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Annual Report of the National Institute of Genetics

No. 29, 1978

(Genetic Stocks Center)

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V. Cytogenetics

GENERAL STATEMENT

The National Institute of Genetics will greet the 30th anniversary of the founding on June 1st of the next year. Thirty years old is, if compared to man, the age to reach the full-fledged manhood. On this memorial occasion we must consider deliberately how the Institute should be directed in the next decade.

Ever since its foundation, the Institute has been well-balanced in its organization being consisted of laboratories of most fields of genetic studies, and researches have been promoted by lively discussions in cooperative atmosphere among the research staffs of different disciplines. Remarkable progress has been achieved recently in the fields of life science. Furthermore, the scale of research has been becoming larger and larger. If we stick to the conventional system and would not take an adequate measure to modulate the situation, we shall be left behind the rapid progress of this science. In order to cope with such circumstances, it is necessary to strengthen the research power not only in the number of researchers but also in their capacities. Far from that, due to the limitation in budgetary allotment, we are obliged to reduce the number of personnels every year. It means a serious damage to the development of our science.

Looking back the calendar of this year, the following are worthy to note. Kihara Institute for Biological Research put its Mishima branch together with the main institute in Yokohama and Prof. H. Kihara, who was the former director of the National Institute of Genetics, left Mishima on February 10th, 1978. It was a great regret that we missed a highly respected leader from Mishima. We had a farewell lunch at the Institute on this occasion, inviting all other honorary members of the Institute. In commemoration of the day Dr. Kihara gave us quite an impressive lecture on his recent work. On the same day we held also a requiem mass to the memory of Dr. F. A. Lilienfeld who passed away last July.

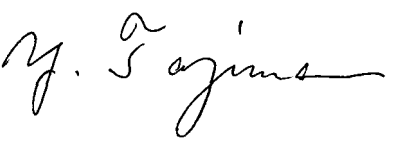
On June 9th Dr. M. Kimura, head of the Department of Population Genetics, was loaded a great honour. He was awarded by the University of Chicago the honorary degree of Dr. of Science for his distinguished contribution in the field of population genetics.

In August the XIVth International Congress of Genetics was held in Moscow. Prior to the Congress, the Genetics Society of America declared to boycott the Congress against the discrimination enforced by the U. S. S. R. Government in issuing visas to Israeli scientists. It caused a confusion for a time. The Congress, however, ended with a great success as the biggest one ever held, having 3,500 participants. From Japan more than 70 geneticists (10 from our Institute), including Professor Kihara, attended the Congress, and I believe that we contributed a great deal to the success of the Congress.

The Institute received three visiting scientists from abroad in this fiscal year, i.e., Mr. Louis Assémat from France (Dept. of Applied Genetics, one year), Dr. A. R. Kasturi Bai from India (Dept. of Morphological Genetics, 9 months), and Dr. B. A. Marcum from U.S.A. (Dept. of Biochemical Genetics, 3 months).

The Institute had also several scientific visitors from Asian countries in this year: in March parties of Science Foundation from Korea and from Thailand, in September a party of Academy of Science and in October another party of Food Science and Technology Investigation, both from the People's Republic of China. They made their efforts to materialize scientific and personnel exchange programs between their own countries and Japan.

The main building for Genetic Stocks Center was completed of its construction in September. The budget for building annex laboratories for breeding mice and rearing silkworms was approved by the government at the end of this year. Thus, necessary arrangements have reached almost completion for bringing the Genetic Stocks Center in its full operation.



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OISHI,* Kugao, Ph. D.

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* Visiting researcher ** Deceased December 19, 1978

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OSHIMA, Chozo, Manager, Head of the Physiological Genetics Department

PROJECTS OF RESEARCH FOR 1978

Department of Morphological Genetics

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)
Cytogenetics in the silkworm (MURAKAMI and IMAI)
Studies on recombination in the silkworm (MURAKAMI)
Genetics of the silkworm (MURAKAMI, FUKASE and OHNUMA)
Radiation and chemical mutagenesis in the silkworm (TAZIMA and MURAKAMI)

Department of Cytogenetics

Studies on chromosomal evolution in rodents and other small mammals (YOSIDA)
Chromosome study on experimental tumors (YOSIDA)
Experimental breeding and genetics of mice, rats and other wild rodents (YOSIDA and MORIWAKI)
Cytogenetical study on sister chromatid exchange in mammalian cells (KATO)
Cytogenetical and immunological studies on mouse plasma cell tumor (MORIWAKI)
Immunogenetical study on the histocompatibility antigens of wild rodents (MORIWAKI)
Cytogenetical study of ants (IMAI)
Cytogenetical study of fishes (YOSIDA)

Department of Physiological Genetics

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)
Genetic studies on the effects of adverse environments on *Drosophila* flies (OSHIMA and WATANABE)

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)
Genetical and biochemical studies on Japanese middle size dog (OGAWA)
Genetics of isozymes in plants (ENDO)
Genetic analysis of developmental mechanisms in hydra (SUGIYAMA and FUJISAWA)

