moths produced the same number of male and female progeny. The possible cause of this phenomenon would be explained as being due to sex-linked (recessive) lethal mutations. In addition to this class mutation, mutational events as an autosomal factor (s) interfering with the X-chromosome inactivation mechanisms in the normal oogenesis should be also considered to be one of possible interpretations for the sex-distortion. These findings suggested that embryonic lethal mutations in the P_1 progeny from the treated males would be included in dominant lethal mutations as chromosomal abnormality, sex-linked recessive lethal mutations and others.

Mutagenicity of Diethylsulphate in Spermatozoa of the Silkworm
I. Delayed Mutagenic Effect

Akio MURAKAMI

A high incidence of mosaic mutations is a remarkable characteristic feature in chemically-induced mutations in the silkworm. It is generally thought that the mosaic mutation would be expressed as a delayed mutagenic (fixation) effect on the induced pre-mutational lesion. However, a question arised whether the delayed mutagenic effect could be separated from a delayed drug-action effect depending on the half-life of chemicals. The latter effect will be more pronounced for chemicals with a long half-life than those with a short one.

The mutagenicity of diethylsulphate (DES) in pupal spermatozoa of the silkworm was investigated to achieve a better understanding of the nature of chemically-induced delayed mutagenic effects. DES was selected by a reason of its short half-life (1.8 hr at 25°C) and strong mutagenicity. It should be of interest to note that DES is extensively used in a variety of ethylation processes in organic synthesis and in various commercial areas. Detection of mutational incidences was made by the egg-color specific locus methods. 0.025 ml per pupa of a freshly prepared DES emersion at different concentrations from 0.1 to 1.0% in 0.85% NaCl solution was injected into the dorsal abdomen of 11-day old male pupa (or 2 days before emergence), which was 0.75 g in body weight.

The result of experiments indicated that DES induced a markedly high incidence of simple mosaics regardless of locus, whereas the incidence of complete mutations was rare. Other type mutants such as complex double and triple mosaics were also detected slightly. Although the testis in pupae at this stage contains various stages of germ-cells ranging from primordial spermatogonia to spermatozoa, the most advanced germ-cell or mature spermatozoon would be only concerned in fertilization. Therefore it would take at least 50 hours between the DES treatment and the syngamy under the experimental condition at 25°C. Considering the short half-life of DES in aqueous media and the pupal age (or germ-cell stage) tested it is very unlikely to suspect that the chemical was mutagenic in either sperm pronuclei or cleavage nuclei. Consequently, the high incidence of mosaic mutations would be interpreted on the assumption that DES which induce premutational damage in spermatozoa is fixed and/or expressed as mutations by passing through one or more cleavage divisions.

Among various types of mosaics, half-and-half type mosaics were detected. If this type mutant were what is called the real one-half type, this mutant would arise from a permanent change in one strand of an already exisiting double strand DNA molecules in mature sperm. The complete mutation would arise from simultaneous two-hit events in the paired nucleotide chains of DNA in the locus. Double mosaics would arise from an independent two-hit event at the *pe* locus in one strand of chromosomes and that at the *re* locus in another strand in the mature sperm. The triple mosaic mutation would arise as the mutational events both in mature sperm and in early cleavage cells.

In any case, the result of the present experiment clearly suggested that about 90% of DES-induced egg-color mutations in mature sperm would have been resulted from the delayed mutagenic effect, but remaining about 10% (half-and-half type mosaics, complex mosaics and completes) would have been resulted from the permanant genetic change in an already exisiting gene DNA of the sperm.

Mutagenicity of Diethylsulphate in Spermatozoa of the Silkworm
II. Dose-Mutagenicity Relationship

Akio MURAKAMI

Analysis of dose-mutagenicity relationship would provide some useful

informations for better understanding the nature of chemically-induced mutations as did for that of radiation-induced mutations. It would also provide an important information to asses the dose-level at which the chemical is likely to be used in view of the beneficial judgement on environmental chemicals. Although a number of reports on the chemically-induced mutations little informations on the dose-mutagenicity relationship have been communicated. In the relations between diethylsulphate (DES) dose-egg-color mutagenicity in silkworm spermatozoa, the frequency was increased slowly at first and then rapidly to the dose level at 0.6%, regardless of the locus. Over this dose level the frequency of mutations was slowly decreased at the *pe* locus or kept at 0.6% level at the *re* locus. The frequency of complete mutations was too rare that the dose-mutagenicity relation curve could be regarded as that of the mosaic mutations for the most part. In other words, the DES dose-response curve of mosaic mutamutations is sigmoidal or logarithmic in shape, regardless of the locus. This observation is well agreed with that in the DES-induced sex-linked recessive lethal mutations of *Drosophila* spermatozoa (Pelecanos and Alderson 1964). The DES dose at which the maximum mutation frequency was shown in silkworm spermatozoa was about 0.6%, indicating that it is about 1.5 times as high as that in *Drosophila* spermatozoa (0.4%) (Pelecanos and Alderson 1964). In *E. coli* an exponential increase was observed in streptomycin-resistant mutations after treatment with mono-functional alkylating agents, MMS and DDVP (Wild 1973).

Reasons why the DES dose-mutagenicity curve was sigmoidal could be interpreted by at least two possibilities as follows: one is characteristics of chemical reaction of DES in ethylation of sperm DNA. Indeed, Sega *et al.* (1973) indicated that in the dosimetric study on mouse sperm DNA the ethylation per nuclectide increased at a rate greater than the first power of the dose. Another is the nature of biological interaction of DES *in vivo*: cellular repairability of ethylated sperm DNA at lower doses and cell killing (or toxic) effect at higher doses. Furthermore, a compromized mechanisms of those possibilities could not be completely ruled out.

Lack of Recombinogenicity of Ethyl Methanesulphonate in the Silkworm Oocytes

Akio MURAKAMI and Isao ARIGA[1)]

In the previous paper, it was reported that a bifunctional as well as monofunctional alkylating agent, Mitomycin C (MC), slightly but significantly induces much more interchange type recombinants than exchange type ones in early growth stage oocytes of the 5th instar silkworm larvae (Ariga and Murakami, J. Sericult. Sci. Japan 44: 154–160, 1975). To account for the finding a tentative interpretation was proposed that MC produces both DNA strand breakages (or cut) in the larval oocytes due to the formation of cross-linking between the paired DNA strands and which would reunion in turn with recombinational repair mechanism. The purpose of the present experiment was intended to test whether a monofuctional alkylating agent ethyl methanesulphonate (EMS) can induce recombinants in the larval silkworm oocytes.

The incidence of recombinations was scored by the egg-color method. 0.025 ml per larva of EMS solution at three different concentration (0.25, 0.50 and 1.00%) dissolved in 0.85% NaCl solution was injected into 5 and 7 day female larvae at the 5th instar heterozygous in the trans type of two egg-color genes, *pe* (V–0.0) and *re* (V–31.7).

The frequency of recombinants recovered was very low and not significantly different from that of the control, regardless of EMS doses and stages at which larvae were treated. It should be noted, however, that at 7 day in the 5th instar (or mature) larvae EMS at a dose of 0.1% slightly but clearly increased the frequency of mosaic or fractional-body mutations when compared with the control in the egg-color specific locus method, but not increased the incidence of complete or whole-body mutations probably corresponding to double strand DNA changes. Such being the case, the dose of EMS used in the experiment might be not insufficient for the induction of recombinants. Current thought indicates that EMS induces a predominant number of single strand breaks (or nick). EMS-induced single strand DNA changes (including DNA strand breaks) would not be converted into double strand changes since the germ-cell at this larval stage has already been finished DNA replication. It is very likely to note that this type single strand damage would be repaired by certain mechanisms, resulting in the low incidence of mosaic mutations.

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From these results in the present and previous experiments, recombinogenicity of the alkylating agents may depend on the number of functional sites and it may require at least two functional sites. In other words, both strand breakages in the complementary DNA strands may be essentially necessary for the induction of recombinations in prophase I oocytes of the silkworm.

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN MICRO-ORGANISMS AND PLANTS

Radiation and Drug Sensitivities of *Bacillus subtilis rec* Strains

Yoshito SADAIE, Hatsuo INUKAI and Tsuneo KADA

Lack of genetic recombination capacity usually makes strains manifest different phenotypes. Recombination-deficient strains are usually sensitive to UV, gamma-rays and certain chemicals. Recombination repair processes are postulated to be efficient mechanisms assuring cellular resistance to a number of DNA-damaging agents. We examined radiation and drug sensitivities of *rec* mutants of *Bacillus subtilis* carrying one of seven distinctive *rec* mutations. The results indicated that the radiation (UV or gamma-rays) sensitivities are roughly correlated to the sensitivities to typical chemical mutagens. The *recE* locus seems to be essential and the *recA* and *recG* loci may be also required generally for DNA repair. Some chemicals such as 4NQO or potassium bichromate are more specific to *recB* or *recD*

Table 1. Drug sensitivities of *Bacillus subtilis rec* strains

Strains	Length of inhibition (mm)				
	MC (0.3 μg)	MMS (119 μg)	4NQO (4.8 μg)	$K_2Cr_2O_7$ (30 μg)	DAPA (194 μg)
rec$^+$	1	0	2	2	2
recA1	7	13	6	8	8
recB2	9	14	11	16	7
recC7	3	4	2	6	1
recD3	8	13	7	16	6
recE4	9	19	5	10	11
recF16	7	14	5	10	8
recG13	7	16	6	9	9
rec$^+$	1	0	2	1	0
rec-43	12	16	12	12	11
rec-45	14	16	8	13	14

Bacteria were streaked radially from the center of broth agar.
Paper disc was placed with indicated amounts of drug on the center of the plate.
After overnight incubation, the length of growth inhibition was measured (Mutrition Res. **16** 165, (1972)).
MC: mitomycin C, MMS: methyl methanesulfonate, 4NQO: 4-nitroquoline-N-oxide, DAPA: sodium-*p*-dimethylaminobenzene-diazosulfonate

mutants. SPO2 lysogens of *recE*, *recF* and *recG* strains are not inducible. These loci may be required for their inducible repair functions. Many of these *rec* strains were kindly supplied by Dr. Dubnau (J, Bacteriology *117*, 488).

Some of the results are described in Table 1.

Prophage Induction in *rec* Strains of *Bacillus subtilis*

Yoshito SADAIE

Isolation and characterization of bacterial recombination-deficient (*rec*) mutants revealed involvement of certain *rec* gene products in prophage induction. The prophage SPO2 inducibility was examined in *rec* mutants of *Bacillus subtilis* Marburg NIG43 (*rec-43*) and NIG45 (*rec-45*) isolated in our laboratory. As indicated in Fig. 1, the *rec-43* mutation locates right to *dna-8132* (Hara *et al.* Nature New Biol. *244*, 200 (1973)) with a co-transfer index of about 75% by transformation and the *rec-45* tightly linked to *recA1*. SPO2 lysogens of these mutant strains did not produce phage

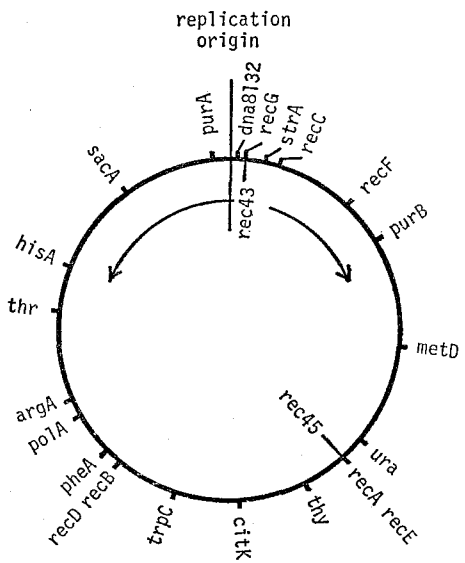

Fig. 1. Genetic map of *Bacillus subtilis* showing *rec* loci and some reference markers.

particles after UV or mitomycin C treatment, though SPO2 transfection of both mutants was normal, indicating possible involvement of *rec-43*$^+$ and *rec-45*$^+$ gene products in destruction of the repressor system of SPO2 (J. Bacteriology, in press).

Recombination-deficient strains so far isolated in other laboratry were obtained, lysogenized and treated with UV or mitomycin C. Among seven distinctive *rec* mutations, only three (*recE4*, *recF16* and *recG13*) conferred the lysogens uninducible. Genetic analysis revealed that the mutations *rec-43* and *rec-45* located very probably in the *recG* and *recE* loci respectively. The *rec-45* and *recE4* lysogens produced very reduced numbers of phage particles both spontaneously and upon artificial induction. The above bacterial strains were kindly supplied by Drs. C. Anagnostopoulos and D. Dubnau. Figure 1 was drawn based on the original maps of Lepesant-Kejzlarova *et al.* (J. Bacteriol. *121*, 823) and Dubnau *et al.* (J. Bacteriol. 117, 488).

Improved Procedures of the *rec*-assay for Rapid Detection of Chemical Mutagens

Tsuneo KADA and Yoshito SADAIE

Since most chemical mutagens produce damages in DNA that are subjected to cellular recombination repair, those substances showing increased lethal activity on Rec$^+$ over Rec$^-$ cells may have damaged cellular DNA and such DNA-damaging agents are very often mutagens. The *rec*-assay utilizing *B. subtilis* strains H17 Rec$^+$ and M45 Rec$^-$ (Mutation Res. *16*, 165, 1972) was useful for detection of upto date more than 30 new chemical mutagens in the environment including phloxin, AF2, Dexon, NBT, etc. In the currently adopted mothod, the Rec$^+$ and Rec$^-$ bacteria are grown overnight in liquid broth, each streaked on the "dry" surface of broth agar, the starting points are covered by a round paper disk containing each drug, the plates are incubated overnight at 37°C, and the inhibition zones are measured.

We have recently performed the following examinations.

A: *Strains*: We collected more than 50 repair-deficient strains of *B. subtilis*, *E. coli* and *Salmonella* possessing impairements in *rec*, *hcr* or/ and *pol* functions and studied about their sensitivities to 30 typical chemical mutagens. A set of H17 Rec$^+$ and M 45 Rec$^-$ strains of *B. subtilis* showed so far the highest and widest sensitivity spectra.

B: *Preparation of bacteria*: Practically no difference in the sensitivity was found in exponential and stationary bacterial cultures. On the other hand, the stocked cultures (at −80°C, with 12.5% glycerol) showed considerably higher sensitivity than freshly grown cultures.

C: Cold incubation: Usually bacteria and drug were placed on the plate at the same time and incubated overnight at 37°C. We recently found that if one lets diffuse the drug first on the plate at cold and then grow the bacteria, the drug inhibition zones were much larger than those in the usual case.

Considering the above observations, the improved new procedures are the followings; by these procedures, the sensitivity increased about 30 times.

Strains H 17 Rec^+ and M 45 Rec^- are grown overnight in broth (meat extract 10 g, polypeptone 10 g, NaCl 5 g, water 1000 ml, pH adjusted to 7.0). One ml of 50% glycerol (W/V) was added into 3 ml of full-grown bacterial broth culture and stocked at −80°C. On the day of experiments, each culture was streaked on the "dry" surface of broth agar (15 g/l of agar added in the above liquid broth) and the drug paper disk is placed. All the plates are kept at 4–5°C for 24 hours then incubated at 37°C for about 20 hours. The length of inhibition zone is then measured.

Table 1

Chemical agent (μg per disk)	Inhibition zones in mm			
	Old procedures		New procedures	
	H17 Rec^+	M45 Rec^-	H17 Rec^-	M45 Rec^-
4NQO (0 02)	0	1	0	15
(0.002)	0	0	0	1
AF2 (0.02)	0	6	0	16
(0.002)	0	1	0	9

AF2 Mutagenicity and Reductase Activity of *E. coli* B/r WP2 resistant to Cr^{+6}

Tsuneo KADA

It has been previously reported that derivatives strains resistant to AF2 [2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide, or furylfuramide] were isolated from *E. coli* B/r WP2 *try* and that supplementation of rat liver homogenate (S9) was recessary for mutations ($Try^- \rightarrow Try^+$) to occur in them (cited in

Mutation Res. *32* 1975, p. 74). As supposed by the above observation and also shown experimentally by Dr. K. Tsuji (Institute of Physical and Chemical Research), cellular reductase activity was found to be deficient in the AF2-resistant bacteria than in the original sensitive bacteria, since DNA-reactive metabolites may be produced by reduction of AF2. The reduction capacity of S9 is thus involved in production of an active form of AF2. We have recently observed that though many derivative strains of *E. coli* WP2 resistant to potassium chromate ($K_2Cr_2O_7$) were also resistant to AF2, about 10% of Cr^{+6} resistant isolants became sensitive to AF2. Since Cr^{+6} gave positive results in the *B. subtilis* rec-assay and showed mutagenec effects on *E. coli* WP2 strains but Cr^{+3} did not (H. Nishioka, Mutation Res. 31, 185, 1975), we suppose that there may exist at least two or three types of reductases whose increased activities are correlated with the cellular Cr^{+6} resistance. The one of them may be involved in reduction of AF2 as well as in reduction $Cr^{+6} \rightarrow Cr^{+3}$.

Ultraviolet Light-induced Mutagenesis in *Bacillus subtilis* Spores

Yoshito SADAIE, Yukio SAITO and Tsuneo KADA

Studies on ultraviolet light (UV) mutagenesis in germinating spores of *Bacillus subtilis* have some unique features. Semi-conservative DNA synthesis begins synchronously about 2 hours after the commencement of germination. It may be interesting to know if photoproducts in spores are mutagenic or not and how pre-mutational damages are fixed. Strains

Table 1. Frequency of His^+ mutations induced by UV irradiation of spores

Strain	UV dose (ergs/mm²)	No. of surviving spores/ml ($\times 10^7$)	No. of His^+ mutants found/ml ($\times 10^2$)	Frequency of induced His^+ mutations/spore ($\times 10^{-5}$)
pol$^+$	0	9.5	1.8	
	300	7.7	11.9	1.36
	900	3.0	60.1	19.8
	1800	0.2	40.8	204.0
polA59	0	9.2	2.7	
	300	3.1	70.1	22.3
	600	0.5	21.5	24.7
	900	<0.01	2.7	>270.0

HA101 (*leu met his*) and HA101 (59) F (*leu met his polA59*) are isogenic and carry Pol^+ and Pol^- characters and a suppressible histidine auxotroph. Their spores were irradiated with UV and plated on selective agar containing small amounts of broth for detection of mutations to histidine-independence. We observed that UV induced mutations in spores and their degree was higher in Pol^- than Pol^+ spores (Table 1). It has been also confirmed in vegetative cells that Pol^- cells produce more reversions than Pol^+ cells. The frequency of mutations declined considerably when UV-exposed spores were incubated in liquid both before plating on selective agar. Therefore, it seems that photoproducts in spores serve as premutational damages and their fixation process is hampered by polymerase I function.

Involvement of a Common Enzyme in the Repair of Deprinated and γ-ray-irradiated DNA of *Bacillus subtilis*

Takehiko NOGUTI and Tsueno KADA

We have isolated from *Bacillus subtilis* cells an enzyme which makes γ-ray-irradiated DNA a better primer for DNA-polymerase I and named "primer activating (PA) enzyme" (Biochim Biophys. Ada *395*, 284 & 294, 1975). The enzyme has been partially purified by DEAE and phosphocellulose column chromatography. It was shown that this enzyme introduced new nicks in DNA of phages T7 in addition to those introduced by γ-ray irradiation. Verly W. G. *et al.* (Nature New Biol. **244**. 1973, 67–69) isolated an endonuclease specific to apurinic sites of DNA and demonstrated its general occurrence in bacteria, plants and animal cells. Because both the apurinic sites and certain portion of γ-ray-provoked lesions are known to be alkali-labile, we examined if the PA enzyme might be also specific to the deprinated sites. Depurination was carried out by heating T7 DNA at 70°C by the method of Lindahl, T. and Nyberg, B. (Biochemistry **11**, 1972, 3610–3617). DNA was then treated with the PA enzyme in a buffered solution (pH 8.0). The priming activity was determined as to the above DNA samples after purification with phenol treatment. In order to observe the effect of our enzyme on the alkali-labile sites, denaturation of DNA was carried out in two ways, treatment with formamide or exposure of a portion of the treated DNA to alkali. The treated DNA was thereafter analyzed with sucrose gradients containing formalin. The profiles obtained with formamide treat-

ment indicated that the enzyme introduced cuts into the heated DNA possessing apurinic sites. The subsequent alkali treatment reduced the molecular weight of the heated DNA close to a level found with the enzyme treated DNA. The results show that the primer activating (PA) enzyme introduces nicks at the alkali-labile region of heated DNA, probably at its apurinic sties. It is thus indicated that certain portion of γ-ray provoked lesions of DNA may be repaired by the apurinic site specific enzymes.

Induction of *wx* Mutant in an Inbred Line of Maize

Etsuo AMANO

The waxy starch (*wx*) locus of maize is one of a few loci suitable for genetic fine structure analysis. Its phenotype can be examined in endosperm and in pollen grains which are in haploid generation. Thus the fine structure analysis of the locus can be done by pollen analysis in which scoring of 10^6 pollen grains is not so difficult.

To obtain useful *wx* mutants, several mutagens have been tried and EMS was found to be the most effective. However, since maize has unisexual flowers, segregation of the mutants by self-pollination can not be expected like barley or rice plant when seeds are treated with the mutagen. To detect *wx* mutants, the test cross with a standard *wx* tester stock is a very convenient and common method. However when hybridized by the test cross, the genetic background may be disturbed; moreover it becomes very difficult to identify a newly induced *wx* mutant gene from the tester *wx* in the segregated progenies. To establish inbred line mutants in maize, useful method has been proposed previously (Ann. Rep. N. I. G. No. 23 pp. 84–85). It utilizes mixed pollen of the tester *wx* and the non-treated parental *Wx* line for pollination of mutagen treated *Wx* plants. If a cob was found to have some *wx* mutant kernels, theoretically a third of the *Wx* kernels or a half of the kernels with the parental *Wx* phenotype on the cob would be heterozygous for the newly induced *wx* mutant gene. By self pollination of such inbred line heterozygotes, three *wx* mutant lines have been established in 1974. Also another additional ten mutant carrying cobs have been detected. These three inbred line mutants induced by EMS were normal in fertility and in other viability characters. These inbred line mutants will be used in studies on fine structure analysis of the *wx* locus and on characterization of the induced mutants.

Induction of Waxy Starch Mutant in Rice

Estuo AMANO

Waxy (*wx*) mutants were obtained at a high frequency in maize by treating seeds with a chemical mutagen, ethyl methanesulfonate (EMS). Ionizing radiations have failed to induce *wx* mutants having good viability or fertility. To study fundamental factors in mutation breeding, seeds of *Oryza sativa* var. Norin No. 8 were treated with EMS to induce waxy starch mutants. Norin No. 8 is one of the typical paddy field rice in Japan and its starch character is non-waxy (*Wx*). Seeds were soaked in 0.05 M or 0.1 M aquous solution of EMS for five hours at 27°C in a shaking incubator. After the treatment, seeds were rinsed three times with water. In these treatments, distilled and deionized water was used. The rinsed seeds were sown in soil of wooden flats in green-house. Some of the seedlings were transplanted to the soil bed in a greenhouse, but most of them were left in flats densely planted. Mature panicles were harvested and dried. Each panicle was threshed separately and more than ten hulled kernels were examined visually. Possible *wx* mutants were further examined by means of iodine staining. Among 1535 panicles thus examined, seven panicles were found to segregate waxy starch kernels. The overall frequency of *wx* mutants was 0.45%, being about a half of that of the maize experiment. Although the value was smaller in rice than in maize, it is still very high for a single locus. According to the results in maize experiments, most of the mutations induced by EMS might be point mutations, but still another deleterious mutations might occur simultaneously in these mutants, because the high efficiency of the mutagen might be also true in other loci. Studies on these simultaneous mutations and on characteristics of the induced mutants will be continued further.

Chromosome of Cultured *Haplopappus* Cell

Etsuo AMANO

The cultured callus cell of *Haplopappus gracilis* ($2n=4$) is soft and fast growing. Five cell lines have been maintained on agar slant medium for more than three years. The medium (EFR) used is a modified Erikson's which is based on Murashige and Skoog's medium and contains 2.4 D (2ppm) and yeast extract (0.3%) besides inorganic salts, vitamines and sucrose (2%).

Chromosomes of these five cell lines and a cell line (HB) of one year old were examined by aceto-orcein staining. Squash preparations were made from freshly growing callus. To examine the ploidy of the cell, anaphase chromosome numbers were converted to metaphase numbers. The results are shown in Table 1. In five out of the six cell lines, most of the cells were diploid. However, in a cell line HL, more than half of the cells examined were tetraploid. When the chromosome number was examined all of the five cell lines were diploid as reported last year. Further examinations were made about two HL derivatives used in an experiment which might not disturb the ploidy. Results included in Table 1 indicate that one of them had the same proportion of ploidy as the parental HL line, but the other was very alike to the other diploid cell lines. Such a fluctuation of the ploidy might be caused by chance during transferring the callus to a new medium, although further investigations are necessary.

In the course of these chromosome studies, certain difference in the karyotype was noticed. The cell lines, HR (originated from root), HS (stem) HNC (spontaneously occured on stem) and HB (flower bud), were all in homomorphic with two submedian and two subterminal chromosomes with satellite. In two cell lines, HL (leaf) and Hse (seed) which were derived from different individual plants, had an identical submedian chromosome pair, but one of the satellite chromosome was different in shape. It seemed to have elongated short arm and lack the satellite, giving a significantly heteromorphic configuration. Such heteromorphism in *H. gracilis* was reported by Kamra (Om P. Kamra 1963 Chromosoma 13: 540–544). Effect of the heteromorphism or of this chromosome on callus culture was not significant

Table 1. Number of chromosomes in cultured *Haplopappus* cells on EFR agar medium.

Cell line	Transfer generation	Cells with chromosome No.			
		4	8	12	16
715 HR	74	58	2		
715 HS	73	78	1		
7110HNC		116	13	1	1
716 HL	68	17	22		1
705 Hse		71			
736 HB		8			
716 HL	(SGC24)	17	21		
716 HL	(SGC48)	114	6		

as far as morphology and growth of the callus were concerned. It may be an useful marker in cytological studies of these cultured cells.