Department of Applied Genetics

Quantitative genetic studies in poultry (KAWAHARA)
Genetics studies in wild populations of Japanese quails (KAWAHARA)
Theoretical studies on breeding techniques (IYAMA)
Behavioral genetic studies in mice (FUJISHIMA)
Genetic studies of trees in natural forest (IYAMA)
Evolutionary studies in wild and cultivated rice species (OKA and MORISHIMA)
Ecological genetic studies in weed species (MORISHIMA)
Genic analysis for isozyme variations in rice (ENDO and OKA)
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 INOUE)
Environmental mutagens and carcinogens (KADA, SADAIE, TUTIKAWA and HARA)
Radiation genetics in mice (TUTIKAWA)
Biochemical factors involved in cellular repair of genetic damage (INOUE and KADA)
Genetics of *Bacillus subtilis* (SADAIE and KADA)

Mutation and differentiation studies on plant tissue culture (AMANO and KADA)
Radiation and chemical interaction in the cells (KADA)
Genetic fine structure analysis in maize (AMANO)

Department of Human Genetics

Genetic and epidemiologic studies on certain malformations in human embryos (MATSUNAGA)
Genetic studies on retinoblastoma (MATSUNAGA)
Clinical cytogenetic studies of congenital abnormalities in man (NAKAGOME and OKA)
Molecular cytogenetic studies of human chromosomes (NAKAGOME and AZUMI)
Studies on human chromosome variants (NAKAGOME, AZUMI and MATSUNAGA)

Department of Microbial Genetics

Genetic regulatory mechanism of DNA replication in *E. coli* (HIROTA, YAMADA, and YASUDA)
Genetic regulatory mechanism of cellular division in *E. coli* (HIROTA and NISHIMURA)
Molecular genetics on DNA replication (YASUDA and HIROTA)
Genetics of bacterial cell envelope (HIROTA, and NISHIMURA)
Synthetic bacterial plasmid (YASUDA, and NISHIMURA)
DNA replication origin of *E. coli* (YASUDA, YAMADA and HIROTA)

Department of Population Genetics

Theoretical studies of population genetics (KIMURA, MARUYAMA, OHTA, and TAKAHATA)
Studies on molecular evolution from the standpoint of population genetics (KIMURA and OHTA)
Theoretical studies on the evolution of multigene family (OHTA)
Mathematical studies on the genetics of structured populations (MARUYAMA)

Department of Molecular Genetics

Studies on the structure of genome RNA of viruses (MIURA and SHIMOTOHNO)
Studies on the primary structure of DNA (SOEDA and MIURA)
Studies on structure and function of messenger RNA (MIURA and SHIMOTOHNO)
Genetical and enzymological studies on *E. coli* RNA polymerase (SUGIURA)
Studies on T_4 RNA ligase (SUGIURA)
Cloning of eukaryotic cell genes and their structural analysis (SUGIURA and SHINOZAKI)

Genetic Stocks Center

Studies and conservation of germplasm resources in rice and wheat species (FUJII, SANO and OKA)
Specificity of mutagen tolerance in higher plants (FUJII)
Exploitation of genetic ability of nitrogen fixation in Graminae (FUJII, SANO, IYAMA and HIROTA)
Studies on genetic differentiation in rice (SANO)
Developmental genetic studies on mouse teratomas (NOGUCHI)
Studies on chromosomal polymorphism in Drosophila (INOUE)
Genetical study of flagellar formation in *Escherichia coli* K-12 (KOMEDA)
Basic studies on the gene purification and the construction of DNA banks (SUGIURA)

RESEARCHES CARRIED OUT IN 1978

I. MOLECULAR GENETICS

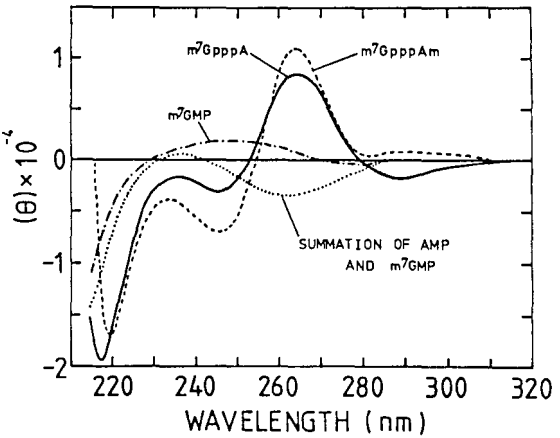
Interaction between Bases Involved in the 5′-terminal Cap Structure of Eukaryotic Messenger RNA

Masao HATTORI and Kin-ichiro MIURA

Most eukaryotic mRNAs and virus RNAs carry a so-called cap at the 5′-terminal as $m^7G^{5'}ppp^{5'}N(m)$. This contains two pyrophosphate linkages and 7-methylguanyilc acid carrying plus charge in its base moiety, being quite different from inner part of an RNA molecule. This structure stabilizes mRNA for exonucleolytic attack and seemes to play any positive role for an initial step in protein synthesis. Thus the knowledge on conformation of the cap structure is required. Here, we synthesized several kinds of the 5′–5′ confronting nucleotide structure (cap in mRNA), including methylated and non-methylated materials, and characterized these compounds by circular dichroism (CD) and ultraviolet absorption spectrum.

m^7GpppA and $m^7GpppAm$ have UV maxima at 258 nm and 257 nm respectively in 0.1 M Na^+ (pH 7) at 25°. Molecular absorbances of these compounds are fairly lower than those which are calculated with each component. After the cleavage of pyrophosphate linkages by venom phosphodiesterase, absorbances of the intense peaks increase by 109% in m^7GpppA and 123% in $m^7GpppAm$. This suggests that both m^7GpppA and $m^7GpppAm$ have base-stacked conformation in a neutral solution, and that the latter stacks more strongly than the former.

CD spectrum of the 5′–5′ confronting nucleotide structure shows typical pattern. CD spectrum of m^7GpppA has peaks at 235 and 265 nm, and troughs at 218 and 289 nm in 0.1 M Na^+ (pH 7) at 26°. As shown in Fig. 1, the spectrum is quite different from a summation spectrum of each component(AMP and m^7GMP). The peak at 265 nm is opposite in sign and increases in magnitude greatly compared to that of the summation spectrum. The long wavelength negative cotton effect which sometimes is characteristic for oligonucleotides containing a guanosine residue is also observed in this case. Except for this negative band, the peaks and troughs of m^7GpppA



are highly sensitive to temperature. By lowering temperature up to 2°, the magnitude of peak at 256 nm increases significantly.

In the same condition (0.1 M Na^+, pH 7, at 25°), CD spectrum of m^7GpppAm is quite similar in shape to that of m^7GpppA except for a long wavelength band. The magnitude of peaks at 265 nm increases appreciably compared to that of m^7GpppA, suggesting that methylation of 2′-hydroxy of adenosine in m^7GpppA strengthens the stacking interaction between N^7-methylguanine and adenine bases.

Structure near the 5′-terminal of Some Eukaryotic mRNAs

Kunitada SHIMOTOHNO, Soh HIDAKA and Kin-ichiro MIURA

The protein synthesis starts at the initiation codon and proceeds in the direction 5′→3′ on the template mRNA. An eukaryotic mRNA carries a "cap" structure at the 5′-terminus, but it is not followed directly by an initiation codon AUG. The part between the cap and the initiation codon may participate with the initiation complex formation. Here we have studied the nucleotide sequence of this region.

Messenger RNAs from cytoplasmic polyhedrosis virus (CPV), which contains 10 double-stranded RNA segments as a genome, were used for nucleotide sequence analysis around their 5′-termini. As the 5′-termini of these RNAs are capped with 7-methylguanylic acid through pyrophosphate lin-

kages [see this report No. 25 (1974)], the cap was deleted by tobacco pyrophosphatase before the 5′-terminal labeling with [^{32}P] phosphate by nucleotide kinase. The [^{32}P] labeled CPV mRNAs were separated by gel electrophoresis. Three smaller mRNAs were extracted from the gel with buffer solution and analyzed. The mRNA was partially digested under limited conditions with alkali, ribonuclease T_1 and U_2. The digests were run in parallel in a polyacrylamide gel and autographed on a X-ray film. The arrangement of guanine, adenine and pyrimidine was deduced. The arrangement of cytosine and uracil was analyzed by mobility shift in two dimensional gel electrophoresis (pH 3.5 and pH 7).

The base arrangement of the 5′-terminal regions in three shorter RNA segments are shown here. Common sequence for these RNAs are surrounded with box or underlined.

CPV mRNA

1 m^{7}GpppAmGUAAAAGUCAGUACCAUACCGGAAAAAGAUGAAAAGGAYAAYG'GA

2 m^{7}GpppAmGUAAAUCCCCGGCGUAAACCGAAUAUCGCGAGACCGYAAAYG

3 m^{7}GpppAmGUAAAGUCCAGUACUAGUUAAAGACAUUUGACAAGGAYGAYYAYG

Six bases arrangements continuing to the 5′-terminal cap were the same for these RNAs, but the arrangements between these and the protein initiation codon AUG were heterogeneous. There are some partially common sequences (underlined) to two RNAs. In number 1 mRNA the nucleotide sequences surrounding the first AUG consist of only purines, so that these area cannot construct a hair-pin structure.

Cucumber mosaic virus (CMV) contains 5 (4 in some strains) single-stranded RNA segments as genome. These viral RNA segments can function as messenger RNAs. These carry the cap structure at the 5′-terminus. The smallest RNA segment (number 5) was analyzed as mentioned above. The base sequence near the 5′-terminus is shown here.

m^{7}G^5′pppG-U-U-U-U-G-U-U-U-G-*A-U-G*-G-A-G-A-A-U-U-G-C-G-U-A-G-A-G-G-G-

The sequence between the 5′-terminus and the initiation codon is quite simple and U-rich. In this area a long common sequence to other mRNAs so far analyzed was not found. A sequence complementary to the 3′-terminal part of ribosomal RNA has been found in the sequence a head of the initiation codon in many prokaryotic mRNAs and some eukaryotic mRNAs. Shine and Dalgarno proposed that the mRNA is bound at first on ribosome by this sequence. However, such sequence was not detected in CMV No. 5 RNA. Thus the above-mentioned Shine-Dalgarno's idea seems not to be common for every eukaryotic mRNA. (A part of this work was published in FEBS Letters **98** (1979) 115–118)