Effects of Fast Neutrons on Dry and Wet Seeds in Maize

Taro FUJII

To investigate the effect of water content upon gamma-ray or neutron-induced mutation, seeds heterozygous for the Yg_2-gene were used. Moisture-stabilized (13%) or 24 hrs water-steeped seeds were exposed to ^{137}Cs gamma-rays or 14 MeV neutrons. Mutation from Yg_2 to yg_2 was detected in the seedlings as color change, green to yellow-green. Although the mutation frequency enhanced by increasing the dose almost in a linear relationship, the average number of *yg*-stripes per leaf differed considerably between dry and wet seed lots of gamma-rays. The frequency in each 3 different doses of wet seeds was about 1.5 times higher than that of corresponding lot of dry seeds. Experiments were repeated twice using similar doses of neutrons in order to check carefully the mutation frequency at respective doses as well as to determine the difference in frequencies between dry and wet seeds. However, unlike the case with gamma-rays, no clear difference in the frequency between wet and dry seeds was observed. Namely, a frequency of 2% was observed with around 20 rad exposures; frequencies of 6–7% at the highest dose lots were observed throughout two experiments using dry and wet seeds. It may be noted that the no change or no recovery in neutron induced mutations was seen in the dry or wet seeds treatments. Though the mechanism of neutron mutagenesis lacking repairability is not clear, the present observation had led to a general conclusion that the effect of neutron is less modifiable by exposure conditions or by environmental changes.

Sex Expression in the Offspring of Gamma-ray Induced Monoecious-like Plants in Cucumber

Taro FUJII

Effect of gamma-irradiation on the sex expression in the F_1 plants obtained from the crosses between original gynoecious strain (MSU) with male flowers of monoecious-like plant (MSU–713–5M) (cf. Ann. Rep. 24, 45) has been studied. F_1 plants came from 6 F_1-fruits were examined so far. Observation was carried out up to 50th nodes in each individual since some plants

did not survive beyond ca. 55th nodes. As shown in Table 1, most of the strains segregated monoecious-like plants. The number of male flowers in segregants from the F_1-fruits No. 1 and 3 was merely 2 or 3 on the basal nodes in each plant. On the other hand, most segregants from F_1-fruit No. 5 had developed many male flowers. For instance, 116 male flowers in total had developed over 19 nodes in some plants; even in the least case of the same strain, 26 male flowers developed over 4 nodes. Differences in the number of male flowers in segregants among the F_1-strains might be correlated to certain genic alterations, since sex expression in cucumber is controlled mainly with 2 genes, *Acr* and *G*. Examination of F_2 and F_3 generations has been done with several F_2 seeds obtained from selfed F_1-segregants up to date, and segregation of monoecious-like plants was observed successively. Thus the monoecious-like behaviour of the radiation treated MSU strain might be due to certain heritable change.

Table 1. Segregation of monoecious-like plants in F_1

F-fruits number	No. of F_1 plants	No. of monoecious-like plants
1	23	6
2	5	0
3	14	2
4	no germination	
5	41	18
1	1	0

VIII. POPULATION GENETICS (THEORETICAL)

Some Models of Allelic Mutation in Molecular Population Genetics

Motoo KIMURA

In order to analyse theoretically how much genetic variability is maintained within a finite population at the molecular level (i. e., at the level of the internal structure of the gene), two models of allelic mutation are considered. In the first model, it is assumed that the number of possible allelic states at a gene locus is so large that whenever mutation occurs it leads to a new, not preexisting allele; both selectively neutral and overdominant cases are considered. In the second model, allelic states are expressed by integers and stepwise production of alleles by mutation is assumed.

Analytical treatments based on the diffusion equation method are supplemented by numerical analyses and Monte Carlo experiments. Formulae are derived which give the effective number of alleles maintained in a population at equilibrium. For details, see "Lectures on Mathematics in the Life Sciences" (1974, American Math. Soc.): 1–23.

Simulation Studies on Electrophoretically Detectable Genetic Variability in a Finite Population

Tomoko OHTA and Motoo KIMURA

Using a new model of isoalleles, extensive Monte Carlo experiments were performed to examine the pattern of allelic distribution in a finite population. In this model it was assumed that the set of allelic states is represented by discrete points on a one-dimensional lattice and that change of state by mutation occurs in such a way that an allele moves either one step in the positive direction or one step in the negative direction on the lattice. Such a model was considered to be appropriate for estimating theoretically the number of electrophoretically detectable alleles within a population. The evenness of allelic distribution was measured by the ratio of the effective to the actual number of alleles (n_e/n_a). The results of the Monte Carlo experiments have shown that this ratio is generally larger under the new model of isoalleles than under the conventional Kimura-Crow model of neutral isoal-

leles. In other words, the distribution of allelic frequencies within a population is expected to be more uniform in the new model. By comparing the Monte Carlo results with actual observations, it was concluded that the observed deviation from what is predicted under the new model with selective neutrality is not in the direction of conforming to the overdominance hypothesis but is, in fact, in the opposite direction. For details see Genetics **76**: 615–624.

Probability of Gene Fixation in an Expanding Finite Population

Motoo KIMURA and Tomoko OHTA

A mathematical theory was developed, based on diffusion models, that enables us to compute the probability of a rare mutant allele eventually spreading through a population when the population size changes with time. In particular, we elaborated the case in which the mutant allele has a definite selective advantage and the population expands following the logistic law. In this case, the probability of ultimate fixation of a single mutant is given by $u=2s(Z/N)$, where s is the selective advantage and Z/N is a factor by which the probability of fixation is modified through population expansion. Analytical expression was obtained for Z/N, and the validity of the formula for u was checked by Monte Carlo experiments. For details, see Proc. Nat. Acad. Sci. **71**: 3377–3379.

Detrimental Genes with Partial Selfing and Effects on a Neutral Locus

Tomoko OHTA and C. Clark COCKERHAM

Gene and genotypic frequencies for a deleterious mutant in mutation selection balance are derived for an infinite population undergoing partial self-fertilization. These provide formulations of mean survival and the mutational load. Obtained also are the average number of mutant genes and affected individuals stemming from a single mutant.

As a concomitant effect on frequencies at a neutral locus the mutational load is distributed disproportionately among the neutral genotypes. For partially recessive mutant genes on the 1, $1-sh$, $1-s$ scale, the effect is to increase the frequency of the heterozygote and to decrease the frequencies of homozygotes at the neutral locus relative to the frequencies expected with

complete neutrality. This apparent overdominance at the neutral locus has been shown to be connected with identity disequilibrium rather than linkage disequilibrium. It increases generally as s and h decrease, and as the proportion of self-fertilization and the degree of linkage increase. The apparent overdominance with complete linkage is generally less than double that for free recombination. For partially dominant mutant genes, $h \geqq 1/2$, the effects on the frequencies of heterozygote and homozygotes at the neutral locus are reversed. For details, see Genet. Res. **23**: 191–200.

Speed of Gene Frequency Changes in Reverse Directions in a Finite Population

Takeo MARUYAMA and Motoo KIMURA

It is shown that in finite populations the average length of time which it takes for an allele to increase its frequency from p to q ($>$p) on the way to fixation is equal to the average length of time which the same allele takes when its frequency decreases from q to p on the way to extinction, although the probabilities of occurrence of these two events can be very different. In addition to the means, the sojourn times in each specified gene frequency interval are shown to be the same for the two reverse processes. For details see Evolution **28**: 161–163 (1974).

A Markov Process of Gene Frequency Change in a Geographically Structured Population

Takeo MARUYAMA

A Markov process (chain) of gene frequency change is derived for a geographically-structured model of a population. The population consists of colonies which are connected by migration. Selection operates in each colony independently. It is shown that there exists a stochastic clock that transforms the originally complicated process of gene frequency change to a random walk which is independent of the geographical structure of the population. The time parameter is a local random time that is dependent on the sample path. In fact, if the alleles are selectively neutral, the time parameter is exactly equal to the sum of the average local genetic variation appearing in the population, and otherwise they are approximately equal. The Kolmogorov forward and backward equations of the process are obtain-

ed. As a limit of large population size, a diffusion process is derived. The transition probabilities of the Markov chain and of the diffusion process are obtained explicitly. Certain quantities of biological interest are shown to be independent of the population structure. The quantities are the fixation probability of a mutant, the sum of the average local genetic variation and the variation summed over the generations in which the gene frequency in the whole population assumes a specified value. For details see Genetics **76**: 367–377 (1974).

A Proof that Certain Quantities are Independent of the Geographical Structure of Population

Takeo MARUYAMA

A proof is given to that certain quantities are independent of the geographical structure of a population. The quantities are: (1) the fixation probability of a mutant; (2) the sum of the quantity $x(1-x)$, where x is the mutant frequency, while the mutant is segregating; and (3) the quantity $x(1-x)$ summed over the generations during which the gene frequency in the whole population assumes a specified value. The independence of geographical structure for the latter two quantities is not exact if there is selection, but is a close approximation. The model is a geographically structured version of Moran's haploid overlapping generation model. The population consists of colonies connected genetically by migration. Each individual has the same negative exponential lifetime distribution. When an individual dies, it is immediately replaced by an individual born in the same colony with a probability proportional to the frequency and fitness of the type giving birth. In a diploid population the guantity $x(1-x)$ is proportional to the heterozygosity. For details see Theo. Pop. Biol. **5**: 148–154 (1974).

IX. POPULATION GENETICS (EXPERIMENTAL)

Similarity of Gene Frequencies in Different Drosophila Species: Evidence for the Neutrality Hypothesis of Protein Polymorphisms

Tsuneyuki YAMAZAKI and Takeo MARUYAMA

Ayala and Gilpin (Proc. Nat. Acad. Sci. **71**: 4847, 1974) applied a genetic distance method to test the validity of the neutrality hypothesis of protein polymorphisms advanced by Kimura and Ohta. Based on the data of several Drosophila species they have concluded that the theoretical prediction of the measure is incompatible with the data and therefore the neutrality hypothesis is proved to be incorrect. Here, however, we show that Ayala and Gilpin's conclusion is unwarrented and more importantly that their measure is indeed consistent with the neutrality hypothesis and the test supports the hypothesis.

Consider two isolated populations (species) which were identical to each other at time t=0 (say), and assume that the two populations proceed independent evolution of gene frequency change under the influence of genetic random drift, natural selection, mutation etc. The frequencies of various alleles at a given locus become different in the two populations as time goes on. To this situation, Ayala and Gilpin used a genetic distance between populations, say 1 and 2, defined by

$$d=\left\{\sum_{j=1}^{k}(x_{1j}-x_{2j})^2\right\}^{\frac{1}{2}}\Big/\sqrt{2}\,.$$

where x_{ij} is the frequency of allele j in population i, and k is the number of alleles at a locus under consideration. The distance d takes values between 0 and 1. If two populations have identical gene frequencies $d=0$, and if two have no allele in common $d=1$.

Assuming no selection (neutrality), no mutation or migration from outside and finite population size (N), Ayala and Gilpin have calculated the statistical distribution of the d-values for a case of three alleles with equal initial frequency (1/3 each). The distributions are graphically presented for $t=0.1$N, 0.2N, 0.5N and N, but no further time. The distributions of d given in their figure are all bell shaped, though for larger t the variance is larger. It is important to note that the distribution is not given for t greater than N,

the population size. There seems to be no reason to assume that history of two separated species is always less than N generations.

In order to test the prediction derived from the neutrality hypothesis, they used data from Drosophila species. The d-values were measured among different species at three different degrees of evolutionary level; among subspecies, among sibling species and among fullspecies. They used three sibling species *D. willistoni*, *D. equinoxialis* and *D. tropicalis*; one nonsibling species *D. nebulosa*; two pairs of subspecies *D. w. willistoni*, *D. w. quechua* and *D. e. equinexialis*, *D. e. caribbensis*. They assayed electrophoretic alleles at 36 gene loci. At each of the three evolutionary levels, the distribution is strongly concaved downward and slightly distorted U-shaped with peaks at the $d=0$ and $d=1$ classes. As the evolutionary degree proceeds from subspecies level to full species level, the $d=0$ class becomes less frequent and the $d=1$ class increases, and the intermediate classes also decreases, (see Fig. 1–A which is reproduced from Fig. 2A in their paper). The graphs of the histogram contrast strongly with the presented predictions derived from the neutrality hypothesis. Ayala and Gilpin claim that contrast between the data and the prediction proves the failure of the neutral hypothesis. They further argue that more consistent explanation is some form of normalizing selection which maintains quasi-stable polymorphic equilibria.

Having strong reservation, we have made calculations for two different cases. The first is exactly the same as Ayala and Gilpin; no mutation, selective neutrality and three alleles of initially equal frequency (1/3 each). The distance d was calculated among ten separated populations. This enables us to make 190 pairwise comparison. The results of the distribution of the d-values are presented in Fig. 1–B for $t=$N, 4N, 5N. We can clearly see a remarkable agreement between the data presented in Fig. 1–A (the same as Ayala and Gilpin's Fig. 2A) and the prediction derived from the neutrality hypothesis. This comparison of Fig. 1–A and 1–B alone is sufficient to unwarrent Ayala and Gilpin's conclusion. The disagreement pointed out by them is simply due to insufficient separation time used for the prediction. When it is taken long enough as we did, the theoretical distribution agrees very well with the data. Our present finding can be regarded as supporting evidence for the neutrality hypothesis.