The Primary Sequence of the Late Region of Polyoma Virus DNA: the Expression of the Late Genes and Comparison with DNA Sequences of SV40 and BKV

Eiichi SOEDA

The nucleotide seqeunce of the late region of the polyoma virus genome has been deduced, which codes for the major capsid protein VP1 and the C-terminal region of the minor proteins VP2 and VP3. The amino acid sequence of VP1 predicted from the nucleotide sequence is in good agreement with the partial N-terminal sequence and amino acid composition of VP1 (Hewick, R. M. *et al.* 1975, Virology 66: 408–419, Fey, G. and Hirt, B. 1975, Cold Spring Harbor Symp. Quant. Biol. **39**: 235–241).

When both nucleotide and amino acid sequences are compared with their counterparts in the related viruses, SV40 (Rogiers, R. *et al.* 1978, Eur. J. Biochem. **85**: 205–223, Pan, J. *et al.* 1977, Nucleic Acids Res. **4**: 2539–2547) and BKV (R. Young, personal communication), extensive homologies are found along the entire regions of the viral genes. Maximum homologies appear to occur in the regions which code for the C-terminal of VP1, on the contrary of the result of heteroduplex analysis (Ferguson, J. and Davis, R. W. 1975, J. Mol. Biol. **94**: 135–149) with SV40 and polyoma virus DNAs.

During the late lytic cycle of polyoma virus (Py), following commencement of viral DNA replication, three viral RNAs appear in cytoplasm of cells. They have sedimentation coefficients of 16 s, 18 s and 19 s, each of which directs *in vitro* synthesis of the viral capsid proteins, VP1, VP3 and VP2, respectively. The main body of 16 s mRNA appears to be located

from 48 to 26 map units of Py genome counterclockwisely whereas the bodies of 18 s and 19 s mRNAs span completely the region of 16 s mRNA, starting from the proximal part of the late gene. The genetic evidence and peptide analysis have presented that the N-terminal of VP1 and the C-terminals of VP2 and VP3 are closely located around 48 map units, possibly coding regions of these proteins overlap with each other with different coding frames, by analogy with SV40. The evidence is consistent with the topographical model of the late region of the physical map (Fig. 1) which shows positions of late mRNA and capsid proteins.

SV40 and Py have very similar growth cycle and genetic organization, suggesting they diverged from a common origin. Heteroduplex analysis of DNAs between two viral species has revealed that the strongest sequence homology occurred in the region where VP1 and VP2/VP3 coding regions of SV40 overlap each other with different coding frames. This led to speculation that the same may be true for Py late gene and the overlap gene would be imposed severe restrictions on genetic drift, thus these region must be highly conserved.

Here we report the nucleotide sequence of the coding regions of VP1, VP2 and VP3 of Py and compare with the counterparts of SV40 and BKV. Conservation of overlap genes and divergency on the sequences of late genes of these viral species will be discussed from the aspect of molecular

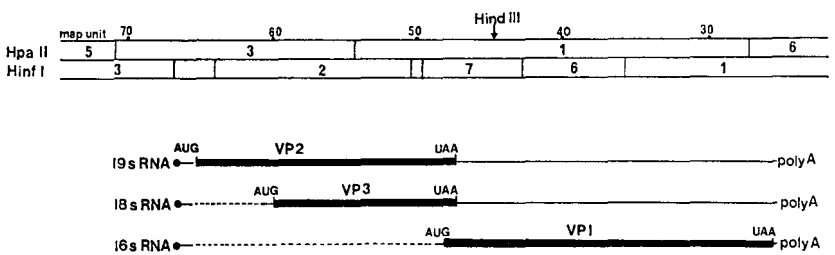


Fig. 1. A topographical model of the polyoma virus late genes.

The cleavage maps of the restriction endonucleases *Hpa* II and *Hinf* I are shown at the top on the linearised late region of polyoma virus DNA. The DNA has been divided into 100 map units, taking the single cleavage site of the restriction endonuclease *Eco*RI as a zero reference point. The three known viral late messenger RNAs (with polyadenylated tails) and their location in terms of the genomes are shown. Coding regions of the three virion proteins are shown using a heavy dotted line and the regions spliced out of the mature late mRNAs using a dotted line.

evolution.

The fine physical map of Py with several restriction enzymes has been defined. The nucleotide sequence of Py late region was deduced by the Maxam and Gilbert procedures, using 3′- and 5′-terminally labelled fragments generated by digestion of Py DNA with restriction enzymes.

In the nucleotide sequence with the same polarity of late mRNAs (Fig.2), there are two long uninterrupted coding frames: One lies at the 5′-end of the sequence and contains the potential coding sequences for VP2/VP3. The other contains 383 consecutive sense codons starting with the ATG and extends from 47.5 to 25.8 map units where the body of VP1 gene has located. The partial N-terminal sequence of VP1 synthesized *in vitro* has completely matched up to the first 20 cycles of sequence with that predicted from the DNA sequence.