However we realize that this can not be taken too strongly for two reasons. The evolutionary time for the Drosophila species is not known and thus we are not certain as to the appropriateness of the generations used in our

calculations. In the absence of mutation, the genetic variability in each population decreases at a rate of 1/2N in each generation and eventually it becomes monomorphic. But the Drosophila populations studied are very polymorphic and it is not unreasonable to assume they are in some equilibrium states. To take this into account, we introduced mutation and kept the populations always in equilibrium states. A single equilibrium population was copied into 5 identical populations and thereafter each population

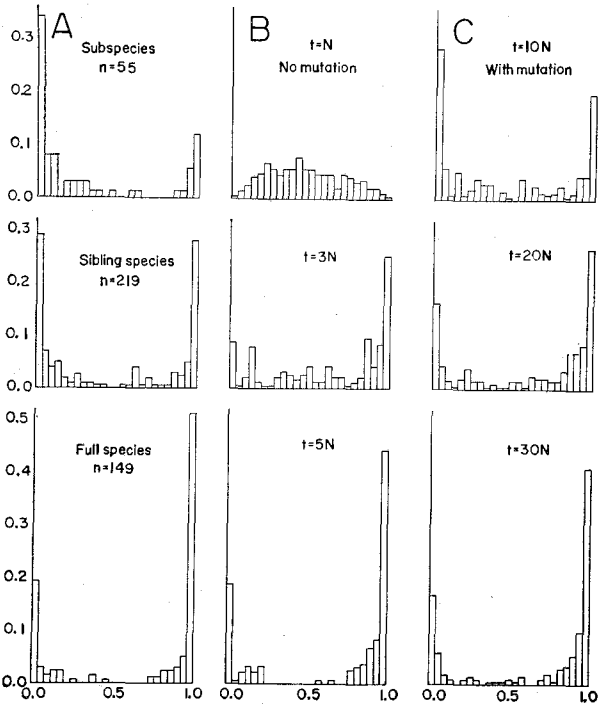

Fig. 1. (A) Distribution of loci relative to genetic distance between subspecies (upper), sibling species (middle), and non-sibling species (lower) of the Drosophila willistoni group. n is the number of pairwise comparison. Redrawn from Fig. 2 (A) of Ayala and Gilpin (1974).
(B) and (C) Distribution of genetic distance simulated by Computer TOSBAC 3400. The population size N=100. (B) is the result of simulation done under the same assumption as that of Ayala and Gilpin (1974): neutrality, no mutation and initially three alleles with equal frequency (1/3). In (C) mutation is incorporated, (4Nu=0.15).

proceeded independent evolutionary process. At three different generation times, the d-values were calculated among populations. We made 20 repications and altogether $10\times20=200$ pairwise comparisons, ($4Nu=0.15$). The distribution of the d-values are presented in Fig. 1–C. It is U-shaped and in early generations the $d=0$ class is more numerous than the $d=1$ class, but the situation is reversed in later generations. There is again a remarkable agreement between the data of Fig. 1–A and the prediction presented in Fig. 1–C. This case of prediction seems more realistic for the presence of mutation and thus each population itself is in a steady state. Therefore we present this analysis as supporting evidence for the neutrality hypothesis.

A similar point has been made by Nei and Tateno (Proc. Nat. Acad. Sci. 72; 2758–2760 (1975).

Negative Correlation between Lethal Genes and Polymorphic Inversions

Takao K. WATANABE and Tsuneyuki YAMAZAKI

It has recently been shown that lethal frequency on chromosome 2 in natural populations of *Drosophila melanogaster* in Japan has increased rather drastically within last ten years: from 15 to 30% in Katsunuma population, and from 10 to 20% in Hiroshima population. Moreover it was noted in Katsunuma population that this increase of lethal was accompanied by a decrease of polymorphic inversions on the same chromosome.

Through examination of all available data on lethal and inversion frequencies on the second chromosome in natural populations of *D. melanogaster*, we have discovered that there is a clear negative correlation between the two quantities. Lethal genes are located more densely on the regions of

Table 1. Viability of inversion heterozygotes with or without induced mutations.

Genotype	No. of cross	Viability ± *s. e.*
+ /In	64	1.000±0.025**
+′/In	58	.980±0.035*
+ /In′	59	.887±0.026*,**

′ indicates EMS treated chromosome.
* significant at 5% level.
** significant at 1% level.

standard gene arrangement than the inverted regions, and this accounts for the negative correlation.

To reveal the underlying mechanism of the phenomena, we have carried out an experiment and found that effect of EMS induced mutations on the inversion-carrying chromosome is more severe than that on the standard chromosome (Table 1). We interpret these results as evidence for coadaptation or position-effect within the inversion chromosomes. New mutations within the coadapted gene complex are quickly eliminated from the population and polymorphic inversions are kept free of mutants through selective elimination.

Electrophoretic Protein Polymorphism in a View of the Stepwise Mutation Model of Neutrality Theory

Takeo MARUYAMA and Tsuneyuki YAMAZAKI

Protein variations are detected by differences in electrophoretic mobility. The mobility is believed to be determined by differences in the numbers of positively and negatively charged amino acids in the protein under examination. Some amino acid alterations, but not all, change the electric charge by unit amount in a positive or negative direction. Taking these properties of electrophoretically demonstrable mutants into account, Ohta and Kimura (Genet. Res. 22; 20, 1973) put forward the "stepwise mutation" model and advanced its theory. They have derived a formula for the probability (H_0) that two homologous genes are of the same electric charge, and therefore operationally identical and the probability (C_k) that two homologous genes taken from a population are alleles which differ by a given amount of electric charge;

$$H_0=1/\sqrt{1+8Nu}, \tag{1}$$

$$C_k=H_0\lambda_1{}^k \tag{2}$$

where N=population size, u=mutation rate and

$$\lambda_1=\frac{1+4Nu-\sqrt{1+8Nu}}{4Nu}.$$

On the other hand, the data in various speices are now available to test this stepwise mutation model. We collected data, from published literature, on about 200 loci. (The data source is given at the end of this paper.)

The homozygosity and the probabilities C_k for $k=1, 2, \ldots, 5$ were calculated for each of the loci. For example, if $P_1, P_2, \ldots, P_n$ are the frequencies of alleles arranged in a sequential order in accordance with electrophoretic mobility, then $H_0=P_1^2+P_2^2+\ldots+P_2^n$, $C_k=P_1P_{k+1}+P_2P_{k+2}+\ldots+P_{n-k}P_n$. Since the C_k in formula (2) depends on the Nu, it is not appropriate to combine data of loci with different Nu values. Thus we classified the loci into four groups of similar heterozygosity; heterozygosity range $0\sim0.2$, $0.2\sim0.4$, $0.4\sim0.6$ and $0.6\sim0.8$.

The purpose of this note is to compare these C_k for the appropriate theoretical expectation derived from the neutrality model. Since the theoretical C_k is a function of Nu, we need to know it for each class. At present the true values are not known, but an estimation can be obtained from observed H_0 value and formula (1). Namely, since

$$\text{Expectation } (H_0)=1/\sqrt{1+8Nu},$$

$$Nu=\frac{H_0^2-1}{8}. \tag{3}$$

Using the observed mean H_0 and this estimate of Nu, we calculated C_1, C_2, $\ldots$, C_5 from formula (2). The comparisons are made in Table 1. Since H_0's are not independent in the present analysis, they are not a subject of the comparison.

Data source: Prakash, S., Lewontin, R. C., and Hubby, J. L. (1969) Genetics 61, 841. Kojima, K., Gillespie, J. and Tobari, N. (1970) Biochemical Genetics 4, 627. Prakash, S. (1969) Proc. N. A. S. 62, 778. Lakovaara, S. and Saura, A. (1971) Genetics 69, 377. Lakovaara, S. and Saura, A. (1971) Hereditas 69, 77. Kojima, K., Smouse, P., Yang, S., Nair, S. and Brnic, D. (1972) Genetics 72, 721. Yang, S. Y., Wheeler, L. L. and Bock, I. R. (1972) Studies in Genetics Univ. Texas Publ. 7213. Rockwood-Sluss, E. S., Johnston, J. S. and Heed, W. B. (1973) Genetics 73, 135. Saura, A. (1974) Hereditas 76, 161. Ayala, F. J., Tracey, M. L., Barr, L. G. and Perez-Salas, S. (1974) Genetics 77, 343. Gillespie, J. H. and Kojima, K. (1968) Proc. N. A. S. 61, 582.

Table 1. Theoretical and observed values of C_k, the probability that two homologous genes are of alleles k-step apart. The numbers in the parentheses are the theoretical values. The mean 8Nu was estimated from the mean H_0 and formula (3). Comparison for H_0 was not made for this reason. A value in data represents the average of the corresponding probabilities for the number of loci given in the rightmost column.

Class Heterozygosity $1-H_0$, between	Mean 8Nu	H_0	C_1	C_2	C_3	C_4	C_5	Number of loci belonging to the class
0 ~0.2	0.085	0.960	0.018 (0.019)	0.002 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	400
0.2~0.4	1.094	0.691	0.118 (0.126)	0.030 (0.023)	0.006 (0.004)	0.001 (0.001)	0.000 (0.000)	58
0.4~0.6	3.114	0.493	0.178 (0.167)	0.060 (0.057)	0.010 (0.019)	0.003 (0.007)	0.001 (0.002)	68
0.6~0.8	6.506	0.365	0.205 (0.170)	0.084 (0.079)	0.019 (0.037))	0.008 (0.017)	0.001 (0.008)	12

X. EVOLUTIONARY GENETICS

On Some Principles Governing Molecular Evolution

Motoo KIMURA and Tomoko OHTA

The following five principles were deduced from the accumulated evidence on molecular evolution and theoretical considerations of the population dynamics of mutant substitutions: (i) for each protein, the rate of evolution in terms of amino acid substitutions is approximately constant per site per year for various lines, as long as the function and tertiary structure of the molecule remain essentially unaltered. (ii) Functionally less important molecules or parts of a molecule evolve (in terms of mutant substitutions) faster than more important ones. (iii) Those mutant substitutions that disrupt less the existing structure and function of a molecule (conservative substitutions) occur more frequently in evolution than more disruptive ones. (iv) Gene duplication must always precede the emergence of a gene having a new function. (v) Selective elimination of definitely deleterious mutants and random fixation of selectively neutral or very slightly deleterious mutants occur far more frequently in evolution than positive Darwinian selection of definitely advantageous mutants. For details see Proc. Nat. Acad. Sci. **71**: 2848–2852.

Distribution of Antigenic Specificities of Mouse H-2 among Wild Rodents and Its Evolutionary Implication

Kazuo MORIWAKI

Serological survey of erythrocyte antigens crossreacting with mouse H–2 alloantisera has been carried out by using various wild rodents collected from Japan, Southeast Asia and India. An improved PVP-hemagglutination method (Kaliss, N. Transpl. **15**: 251, 1973) which modified PVP specification from K60 to K90, was employed in this work. Alloantisera for various H–2 specificities given by Transplantation Immunology Branch, NIH (U. S. A.), have been diluted serially with 0.6% PVP solution containing 5% BSA and phosphate buffered isotonic saline. The mixture of antiserum and red blood cells kept in the microtiter plate was incubated at 37°C for one hour and 5°C for 15 hours. Degree of hemagglutination was estimated

under the microscope. The results of the present survey are summarized in Table 1.

The remarkable findings in the present study are as follows: (1) *Mus musculus molossinus* almost failed to show crossreaction with H–2 specificities (D 3, 4, 13, 19, 41, 42, 43, 44 and K 5, 23) which appeared in the laboratory mice, *Mus musculus musculus*. In the feral population of *M. m. musculus* subspecies collected from Europe, several H–2 specificities have been demonstrated as well (Mickova, M. & P. Ivanyi, In "Immunogenetics of the

Table 1. Cross reaction with various rodent erythrocytes of anti-H_2 alloantisera

	Maximum dilution (Log_2) of antisera for positive hemagglutination				
Alloantisera	C 3b	D 4	D 5AF	D 13	D 23
Antigen specificities	D3, 19	D 4	K5, 52	D4, 13, 41 42, 43, 44	K 23
Control					
B10 D2	9.0	13.0		13.0	
B10 BR			13.0	0.0	7.0
Species tested					
Family Muridae					
Subfamily Murinae					
Mus musculus molossinus	2.0	1.4 (8)	0.0 (8)	0.0 (10)	0.0 (8)
M. caroli		0.0	0.0	0.0	0.0
M. dunni	4.0	5.5	5.5	5.7 (3)	3.0
M. platythrix	0.0	2.5 (4)	2.8 (4)	5.1 (8)	2.5 (4)
Rattus rattus	0.0	0.0	0.0	4.3 (4)	0.0
R. sabanus	4.0			3.5	
R. flavipectus				5.7 (3)	
R. norvegicus (LE)	0.0	0.0	0.0	0.0	0.0
(WKS)	0.0	0.0	0.0	0.0	0.0
Millardia meltada			0.0		
Vandeleuria oleracea	0.0	0.0	0.0	0.0	0.0
Micromys minutus		0.0	0.0	0.0	0.0
Family Cricetidae					
Subfamily Cricetinae					
Peromyscus leucopus				3.0	
Cricetus griseus	0.0	0.0	0.0	0.0	0.0
Subfamily Microtinae					
Microtus montebelli	0.0				
Clethrionomys rufocanus bedfordiae	4.0	0.0	0.0	0.0 (3)	0.0

Log_2 value represents mean of two individuals, unless number is indicated in the parenthesis.

H-2 system" 1971). (2) Unexpectedly, some species belonging to genus *Rattus* and *Mus* other than *Mus musculus* have exhibited the considerable degree of crossreaction with the mouse H-2 antigens. (3) Most rodent species other than genus *Rattus* and *Mus* could not show any detectable positive hemagglutination.