The nucleotide sequence is furnished with several structural futures including the potential recognition sequences for ribosome binding and splicing of mRNA proceeding the VP1 initiation site and AATAAA sequence near the polyA tails of late mRNA. The region preceeding the VP1 initiation site is characterized by a long inverted repeat sequence, which possibly forms a hairpin loop in the mRNAs. The similar sequence which can form a part of the loop is also found in the leader sequence late mRNA by 900 nucleotides upstream of the body. Such a structure might be utilized in the recognition events involved in the processing which leads to mature 16 s mRNA.

When both the nucleotide and amino acid sequences of these viral species are aligned, extensive homologies are found throughout most of the VP1 genes (Fig. 2). Heteroduplex analysis with covalently linked DNAs of SV40 and Py suggests that the strongest homologies are located around the N-termini of the VP1 genes. The present data, on the contrary, show that most of the larger homologous nucleotides are found in regions of the genes coding for the C-termini. This disparity may be a reflection of the two different methods of analysis. The overlap region of VP1 with VP2/VP3 of polyoma virus shorter by 89 nucleotides than that of SV40 and shows very limited homology with SV40. This leads to the suggestion that the overlap segments both viruses have been free from stringency imposed on drifting during evolution and that the proteins VP2/VP3 of polyoma virus have possibly been truncated by the appearance of a termination codon within the sequence.

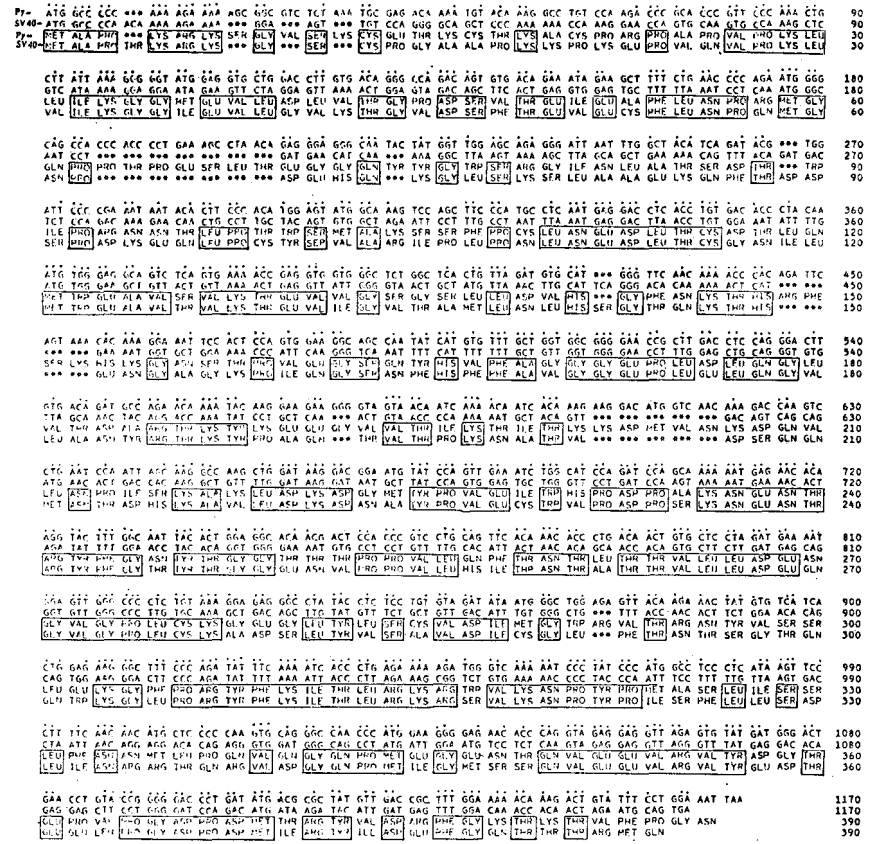


Fig. 2. Comparison of nucleotide and amino acid sequences of the VP1 gene and gene products of Py and SV40.

The VP1 sequences of Py and SV40 are aligned so as to maximise homology. Identical peptides and nucleotides, at corresponding positions, are indicated by the boxes or dots, respectively.

A Simple Method for the Identification of Altered Subunits in Mutant RNA Polymerases of *Escherichia coli*

Masahiro SUGIURA, Nozomu ITO and Mie SUZUKI

DNA-Dependent RNA polymerase from *Escherichia coli* is a multi-subunit enzyme with a subunit composition of $\alpha_2\beta\beta'\sigma$. In order to study the

structure, function, and genetics of this enzyme and its subunits, methods for reconstitution of the active enzyme from its isolated subunits have been developed. These methods are, however, laborious and time-consuming and hardly applicable to unstable mutant RNA polymerases. During the course of isolating temperature-sensitive mutants of RNA polymerase, we developed a simple procedure to identify altered subunits. The procedure consisted of mixing a mutant enzyme with an excess of a wild-type subunit followed by denaturation and renaturation. This gave active enzyme molecules in which the majority of a subunit species was replaced by a corresponding wild-type subunit. When enzyme containing temperature-sensitive β' subunit was mixed with an excess of each separate wild-type subunit followed by denaturation and renaturation, only the mixture containing wild-type β' subunit was temperature resistant. Likewise, the rifampicin-resistant enzyme exchanged specifically with wild-type β subunit became rifampicin sensitive. Thus results indicated that the method was applicable to identify altered subunits in mutant RNA polymerases. The subunit replacement experiments were also carried out successfully using crude enzyme preparations. This work was published in Analytical Biochemistry **84**, 337–339 (1978).