One of the possible explanation for those findings might be given from the evolutionary view point of H-2 genes. The broad distribution of H-2 antigens in genus *Mus* and *Rattus* allows us to assume that those antigens appeared in a common ancester of the both genera. Though these antigens have been retained until today in such species as *Mus musculus musculus*, *M. dunni, M. platythrix, Rattus rattus, R. flavipectus* and *R. sabanus*, they seem to have been altered in *M. m. molossinus*. Considering that the Europian sbuspecies is inhabiting in rather peripheral region than the Asian one in the geographical distribution of this species, the former, *M. m. musculus*, could be somewhat older than the latter, *M. m. molossinus*. Probably, the alteration of the H-2 genes might have occurred during the differentiation process of those subspecies from *musculus* to *molossinus*.

XI. HUMAN GENETICS

Indirect Inguinal Hernia: A Multifactorial Threshold Trait

Ei MATSUNAGA, Shigenori SAWAGUCHI* and Toshiro HONNA*

Among 1,723 probands who had been operated because of indirect inguinal hernia, the ratio of males to females was 2.8: 1, being the same as that in the general population, and in both sexes the ratio of bilateral to unilateral hernia was 1: 4. Analysis of family history showed that the incidence of hernia among parents and elder siblings relative to that in the general population varied to some extent according to the sex of the probands and the laterality of hernia. The relative incidence tended to be higher, though slightly, if the proband was a female than if it was a male, and if the condition was bilateral than if it was unilateral, suggesting that predisposition to hernia is determined by polygenic inheritance with a threshold beyond which individuals are at risk. However, no indication was found for an increase in parental consanguinity. The heritability of liability to indirect inguinal hernia was estimated as $64 \pm 3\%$ from the regression coefficients of parents on probands. There were more twins among the probands than would be expected, suggesting that twins are especially prone to hernia. Details of the study will be published in the Jap. Journ. of Human Genet.

Possible Genetic Consequences of Relaxed Selection against Common Disorders with Complex Inheritance

Ei MATSUNAGA

With increasing success in medical treatments for many common disorders with complex inheritance, it is expected that the incidence of patients requiring treatments will gradually rise from generation to generation. While this is theoretically true for a long range of future, the pattern of possible increase in the frequency of a disorder resulting from complete cure has been examined with special reference to the next few generations. It was shown that the maximum possible increment will take place in the first generation, with additional increment rapidly diminishing with subsequent generations.

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For congenital heart disease, for example, the restoration of full fertility to all of the patients would incur an increase in the frequency at birth of the disease by about 10% after five to ten generations. Details of the study will be published elsewhere.

Isozyme Variations in Man

Tomotaka SHINODA, Ei MATSUNAGA and Jushiro KOSHINAGA*

In order to assess the degree of genetic variation of individuals some 30 enzyme loci were analyzed using tissue extracts by means of gel electrophoresis and specific histochemical staining. These loci were randomely selected. Enzymes tested in this study included several groups such as kinases, dehydrogenases, oxidases, reductases, transaminases and deaminases.

Of 33 different loci examined, 8 were found to be polymorphic with practically the same frequencies as in the previous study. Besides these, some variations in zymogram were seen for several enzymes, but it was not certain if these variations were genetic in origin. The sample size was relatively small and some enzymes easily produced artifacts which had the similar substrate specificity but had different mobility in gels. A part of the data was published in Japan. Journ. Human Genet., **19**: 243–250.

Studies of Q-variants among Patients with Down's Syndrome and Their Families

Kazuso IINUMA and Ei MATSUNAGA

Q-variants of chromosome no. 21 detected by fluorescent staining were classified as either 'F' or 'f', according to the intensity of fluorescence in the regions of the short arms or satellites. A total of nine cases with standard trisomy 21 and their parents were analysed under the classification and it was possible to identify one case suggesting nondisjunction at the second meiotic division in the mother. There was another case where maternal nondisjunction was most likely. Meiotic errors at maternal second division were excluded in 3 cases and paternal first division in 2 cases, respectively. The results were non-informative in two cases. It was found that one of the last cases had a *de novo* translocation t(6q+; 15q−). Q-variants of chromosome no. 15 of the patient and his parents were analysed. The derivative

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no. 15 had a medially fluorescing satellite and this characteristic satellite was also observed in one of the mother's no. 15. Thus, the formation of the translocation was considered to have occurred in the maternal germ cells (Jap. J. Hum. Genet., **20**: 147, 1975).

It was examined whether 'F' of the no. 21, defined as an intense fluorescence of the short arms or satellites, has any correlation with the predisposition to meiotic nondisjunction of chromosome no. 21. Thirty-seven males and 27 females, whose karyotypes were proved to be normal, were selected as a control group. Twenty-seven parents of standard 21-trisomic infants were also confirmed to have a normal karyotype. The third group were 5 boys and 14 girls with standard trisomy 21. The frequencies of 'F' in the control group, the parents, and 21-trisomic infants, were 0.18, 0.20 and 0.19, respectively. No significant differences in the frequencies of 'F' were observed among these groups.

A part of this study was reported in the 19th annual meeting of the Japan Society of Human Genetics (1974).

Non-random Distribution of Exchange Points in Patients with Structural Rearrangements

Yasuo NAKAGOME and Hideaki CHIYO

A total of 248 breakage points from 130 unrelated cases with structural rearrangements were studied to determine whether they were preferentially located within any particular type of band. Thirty of them (15 cases) were identified by the authors and 218 (115 cases) were collected from 51 different publications. Only cases with identification of each breakage within a single band were included in the study. Positions of breakages were classified into light, variable and dark bands, based on their G-band patterns. Alternatively, sites of breakages were classified according to their positions within a chromosome arm, i. e., terminal, centromeric and intermediate parts. Expected numbers of breakages were based on an assumption that each unit length of band had an equal chance of being broken.

Of 248 breakages, 178 were from reciprocal translocations, 32 from inversions and 38 from various other types of rearrangements. In reciprocal translocations, breakages were more frequently observed in the light and variable bands than expected numbers, while those in dark bands were rare ($p \ll 0.001$). In inversions, an excess of light bands and a lack of dark bands were ob-

served ($p<0.005$). There was no excess of variable bands. In other types of rearrangements, the observed numbers of breakages in each type of bands were very close to the expected.

In both reciprocal translocations and rings, the middle part of a chromosome arm seemed to have less chance of being the site of an exchange in contrast to terminal and centromeric parts (for details: Nakagome & Chiyo, Amer. J. Hum. Genet. in press).

A 7p Monosomy Resulting from a Stable Dicentric Chromosome —with Reference to the Mechanism of Inactivation of a Centromere—

Yasuo NAKAGOME, Fumio TERAMURA, Kenkichi KATAOKA and Fumitoshi HOSONO

Partial monosomies of any of C group chromosomes have rarely been described. They included rings of nos. 6, 7 and 9 as well as partial monosomies of 9p, 10p, 11q and 12p due either to a translocation or a deletion.

Propositus was a 3-month-old girl. Main clinical features were: failure to thrive, markedly retarded development, prominent forehead, hypertelorism a downward slant of eyes, low set and malformed ears, high arched palate, micrognathia, funnel chest and pes varus.

Cytogenetic examinations were carried out by the standard leukocyte-culture technique. Her two brothers and parents showed a normal karyotype. The propositus had only 45 chromosomes. One chromosome was missing from each of the C and the D group and there was an extra "no. 2" chromosome. Metaphases were examined further by various banding techniques. The detailed studies of the G-band pattern revealed that the extra "no. 2" consisted of a no. 7 and a no. 15. The break points were in a 7p21 and a 15p11 bands respectively, *i. e.*, the derivative chromosome had a tdic (7; 15) (p21; p11) constitution. The patient was monosomic for both the 7p22 and a part of the 7p21 bands.

Although the derivative chromosome had two centromeres, none of them showed a typical appearance of a dicentric chromosome. By the C-band technique, intensely stained spots were observed at two different sites on the derivative chromosome, both of them being corresponded to the presumed sites of centromeres. The Cd-band method stained only one of them, *i. e.*, the centromere derived from the no. 7. It was assumed that the centromere

derived from the no. 15 lost its component parts and was inactivated. (for details: Nakagome *et al.*, Clin. Genet. in press).

Structural Studies on Human Immunoglobulins

Tomotaka SHINODA

Sequence analyses were carried out on a variety of human immunoglobulins of different types and classes, which were isolated from plasmas or urines of patients with multiple myeloma or heavy chain disease. These results ununbiguously indicated that sequences of the first 110 residues were randomly varied from one to another regardless of the types of the L chains or the classes of the H chains. However variable in this region, these were classified into several subgroups based on certain degree of sequence similarlity among them. Subgroups for the L chains were type-dependent whereas those for the H chains were independent of its classes. In connection with subgroup specificity, we found a L chain whose variable region sequence shared a part of two different subgroups. Thus, it was reasonably assumed that such a sequence had arisen as a consequence of crossing-over between two parent subgroups genes.

Assuming that each subgroup was encoded by a few different germ line genes, then at least 12 major genes should be considered for the variable regions of the L and H chains. Similarly, at least 14 different genes can be assumed for the constant regions of immunoglobulins. Still it seems difficult to interpret the mechanism of structural variabilities of immunoglobulins, unless some new concepts,for example, two gene–one immunoglobulin are introduced. Our data suggested that much variabilities in sequences had resulted from somatic events rather than from those in germ line genes. Parts of data were published in J. Biochem., **75**: 23–44, **77**: 1277–1296.

XII. BEHAVIORAL GENETICS

Circadian Locomotor Rhythm of an Adult Fly of *Drosophila melanogaster*

Chozo OSHIMA

Many kinds of actograph have been designed in order to record the circadian locomotor rhythm of insects. In this experiment, an electric actograph was originally designed; especially a Light Emitting Diode (LED) and a Sharp Photo Diode (SPD) were used as a senser part. A small glass box ($5 \times 10 \times 50$ mm) was put between these Diodes for a beam of red light to penetrate it. When a fly moved and crossed the beam, the voltage on SPD was changed. The small change was enhanced by an amplifier and recorded as a single vertical line on film in Micro Pen-oscillograph. The film band is moving continuously with a definite speed. The senser part was put in an incubator, controlling temperature and light conditions. The environment of this experiment was set up as follows; temperature was constant at 24°C and circadian light (1500 lux) and dark condition LD 13: 11 (L consisted of dawn-1 hr, dusk-2.5 hr and light-9.5 hr periods) was repeated every day by the program photocontroller. A small glass box has a small amount of poor medium, containing only red sugar and agar, and the unstop was covered with gauze. A fly could survive for 2–3 days and if the culture medium was exchanged with new one every several days, the circadian locomotor rhythm of a fly during a considerable time could be recorded.

Among individual circadian locomotor rhythms of many wild flies in a natural population, a fairly amount of variations would be found on period between acrophases and locomotor activity (amplitude). However, the circadian locomotor rhythm of a wild male fly, extracted from Ishigaki-jima population, was recognized to be bimodal from results, obtained by the actograph mentioned above, and the high locomotor activity was displayed most frequently at dusk period and another locomotor activity was displayed before or at dawn period. Such a circadian locomotor rhythm would be regulated by an endogenous function of the bioclock in brain and it is possible to assume that its function would be daily readjusted at dusk period.

This work was supported by a grant (No. 930403) from the Ministry of Education.

Effects of Light, Temperature and Noise Environments on Longevity and Productivity of *Drosophila melanogaster*

Chozo OSHIMA and Takao K. WATANABE

Many flies of one day age of Oregon R strain were collected, and many sets, consist of one female and two males, were respectively introduced into small vials, which have standard corn meal, sugar and yeast medium. These 200 vials were divided into four groups and each group was put separately under different light and temperature environments; 1) constant light (LL) 2000 lux and constant 25°C (C), 2) constant light (LL) and circadian fluctuated between 20 and 30°C (F), 3) circadian light and dark (LD 12: 12) and constant 25°C (C), 4) circadian light and dark (LD 12: 12) and fluctuated between 20 and 30°C (F). Flies of each vial were transferred into a new vial two times a week and dead females were scored. When a male fly was found to be dead, it was supplied with a new one of the same age. Such procedures were carried for 30 days. On the other hand, all vials were put under constant light (LL) and temperature (25°C) and all emerged flies were counted to estimate productivity under different environments. The experiments were replicated for several times for estimating the longevity under different environments and the results were summed up in Table 1.

Table 1. Longevity (LD 50) of female flies under four kinds of different environments.

Environment Light	Temperature	Number of replications	LD 50 (day)	Ratio
LL	C 25°C	4	16.7±1.1	1.00
LL	F 20~30°C	2	17.2±0.2	1.03
LD 12: 12	C 25°C	3	21.2±2.3	1.27
LD 12: 12	F 20~30°C	3	25.3±1.6	1.52**

The next experiment was carried in order to estimate the effect of noise (2000 helz, 100 phone) on longevity of flies. The material and method were the same to the previous experiment, but two kinds of noise environment; the circadian noise and quiet condition (NQ 12: 12) and the constant quiet condition (QQ) were added to the constant light condition (LL). The experiment was replicated two times and the results were presented in Table 2 The productivity was similarly estimated to the previous experiment.

This work was supported by a grant from the Ministry of Environmental Agency.

Table 2. Longevity (LD 50) of female flies under two kinds of different environments.

Environment		Number of replications	LD 50 (day)	Ratio
Light	Noise			
LL	QQ	2	23.10±4.1	1.00
LL	NQ 12:12	2	22.40±1.9	0.97

Two characters, longevity and productivity (number of eggs), are assumed to be highly correlated, and the results showed the assumption to be correct. However, the experimental results, obtained at different time, could not be compared with each other. In the previous experiment, the circadian light and dark and fluctuated temperature environments were most favourable for the longevity of fly, and the mean number of offspring per female for life time was about 1.3 times more than that under the constant light and temperature condition. On the other hand, the circadian noise and quiet environment was not so unfavourable for the longevity of fly, and the mean number of offspring per female for life time was unexpectedly more than that under the constant quiet condition.

Effects of Light and Noise Environments on Adult Emergence Rhythm of *Drosophila melanogaster*

Chozo OSHIMA and Takao K. WATANABE

One thousand and five hundred eggs, laid for 12 hours (first period) and another 1500 eggs laid for following 12 hours (next period) by many fiies of Oregon R strain, were divided into three groups respectively and each group (50 by 50 eggs put on culture medium of 10 vials) was allowed to develop in incubators, having three kinds of light (about 2000 lux) environments; constant light condition (LL), circadian light and dark condition (LD 12: 12) and mixed circadian light and constant dark conditions (LD 12; 12 for early 7 days and DD). The temperature was kept constant at 25°C in all cases. After 7.5 days, adult flies of the first group began to emerge and they were scored two times a day.

Frequency curves of adult emergence, connecting several scoreing points of two groups, were parallel under the constant light condition and the distance between peaks of these emergence rhythms was half a day. However, these

This work was supported by a grant from the Ministry of Environmental Agency.

frequency curves was transformed under circadian and mixed light conditions. About 50 percent flies of the first group emerged for light period of 8.5 day and about 50 percent flies of the next group emerged for light period of 9.5 day. The emergence rhythms of two groups under the constant light condition were very similar, but the phases of such rhythms were shifted faster or slower under circadian and mixed light conditions and the distance between peaks was spread to be a day. Such a phase shift was also observed under the mixed light condition and then, it is assumed that the developmental rhythm during larval and early pupal periods was individually readjusted every day to the circadian light and dark condition. Consequently, the synchronization of adult emergence of many flies was enhanced. The adult eye was not jet developed during the period, then, the readjustment of rhythm would be controlled by a bioclock located in a main lobe of the brain.

Materials and procedure of the noise experiment were similar to those of light experiment mentioned above. One thousand and five hundred eggs were allowed to develop in incubators, having constant light (LL) and temperature (25°C) and three kinds of noise environments; circadian noise and quiet condition (NQ 12: 12), mixed circadian noise and constant quiet (NQ 12: 12 for early 7 days and QQ) and its reversed conditions (QQ for early 7 days and NQ 12: 12). The noise was pure sound having 2000 helz and 100 phone. The unstop of vial was covered with gauze for penetrating the sound. About 70 percent flies of the first group emerged for quiet period of 8.5 day, but about 60 percent flies of the next group emerged for noise period of 9.5 day. The phase of emergence rhythm was not so remarkably shifted under the circadian noise and quiet condition as shown in the light experiment. However, the readjustment of a bioclock in this case was presumed to be clearly weakened by the constant light condition. The grade of synchronization of adult emergence in two conditions (NQ 12: 12, NQ 12: 12 for early 7 days and QQ) was much higher than that of another condition (QQ for early 7 days and NQ 12: 12). As a matter of fact, both light and noise environments accelerate the development and they would have a bad effect for the life of fly, especially when they disturve the circadian rhythm of bioclock.

Strain Difference in Larval Phototactic Behavior of the Silkworm

Akio MURAKAMI and Kosuke KOTOKU[1]

A comparison of phototactic behaviors between strains (or stocks) showing a different phototaxis may help to better understand the genetical control mechanism of such a biological phenomenon. From this view point, the senior author has been carrying out extractions of the different phototactic strains among 200 silkworm stocks being maintained in his institute. Consequently, four silkworm stocks manifesting differential phototactic responses at the mounting stage under a light condition in an ordinary silkworm rearing room were extracted: two stocks, *pe*;*ok* and *nb*, are gathered in crowds to the light part (photopositive) and other two stocks, *Ze*;*pe*;*re* and *st*, are gathered to the dark part (photonegative). The other stocks showed no clear cut phototactic behaviors.

Using those photosensitive stocks, further experiments have been carried out to throw light upon the phototactic behavior in relation to the growth of larvae throughout the whole larval stage from newly hatched larvae to mature larvae. The measurement of the larval behavior was made by a mean walking distance (in centimeter) from the center (or neutral part) in the silkworm phototatic measurement box to either light or dark direction. The box was composed of two parts: one part is illuminated with an ordinary fluorescence light (20 W) and another is covered with dark paper to shade the light.

The result of experiments indicated that larval stages other than the stage of mature larva are insensitive to phototactic response. At the stage of mature larvae, however, the previous observation was confirmed that *pe*;*ok* and *nb* strains showed the positive phototaxis and the *st* and *Ze*;*pe*;*re* strains showed the negative one. Among them the *pe*;*ok* and *st* strains showed so clear cut phototaxis that factor (s) manifesting their phototactic response seemed to be controlled by a few major genes. Not yet completely analyzed, however, the result of analysis of phototactic behaviors in hybrids between photopositive and negative strains indicated that phototactic response of the F_1 hybrid was somewhat photonegative rather than neutral, regardless of parent phototaxis and the F_2 (or F_1) extended to both directions. This suggests that photonegative nature is more dominant than photopositive

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one and that factor (s) manifesting phototactic response is controlled by polygenes.

Strain Differences in Discriminated Avoidance Learning Curve in Mice

Tohru FUJISHIMA

Fifteen inbred strains of mice were tested for discriminated avoidance responses (in percentage) with the aid of an automated Y-maze apparatus, and the performance of each strain was shown by a learning curve. The operating procedure was to use a lamp and a buzzer for 5 seconds as the conditioned stimulus, which was immediately followed by an electric shock for 5 seconds as the unconditioned stimulus, with intertrial intervals of 30 seconds. Two daily sessions, each consisting of 50 trials, were carried out.

The results indicated that C3H/He acquired the highest learning effect (52.3%), while D103 did not show any acquisition of learning effects, and C57L, C57BR and DBA/2 were superior in reminiscence. It was also found that, although one might expect discrimination to be more difficult to learn than avoidance, some strains showed the reversed trend; SWM and RF prefered avoidance to discrimination, while D103 prefered discrimination to avoidance. On the other hand, C3H/He and C57BL/6 learned approximately equally both of the learning tasks.

Diallel Cross Experiment on the Discriminated Avoidance Learning Abilities of Mice

Tohru FUJISHIMA

A diallel cross experiment was conducted with four inbred strains selected for the memory patterns of discriminated avoidance learning abilities measured with an automated Y-maze apparatus. The strains used were C3H (a high and high line), SWM (a high and low line), C57L (a low and high line) and D103 (a low and low line) for the short-term and long-term memory effects obtained from discriminated avoidance learning tests, respectively.

The data generally indicated that the memory pattern found in the progeny was consistent with that of the parents. This suggests that the memory pattern as measured by the present method is genetically controlled. The general and specific combining abilities had significant variances, but the

maternal effects were insignificant. For the general combining abilities, SWM was the highest (6.42%) and D103 the lowest (−6.13%) in short-term memory effect; C57L was the highest (4.58%) and D103 the lowest (−5.80%) in long-term memory effect.

Complete Blindness of an Inbred Strain of Mice, D103/Ms

Tohru FUJISHIMA

A mouse inbred strain, D103/Ms, derived from strain DM, has a visual sensory defect though its eyes appear to be normal. The sensory defect was investigated by inserting a bi-polar electrode into the non-specific sensory system of the cerebral cortex and observing the evoked electroencephalogram responses to light. It was compared with C3Hs (adult), which had been reported to have visual sensory defects, and also with DM, the parental strain of D103.

The result showed that D103 had a complete blindness, while C3H/He was not so completely blind as D103, and DM had no visual defects. It was also found from a preliminary crossing experiment that the blindness gene of D103 was recessive.

XIII. APPLIED GENETICS

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

Hiko-Ichi OKA and Hiroko MORISHIMA

As reported last year (Ann. Rep. 24, p. 66), isogenic lines having various gene markers in the genetic background of Taichung 65 (T65) were isolated, each from recurrent backcrossing with T65 repeated 7 times or more. The genes incorporated are: *wx* (or *gl*, glutinous), d_2 (Ebisu dwarf), *lg* (liguleless), *Ph* (phenol reaction), *Rd* (red pericarp), *g* (long empty glume), d_1 (Daikoku dwarf), *la* (lazy habit), *ne* (neck leaf or bract development), *bc* (brittle culm), *gb* (glabrous leaf), etc. The purpose of this work is to use the isogenic lines for genic analysis and to investigate the pleiotropic effects of respective genes.

For the latter purpose, the effects of those genes on heading date and on the size of various organs of seedlings and adult plants were observed. The *ne* and d_2 genes delayed heading about seven and four days, respectively. Genes *g*, *gb* and d_1 also delayed heading slightly. The dwarfness genes (d_1 and d_2) reduced the size of organs giving rise to particular allometric patterns. In addition, it was found that *Ph* reduced the number of secondary rachilla per rachis, *g* reduced primary rachilla per panicle, *bc* increased secondary rachilla per rachis but reduced primary rachilla per panicle, etc. However, these genic effects were generally small in magnitude and could be overlooked without a careful statistical test. Therefore, these marker genes excepting those causing dwarfism seem to be useful in genic analysis for various quantitative characters.

Reciprocal translocation homozygotes of T65 under observation are 30 lines, each having been purified by three times backcrossing to eliminate radiation-induced mutant genes. They were each crossed with isogenic lines carrying *lg* and *g*, and *ne* and *bc*, respectively, to identify the chromosomes involved in translocations. The seeds of these isogenic lines will be mailed to research workers interested in rice genetics upon request.

Genic Analysis for the Sterility of Hybrids between *Oryza sativa* and *O. glaberrima*

Hiko-Ichi OKA and Hiroko MORISHIMA

The F_1 hybrid between the two rice species is highly pollen-sterile, though their chromosomes normally pair in meiosis. In order to look into the genetic basis of the sterility, isogenic F_1-sterile lines having the genetic background of *sativa* and *glaberrima* parents, respectively, were isolated from B_8F_2 plants. Their crossing experiments are under way. The results so far obtained indicate that the F_1-sterility genes from both parents cause deterioration of the gametes through an interaction of their effects on the sporophyte and gametophyte; gametes of both sexes carrying *a* deteriorate in the plant with *A*. Evidence supporting this hypothesis has been accumulated, while the presence of other types of genes was also suggested.

A Competition Experiment between Rice Cultivars of Different Ages

Hiko-Ichi OKA and Hiroko MORISHIMA

It has been advocated by Sakai (1965, *in* The Genetics of Colonizing Species, etc.) and coworkers that competition does not take place between plants with the same genotype. However, this assertion should be interpreted as: plants with the same genotype and at the same age do not express the effect of competition since they have the same competitive ability, even though they compete each other. Two pure lines of rice (*Oryza sativa*), Taichung 65 and Peiku, were each seeded on May 8 (early) and May 28 (late), and the seedlings raised separately were mix-planted at a spacing of 20×20 cm in different combinations. The results showed that when early and late-seeded plants of the same line were mix-planted, the early plants had an advantage in competition over the late ones. Peiku has a stronger competitive ability than Taichung 65. This relation was reversed when early Taichung 65 and late Peiku were grown in mixture. The experimental results suggest that competition depends upon the growth rate at a certain stage when the leaves of neighboring plants come into contact.

Mortality and Adaptive Mechanisms of Oryza perennis Strains

Hiko-Ichi OKA and Hiroko MORISHIMA

Six strains of *Oryza perennis* Moench and two strains of cultivated rice

(*O. sativa* L.) were tested in various "semi-natural" (partly weeded non-cultivated field) conditions and in a paddy field (control) for survivorship, phenotypic plasticity, and seed maintenance in the soil, at the Central Luzon State University, Philippines. The *perennis* strains, ranging from perennial to annual types, showed wide variations in the characters observed. The perennial type showed lower mortality in the seedling stage but a higher mortality in the adult stage than annual types. The perennial type also showed greater phenotypic plasticity in seedling height and a lower degree of it in panicle length than annual types. Examinations of soil samples about one month after maturity proved that annual strains maintained more seeds in the soil than perennial ones. This difference was also recognized from the number of plants occurring in the next season. Further, experiments made at Misima showed that the seeds of *O. perennis* remained alive for more than three years in water or in moist soils at 25°C. When newly harvested seeds were dibbled or scattered on the soil surface in a greenhouse, a part of them germinated in ten months as their dormancy was overcome. It was pointed out that various attributes were inter-correlated resulting in differentiation of the plants in their adaptive strategies.