Joining of 3′-Modified Oligonucleotides by T4 RNA Ligase. Synthesis of a Heptadecanucleotide Corresponding to the Bases 61–77 from *Escherichia coli* $tRNA^{fMet}$

Eiko OHTSUKA, Satoshi NISHIKAWA, A. F. MARKHAM, Seiji TANAKA, Tetsuo MIYAKE, Toshiaki WAKABAYASHI, Morio IKEHARA and Masahiro SUGIURA

Chemically synthesized fragments corresponding to the 3′ end of $tRNA^{fMet}$ from *Escherichia coli* were joined by T4-induced RNA ligase to yield a heptadecanucleotide (bases 61–77). The 3′ terminus of C-C-A was modified by introduction of the ethoxymethylidene group to prevent intra- and intermolecular self-joining reactions at the 3′ end. The terminal trimer was phosphorylated using polynucleotide kinase and joined to C-A-A with RNA ligase. The hexamer [C-A-A-C-C-A (ethoxymethylidene)] corresponding to bases 72–77 was obtained in a yield of 60%. An undecanucleotide (bases 61–71) which had been synthesized in a yield of 34% by similar enzymatic joining of U-C-C-G-G to pC-C-C-C-C-G was allowed to react

with the 5′-phosphorylated hexamer (bases 72–77) using an excess of RNA ligase to yield the heptadecanucleotide U-C-C-G-G-C-C-C-C-C-G-C-A-A-C-C-A (bases 61–77). The product was identified by homochromatography and nearest neighbor analysis. This work was published in Biochemistry **17**, 4894–4899 (1978).

Cloning and Characterization of Tobacco Chloroplast 23S and 16S rRNA Genes

Masahiro SUGIURA and Jun KUSUDA

Previously we have shown that tobacco chloroplast 23S and 16S rRNA hybridized with two EcoRI fragments of tobacco chloroplast DNA, 1.9×10^6 (I) and 2.8×10^6 (E) dalton fragments, and have cloned these two fragments using the *E. coli* plasmid pMB9 (this report No. 28, 1977).

In order to know whether these two fragments are contiguous, we then attempted to construct a recombinant DNA molecule containing both fragments. The chloroplast DNA was digested partially with EcoRI in the presence of low concentration of distamycin A. Hybridization of ($5'^{32}$P) rRNA to a Southern image of the partial EcoRI digests produced a series of radioactive bands. The partial EcoRI fragments ranged from 4.5×10^6 to 6×10^6 daltons were extracted from a preparative gel electrophoresis and cloned using pMB9 as a vector and *E. coli* HB101 as host bacteria. Two hundreds and seventy one transformants were obtained from 0.2 μg each of pMB9 and the chloroplast DNAs and 12 clones were found to contain rRNA sequences. Ten out of the 12 clones contained both E and I fragments and an additional fragment of 0.46×10^6 daltons. One of the 10 clones, TCP6 was used for further studies. The plasmid pTCP6 DNA was partially digested by EcoRI and analyzed by agarose gel electrophoresis. It was found to contain the three fragments in the order of E - I- 0.46×10^6 fragment. This clearly indicates that the E and I fragments are contiquous.

In order to locate 23S and 16S rRNA sequences on the E+I fragment, the Southern image of pTCP6 EcoRI fragments was hybridized separately to ($5'^{32}$P) 23S and 16S rRNAs. The autoradiographs showed that 23S rRNA hybridized to E fragment whereas 16S rRNA hybridized mainly to the I fragment and slightly to the E fragment. There results and molecular weights of the rRNAs could give the approximate location of the rRNA genes within the fragments E and I.

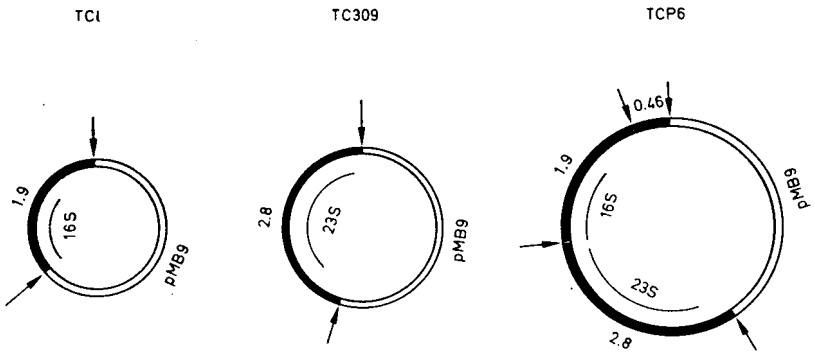


Fig. 1. EcoR1 restriction maps of recombinant plasmids containing tobacco chloroplast rRNA genes. Arrows, EcoR1 sites; Numerals, Molecular weights ($\times 10^{-6}$ daltons)

This work was published in Molec. Gen. Genetics, **172**, 137–141 (1979) and was presented at the 8th ICN-UCLA Symposia on Molecular and Cellular Biology, "Extrachromosomal DNA", Keystone, U.S.A., March 1979.

Gene Controlling DNA Initiation in *Escherichia coli*: *dnaA*$^+$ and Suppressor Genes of *dnaA*$^-$

Yutaka TAKEDA and Yukinori HIROTA

It has been known that the *dnaA* gene product is required in the initiation step of *Escherichia coli* chromosome replication. In this communication, we report the cloning of genes containing *dnaA*, and the genes which suppress the temperature sensitive (ts) mutation of *dnaA*. Restriction maps of these chromosomal regions were also described.

Whole *E. coli* chromosomal DNA of a mutant of *E. coli*, W3110, and a plasmid DNA, pMB9 (Bolivar *et al.* Gene, **2**, 75–93, 1977) or pCR1 (Covey *et al.* Molec. gen. Genet., **145**, 155–158, 1976), were digested with EcoRI and ligated with T4 DNA ligase. The $CaCl_2$-treated cells of CRT46*recA* (a *dnaA* ts mutant) were transformed with the ligation mixtures and the colonies which became thermoresistant and drug resistant were selected. From these transformants, two plasmids were isolated: one was constructed by ligation with pMB9 and the other with pCR1, and those DNAs were designated as pYT47 and pYT48, respectively. These plasmids had the same EcoRI

fragment of *E. coli* DNA whose molecular weight was 5.5×10^6 daltons. After the transformation of CRT46*rec*A with pYT47 or pYT48 DNA, the frequency of appearance of drug resistant colonies at 30°C and at 42°C was the same.

Another plasmid, pYT46, was isolated by selection of Kanamycin resistant transformants at 30°C (ligated with pCR1). After the transformation experiment of CRT46*rec*A with pYT46 DNA, Kanamycin resistant transformants appeared at 30°C, but not at 42°C. All the transformants thus obtained at 30°C showed temperature resistance. Therefore, we thought that pYT46 had a supperssor of *dna*A which required a long time to express its temperature resistance. pYT46 had an EcoRI fragment of *E. coli*, whose molecular weight was 3.5×10^6 daltons. pYT46, pYT47 and pYT48 could not complement *tna*, *rbs*, *unc*A, *asn* and *pyr*E mutations.

A lambda phage carrying *dna*A gene (λ *dna*A) was isolated by a conventional *in vivo* recombination from λ *i*21*tna* by Murakami and Yamagishi (personal communication). This phage also had *tna* gene which located close to *dna*A.

pYT46, pYT47 and λ *dna*A DNAs were digested with the following restriction enzymes; EcoRI, HindIII, BamHI, BglII, KpnI, PstI, SalI, XhoI, HpaI and the digests were analyzed by agarose and polyacrylamide gel electrophoresis. Restriction maps were constructed by digestions of the DNAs with various combinations of restriction endonucleases. The restriction maps of *E. coli* DNA region of pYT46, pYT47 and λ *dna*A were shown in Fig. 1. Compared with each other, we concluded that three DNAs had no overlapping region. It is likely that these DNAs were originated from three different regions of *E. coli* chromosome. We thought that λ *dna*A had the structure gene of *dna*A, because λ *dna*A had also *tna*, a linked gene of *dna*A on the chromosome map of *E. coli*. pYT46 and pYT47 had suppressor genes which were different from *dna*A. The molecular nature of these suppressor gene products were not known. Increase of the gene dose might cause the suppression of *dna*A ts mutation, because cloning vectors, pMB9 and pCRl, had 10–20 copies per cell.

The suppressor gene product of pYT47 was characterized, as follows. From pYT47, two deletion mutants, pYT62 and pYT63, were isolated after the digestion of pYT47 DNA with KpnI or PstI and ligation with T4 DNA ligase. These plasmids having different deletion could not suppress *dna*A

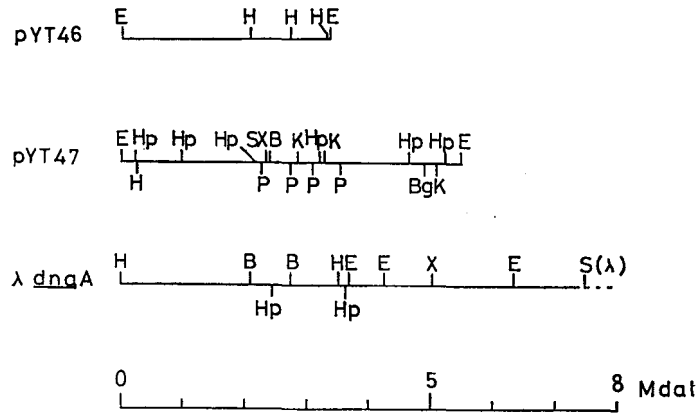


Fig. 1.

ts mutation. The deleted regions of pYT62 and pYT63 were shown in Fig. 2. pYT47, pYT62 and pYT63 were introduced into a minicell-producing strain LC130. Minicells produced from these strains were purified through sucrose gradient centrifugations 3 times, and incubated at 37°C for 30 minutes to degrade host mRNA. Some proteins newly synthesized from plasmid DNA were labelled with [^{35}S]-methionine and analyzed with two dimensional gel electrophoresis described by O'Farrell (J. Biol. Chem., **250**, 4007–4021, 1975). pYT62 could not synthesize two proteins whose molecular weights were 68,000 and 20,000 daltons, and pYT63 could not synthesize only one protein of 68,000 (Fig. 3).