Competitive Abilities of Barnyard Grass and Dallis-Grass against Finger Grass

Hiroko MORISHIMA

In an attempt to look into the adaptability of weedy plants, five strains of barnyard grass (*Echinochloa crusgalli*) and three of dallis-grass (*Paspalum dilatatum*) were tested for their competitive ability against the finger grass (*Digitaria sanguinalis*). The field plot to be used was tilled and the soil was pulverized on April 20 (1974), and young seedlings of barnyard grass and dallis-grass were transplanted on May 17 (early planting) and June 10 (late planting). Four treatments were made each with two replications, *i. e.*, weeding by hand, no weeding, early seeding (broadcasted, April 24) of finger grass, and late seeding (May 22) of finger grass. At maturity in September, the barnyard grass and dallis-grass strains were measured for plant height and panicle number per plant. The fresh weight of other weeds per square meter (mainly finger grass) was also recorded.

The barnyard grass strains significantly differed in the competitive ability against finger grass and other weeds. Those from copper-polluted sites

tended to have lower competitive ability than those from unpolluted sites. The dallis-grass strains showed no significant differences among them, but generally expressed a higher degree of phenotypic plasticity than barnyard grasses.

Copper Tolerance of Barnyard Grass Strains

Hiroko MORISHIMA and Hiko-Ichi OKA

Seeds were collected from barnyard grass (*Echinochloa crusgali*) populations growing on a slag deposit at Hanaoka copper mine (Akita-ken), paddy fields at Morita (Ohta, Gunma-ken, where the people have suffered from copper pollution of the Watarase River from Ashio mine since 1880's), and in other unpolluted places. Young seedlings from the seeds were cultured in testtubes containing Hyponex solution and $CuSO_4$ at the rates of 0.5 and 2 ppm copper. Root elongation was found to be a good indicator of the reaction to copper toxicity. The lines tested were 34 from polluted and 26 from unpolluted sites. Lines from the same population showed a wide range of reactions suggesting that barnyard grass populations would be highly heterogeneous. Nevertheless, it was recognized that populations at polluted sites had a higher mean tolerance and a higher frequency of tolerant plants than those at unpolluted sites.

Perhaps, metabolic systems increasing copper tolerance have been selected in those plants growing in the polluted area. This work was undertaken as a part of a research project financially supported by the Bureau of Environment, "Genetic influences of environmental pollution on plants and animals", and is still under way.

Variations in Heavy Metal Tolerance of Weedy Plants in the Paddy Field

Hiroko MORISHIMA and Hiko-Ichi OKA

In order to observe variations in the tolerance of paddy weeds to copper-polluted soils, soil samples were taken from paddy fields at 8 polluted (containing more than 200 ppm copper) and 4 non-polluted sites nearby Takara copper mine (Tsuru, Yamanashi-ken). The soil samples taken in late May (before transplanting rice) were put in trays and kept moist in a greenhouse to obtain plants from the buried seed population. The seedlings of paddy

sedge (*Cyperus difformis*) at about one month after germination were transplanted into pots containing field soil (control) and toxic soil (a half-to-half mixture of field soil and the soil from a precipitation pond of the mine, containing 1,160 ppm copper and 6,080 ppm zinc). The growth, root development and seed production of the sedges were then observed. This experiment was repeated twice in 1973 and 1974. The results in both years consistently showed that the plants from polluted sites apparently had higher tolerance to the toxic soil than those from unpolluted sites. Some plants from polluted sites tended to grow better on the toxic soil. This suggests that tolerant genotypes have been selected in response to the pollution of soils by the mine.

In parallel to this, the plant species obtained from the buried seed populations (15 sites in total) were recorded and association analysis was made. The association of major paddy-weed species was found to be almost at random. Yet their relative frequencies significantly differed between polluted and unpolluted sites. Polluted soils carried a larger number of *Fimbristylis miliacea, Vandellia angustifolia* and *Rotala indica* plants but a smaller number of *Mazus japonicus* plants than unpolluted soils. *Cyperus difformis* seemed to be more frequent in unpolluted than in polluted soils, though it is highly tolerant to heavy metals as mentioned above.

The Mechanisms of Weediness Found in Dallisgrass Populations

Hiroko MORISHIMA

The dallisgrass, *Paspalum dilatatum* Poir., is an apomictic perennial grass. It has been a weed in some areas of Japan long before its introduction as a forage crop. Recently, its remarkable spread was noticed in southern Kyushu. They grow in the habitats disturbed by man, such as roadside, dikes of farm field, waste places in town, etc. In order to look into the adaptive mechanisms of this species, seeds were collected from natural populations in four localities (two in Miyazaki-ken, one in Kumamoto-ken, and one in Sizuoka-ken), and the plants from the seeds were observed at Misima. The following points were concluded from the results.

(1) Genetic variability as shown by character variations: 180 lines belonging to 20 populations were tested in a plant-to-row experiment to take measurements of several morphological traits. The amount of genetic variations observed was obviously smaller than those in other sexually reproducing

grassy plants. Yet, the differences between localities, between populations within locality, and between lines within population (between individuals of a natural population) were in most cases significant. Parent-offspring regressions also indicated the existence of intra-populational genetic variability. It was pointed out, further, that intra-populational genetic variance was negatively correlated to phenotypic plasticity among populations.

(2) Breeding systems and colonizing ability: It was assumed that sexual reproducing potential can be roughly estimated from pollen development. The sexual ability estimated in each population was negatively correlated with seed propagating ability, and was positively correlated with intra-populational variability. This suggests that the genetic variations observed in populations were at least partly due to segregation after sexual reproduction. It was commonly found in three localities that periphery populations contained a larger amount of genetic variations than those remaining at the center of distribution. Releasing genetic variability might have been necessary for the plants to colonize themselves in new habitats.

(3) Responses to environmental stresses: With a part of the collected materials, responses to various environmental stresses were tested. The plants under a stress condition (walked over in summer, non-weeded) better survived winter than those under a protected condition. A mix-planting experiment with "Suzumenohie" (*Paspalum thunbergii* Kunth; a closely related species native to Japan) showed that dallisgrass was a strong competitor at a relatively low density of planting. In competition with the finger grass (*Digitaria sanguinalis* L.), dallisgrass showed a high mortality and phenotypic plasticity as compared with barnyard grass (*Echinochloa crusgalli* Beauv.).

It was suggested from these observations that the apomictic dallisgrass has a different adaptive strategy from that of annual weeds. Dallisgrass seems to have a "general-purpose genotype" consolidated by virtue of heterozygosity. Its weediness would depend upon colonization by its apomictic seeds, tolerance to various environmental stresses, and pronounced phenotypic plasticity. The adaptive genotype is multiplied by apomixis without change.

Developmental Instability and Phenotypic Plasticity in *Arabidopsis thaliana*

Sujit BAGCHI and Shin-ya IYAMA

In order to inquire into the relationship between developmental instability and phenotypic plasticity, high and low instability lines of *Arabidopsis thaliana*, derived from the progeny of gamma-ray irradiated seed were grown under different environmental conditions. Twelve lines each of high and low instability groups for days to first flower were tested under five different conditions (combinations of nutritional levels and temperatures). Also, 10 high and 11 low instability lines for plant height were tested under seven conditions (Table 1). Developmental instability was measured by within-line standard deviation. The sensitivity to environment or phenotypic plasticity of a line was expressed by the coefficient of regression of its performance on environmental values which were given by the average of all lines grown under the environments.

The response of each line to environments varied among the lines, but showed no significant difference between high and low instability groups ($b=1.02$ and 0.98 for plant height and $b=0.997$ and 1.003 for days to flower). This indicates that developmental instability is a different property from phenotypic plasticity. The expression of developmental instability differed according to environments. As to plant height, developmental instability was least under a varying temperature condition, and showed higher values under constant temperature conditions (Table 1). The value obtained from

Table 1. Average within-line standard deviations of high and low instability line groups under different environments.

Environments			Days to first flower			Plant height		
	Nitrogen	Temp.	High	Low	Mean	High	Low	Mean
E_1	0ppm	25°C	2.94	2.30	2.62	1.91	1.60	1.75
E_2	40	25	2.14	1.81	1.98	2.33	1.90	2.11
E_3	30	25	2.16	1.98	2.07	2.44	1.74	2.09
E_4	30	20	3.02	2.43	2.73	6.21	4.67	5.44
E_5	30	30	2.07	1.61	1.83	4.22	3.49	3.86
E_6	30	20/30	—	—	—	1.56	1.43	1.49
E_7	30	(10/30)*	—	—	—	3.45	3.53	3.49
Average			2.47	2.02	2.25	3.16	2.62	2.89

* Approximate range in uncontrolled glasshouse.

a 10–30°C treatment (in a glasshouse), however, was high perhaps because of too wide temperature range. It seems that developmental instability is a genetic character and expresses itself under constant temperature and unfavorable condition.

Varietal Difference in Cadmium Uptake Ability in Fodder Turnip, *Brassica rapa*

Shin-ya IYAMA

To investigate genotypic differences in cadmium uptake ability, ten varieties of fodder turnip were tested. Five plants per variety were grown individually in a pot containing 20 ppm Cd in 4.5 kg field soil together with untreated control. At maturity, they were harvested and their leaves and roots were dried separately. The samples were ashed by a low temperature dry asher and then amount of extracted cadmium was measured by atomic absorption spectrophotometry. The plants from Cd-containing soils apparently had a higher cadmium content in both the leaves and roots than the control plants. Average cadmium contents in the control were 0.62 ppm in leaf and 0.21 ppm in root, whereas the treated plants had a mean of 28.51 ppm with a range from 20.13 to 40.73 ppm in the leaves and a mean of 9.17 ppm with a range from 6.41 to 14.62 ppm in the roots. Analysis of variance of the data showed that there were significant differences among varieties in cadmium contents in both leaf and root, suggesting that the cadmium uptake ability is a genetic trait. No correlation was found between cadmium content and total dry weight.

The Effect of Intensive Inbreeding on the Weights of Various Organs in Japanese Quails

Takatada KAWAHARA

When the Japanese quails are propagated by successive full-sib mating, complete loss of reproductive ability is observed in between the third and fifth generation (F=50–67%). Body weight also decreases with inbreeding. This report deals with the difference in the weight of various organs found between inbred and non-inbred strains. Data were collected from domestic Japanese quails at three levels of inbreeding, namely, non-inbred control (F=0%), and second (F=38%) and third (F=50%) generations of full-sib

mating. Female and male birds at the age of 25 weeks were observed. Their numbers were 35, 55 and 37 for the three groups, respectively. The weights of 16 different organs were measured seperately after dissection. The effect of inbreeding was shown by linear regression of the weight of an organ on inbreeding coefficient (F).

The mean reduction of organ weight due to 10% increase of F was obtained as follows: −4.80 g in total body weight (mean weight for female and male: 123.65 g), −0.10 g in bone (4.00 g), −2.64 g in muscle (73.78 g), −0.02 g in heart (1.07 g), −0.04 g in lung (1.16 g), −0.13 g in liver (3.93 g), −0.07 g in intestine (2.51 g), −2.56 mg in spleen (42.96 mg), −0.03 g in kidney (1.17 g) and −0.01 g in brain (0.67 g). The weight of sexual organs also decreased with inbreeding at the rate of −0.13 g in testis (mean wt.: 2.47 g), −0.95 g in ovary (4.83 g), and −0.21 g in oviduct (5.65 g) per 10% increase of F. All these regressions were significant at 5% or higher level. The regression obtained in gizzard (−0.01 g), eye balls (−0.001 g) and pancreas (+0.001 g) were insignificant.

The result of this experiment indicates that the effect of inbreeding differs according to organs; it was strongest on spleen and testis.

Properties Concerning Egg Production of Japanese Quails

Takatada KAWAHARA and Shin-ya IYAMA

Variation pattern in the traits concerning egg production of Japanese quails was investigated. Phenotypic and genetic correlations among various traits were estimated in a random bred population kept in this Institute. The characters measured at the age of 23 weeks were: egg weight (X_1), albumen weight (X_2), yolk weight (X_3), shell weight (X_4), shell thickness (X_5), egg length (X_6), egg width (X_7) and body weight (X_8). Those at sexual maurity were: body weight (X_9), egg weight (X_{10}) and age in days (X_{11}). Hen-day egg production rate (X_{12}) was recorded up to 120 days after the first egg. The birds were killed at the age of 25 weeks in order to measure the weights of oviduct (X_{13}) and ovaries (X_{14}). Data were collected from 305 female birds originated from 68 single pair matings between male and female randomly chosen from the population.

Principal component analysis was applied to the correlations obtained. On the whole, extracted components based on the phenotypic and genetic correlations were in good accordance with each other. First four com-

ponents explained 70 to 80% of total variations found in the population as shown in Table 1. The first component was apparently a size factor. The second one would reflect egg production, poor egg production (X_{12}) being associated with poor development of ovaries (X_{14}) and late sexual maturity (X_9, X_{10}, X_{11}), and *vice versa*. In the third component, large number of eggs produced was associated with large body (X_8, X_9) and small eggs (X_1, X_2, X_3, X_6, X_7), suggesting that the component would be related to another pattern of egg productivity. The fourth one was assumed to reflect mainly egg shell property.

Table 1. First four principal components extracted from the phenotypic and genetic correlations among various traits of Japanese quails.

Character	Principal components based on							
	Phenotypic correlations				Genetic correlations			
	I	II	III	IV	I	II	III	IV
X_1	.411	−.039	−.168	.032	.409	.040	−.148	.016
X_2	.380	−.024	−.131	.013	.360	.073	−.136	.111
X_3	.334	−.030	−.258	−.059	.289	−.023	−.065	−.340
X_4	.300	−.094	.096	.437	.290	−.124	−.122	.273
X_5	.044	−.100	.314	.735	.063	−.026	.076	.632
X_6	.325	−.034	−.055	.063	.312	−.036	−.011	.145
X_7	.359	−.029	−.249	.042	.327	.169	−.279	.065
X_8	.269	−.054	.377	−.238	.226	.011	.551	−.114
X_9	.196	.351	.459	−.264	.211	.141	.576	−.109
X_{10}	.198	.513	.073	.063	.239	.531	.051	.069
X_{11}	−.005	.638	.091	.108	−.066	.585	.110	−.183
X_{12}	−.008	−.320	.550	−.010	−.008	−.172	.431	.425
X_{13}	.242	−.042	.167	−.127	.362	−.185	−.058	−.116
X_{14}	.200	−.277	.140	−.316	.199	−.489	.138	−.341
PVAC*	37.74	11.21	9.88	9.22	42.70	14.23	12.78	11.89

* Percent of variance accounted for by the component.

Bilateral Asymmetry in the Transverse Processes of Cervical Vertebrae in Chicken

Takatada KAWAHARA

In order to elucidate the occurrence of developmental instability in chicken, observations of transverse processes have been continued since 1963 (Ann. Rept. 15 & 19). Based on the data so far obtained, a concluding review on

the problem is presented in this report. The asymmetry was classified into two categories, directional and fluctuating. Directional asymmetry was measured in each strain by the mean left-right difference in transverse process length. Fluctuating asymmetry was measured in each bird as the residual value given by (left-right difference)−(mean left-right difference). A total of 507 female birds of White Leghorn at the age of 16 months were observed, which belonged to a closed flock strain (F=6%), four inbred lines (F=57−64%), and five strains of F_1 hybrid between the inbreds.

In directional asymmetry, significant differences were found between the four vertebrae, but were not found between strains. The left transverse processes were usually longer than the right, the mean difference being 0.126 mm. In fluctuating asymmetry, the 10 strains significantly differed. The F_1 hybrids were less asymmetric than their inbred parents. This will be expected if the fluctuating asymmetry results from developmental instability and is mitigated by heterozygosity.

The values showing directional and fluctuating asymmetry were positively inter-correlated among strains (r=0.696), though their correlations with the length of transverse processes itself were insignificant. The asymmetry values showed no significant correlations with body weight, with which the length of transverse processes was correlated (r=0.789). (Published in Jap. J. Genet. **49**: 1–9, 1974).

XIV. MISCELLANEOUS

A Modified Analysis of Two-Treatment Switchback Design with Different Numbers of Individuals in Replications

Tohru FUJISHIMA

An attempt was made to propose an analytical procedure for the two-treatment switch-back design with replications consisting of unequal numbers of individuals.

Let Y_{ijkmn} be the performance of the k^{th} individual of the m^{th} treatment in the n^{th} experimental period of the j^{th} treatment sequence group in the i^{th} replication. Then the statistical model for Y_{ijkmn} may be expressed as follows:

$$Y_{ijkmn}=\mu+B_i+S_j+(BS)_{ij}+c_{ijk}+T_m+P_n+(BT)_{im}+(BP)_{in}+(ST)_{jm}+(SP)_{jn}+(TP)_{mn}+e_{ijkmn},$$

where μ is general mean, B_i is i^{th} replication effect, S_j is j^{th} treatment sequence group effect, c_{ijk} is ijk^{th} individual effect, T_m is m^{th} treatment effect, P_n is n^{th} experimental period effect, and e_{ijkmn} is random error, and $(BS)_{ij}$, $(BT)_{im}$, $(BP)_{in}$, $(ST)_{jm}$, $(SP)_{jn}$ and $(TP)_{mn}$ are the corresponding interaction effects to the respective elements. All elements are fixed constants, except c_{ijk} and e_{ijkmn} which are random variables.

Now we compute for each individual a quantity

$$d_{ijk}=Y_{ijkm1}-2Y_{ijkm'2}+Y_{ijkm3} \qquad (m\neq m'),$$

and calculated the following sums of squares:

$$TSS=\left(\sum_i\sum_j\sum_k d_{ijk}^2\right)/6,\quad TCT=\frac{1}{6}\sum_i\sum_j\frac{\sum_k d_{ijk}^2}{n_{ij}},\quad SSt=\sum_j\left(\sum_i\frac{\sum_k d_{ijk}}{n_{ij}}\right)^2/6\cdot b,$$

$$SSb=\sum_i\left(\sum_j\frac{\sum_k d_{ijk}}{n_{ij}}\right)^2/2\cdot 6,\quad CT=\left(\sum_i\sum_j\frac{\sum_k d_{ijk}}{n_{ij}}\right)^2/2\cdot 6\cdot b,$$

and $$tSS=\sum_i\sum_j\left(\frac{\sum_k d_{ijk}}{n_{ij}}\right)^2/6$$

where n_{ij} denotes the number of individuals of the j^{th} treatment sequence group in the i^{th} replication and b the number of replicaions.

Thus, we obtain the analysis of variance table given in Table 1. This procedure was found to be useful after a trial with the data for milking at unequal intervals in dairy cows.

Table 1. Analysis of variance for two-treatment switch-back design with unequal cell sizes.

Source of variation	d. f.	Sum of squares	Expected mean square
Treatments	1	SSt−CT	$6\bar{e}^2+b(TP)^2/6+3b(SP)^2/2+8bT^2/3$
Blocks	b−1	SSb−CT	$6\bar{e}^2+3(BP)^2$
Treat. X blocks	b−1	tSS−SSt−SSb+CT	$6\bar{e}^2+8(BT)^2/3$
Error	N..−2b	TSS−TCT	$6e^2$
	N..−2b		$6\bar{e}^2$

$6\bar{e}^2=(1/2b)\sum_i\sum_j(1/n_{ij})6e^2$

$T^2=\sum_m T_m^2$, $(SP)^2=\sum_j (SP)_{j2}^2$, $(TP)^2=\sum_m (TP)_{m2}^2$,

$(BP)^2=\sum_i (BP)_{i2}^2/(b-1)$, $(BT)^2=\sum_i\sum_m (BT)_{im}^2/(b-1)$.

Pollen Characters of Chrysanthemum Species

Shuzo NAGAMI[1)]

The pollen characters, especially the diameter and spine number, of 16 *Chrysanthemum* species were studied, by using a scanning electron microscope (Table 1). The conclusions are as follows: (1) All pollen grains of these species were spherical (mean value of diameter, 27.7 μ), and had small spines on the surface (mean value of spine number on the outline of microscopical figure, 16). (2) Gradual increase of the diameter was observed in proportion to the increase of *Chrysanthemum* polyploidy, but no increase was found in spine number (NAGAMI, 1972). (3) In the pollen characters, relationships of L>D>N were found, but no remarkable relationships between the characters and distributional conditions were observed.

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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 characters (pd: pollen diameter, sn: spine number) of chrysanthemums. * exceptional data.

	species name	SN	d	cn	pd	sn
1.	*C. Makinoi*	D	CJ—f	2X	22.4μ	20
2.	*C. boreale*	D	CJ—f	2X	21.5	16
3.	*C. nipponicum*	N	NJ—c	2X	22.8	14
4.	*C. indicum*	D	CJ—f	4X	28.0*	15
5.	*C. indicum var. hexaploid* (H)	D	CJ—c	6X	24.6	13
6.	*C. indicum var. hexaploid* (Y)	D	CJ—c	6X	28.3	13
7.	*C. japonense*	D	CJ—c	6X	29.4	16
8.	*C. Shiwogiku* (T)	D	CJ—c	8X	26.2	21*
9.	*C. Shiwogiku* (A)	D	CJ—c	8X	26.4	17
10.	*C. Shiwogiku var. kinokuniense*	D	CJ—c	8X	26.6	14
11.	*C. Weyrichii*	L	NJ—c	8X	28.5	17
12.	*C. japonense var. octoploid*	D	CJ—c	8X	29.0	19
13.	*C. japonense var. crassum*	D	SJ—c	10X	36.0	14
14.	*C. arcticum ssp. Maekawanum*	L	NJ—c	10X	29.6	17
15.	*C. pacificum* (M)	D	CJ—c	10X	31.5	15
16.	*C. pacificum* (K)	D	CJ—c	10X	32.1	13

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ABSTRACTS OF DIARY FOR 1974

December ('73)	22	110th Biological Symposium
March	22	218th Meeting of Misima Geneticists' Club
April	23	111th Biological Symposium
May	10	219th Meeting of Misima Geneticists' Club
	25	112th Biological Symposium
June	6	220th Meeting of Misima Geneticists' Club
	29	221st Meeting of Misima Geneticists' Club
July	12	222nd Meeting of Misima Geneticists' Club
	27	113th Biological Symposium
August	30	223rd Meeting of Misima Geneticists' Club
	31	224th Meeting of Misima Geneticists' Club
September	3	114th Biological Symposium
	25	115th Biological Symposium
October	22	116th Biological Symposium
	22	117th Biological Symposium
December	2	225th Meeting of Misima Geneticists' Club
	13	118th Biological Symposium

FOREIGN VISITORS IN 1974

February	19–20	NACE, G., University of Michigan, U.S.A.
April	22–23	GRACE, T.D.C., C.S.I.R.O., Australia
May	18	SUEOKA, N., University of Colorado, U.S.A.
August	23	GROSSMAN, M., University of Illinois, U.S.A.
	30	WOLLMAN, E. L., Institut Pasteur, France
September	24–26	ABRAHAMSON, S., University of Wisconsin, U.S.A.
October	21–23	PETIT, C., Université de Paris, France
October	22–23	ROWND, R. H., University of Wisconsin, U.S.A.
		CURTISS, R. III, University of Alabama, U.S.A.

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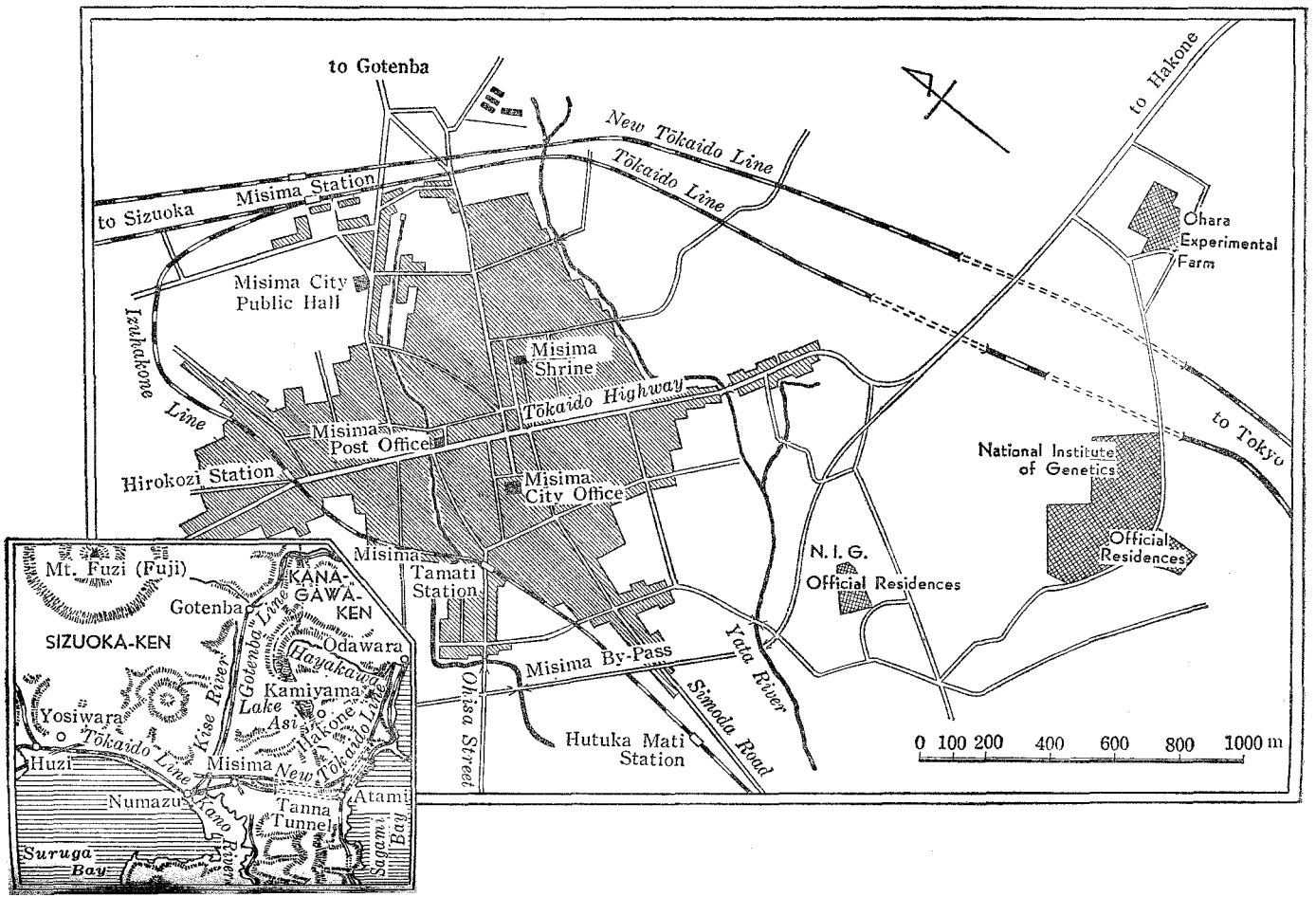
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