Six independent mutants which could not suppress *dna*A ts mutation were isolated from pYT47 after mutagenesis with N-methyl-N′-nitro-N-nitroso-

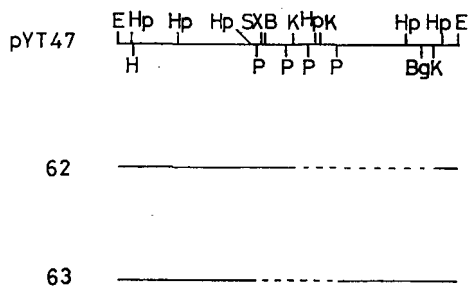


Fig. 2.

guanidine or hydroxylamine. The proteins coded by these mutant plasmids were analyzed with two dimentional polyacrylamide gel electrophoresis. Five of these mutants showed the same pattern as pYT47, while only one mutant, designated pYT74, synthesized a protein having molecular weight of 68,000 daltons with altered isoelectric point. This change of isoelectric point of 68,000 protein was recovered in pYT81, a spontaneous revertant of pYT74 which could suppress a *dna*A ts mutation (Fig. 3). We concluded that the protein having molecular weight of 68,000 daltons was a suppressor protein of *dna*A ts mutation, because the suppression of *dna*A

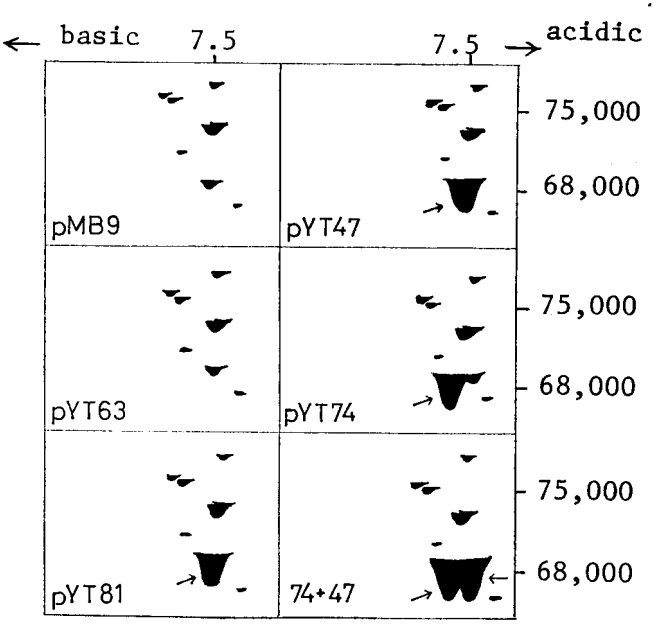


Fig. 3. Pattern of two dimensional gel electrophoresis.

Purified minicells carrying plasmids were treated with Triton-X 100 after sonication. Soluble fractions were used as samples for electrophoresis. Electrophoresis were done as described by O'Farrell. All proteins were stained with Coomassie brilliant blue R250. Proteins newly synthesized from plasmed DNA were labelled with ^{35}S-methionine and detected by autoradiography.

In this figure, spots of proteins after staining were shown. Arrows indicate mutant and wild type proteins having a molecular weight of 68,000. This spot was radioactively labelled.

ts mutation was caused by the change of isoelectric point of the protein. Recently, Murakami and Yamagishi identified the gene product of *dna*A, whose molecular weight was about 50,000 daltons (personal communication). Therefore, the gene product of a suppressor gene on pYT47 was different with the *dna*A protein.

II. MICROBIAL GENETICS

Properties of Penicillin Binding Protein (PBP) 1a, 1b and 3

Toshihide TAMURA, Hideho SUZUKI, Yukinobu NISHIMURA and Yukinori HIROTA

The function of PBPs of *E. coli* K12 in growth and division have been speculated by analysing mutants defective in one or more PBPs (Suzuki *et al.*, *Proc. Nat. Acad. Sci.*, **75**, 664–668, 1978). Using membrane fraction of these mutants, attempts were made to search for possible enzymatic activities of each PBPs in terms of murein synthesis in the subcellular system. The findings to date are that at least PBP-1b (the gene product of *pon*B$^+$) may play a principal role in murein synthesis *in vitro*, and that other PBPs be auxiliary in directing murein synthesis to morphogenesis or division of a cell. As a consequence of these studies, purification of the individual PBP was attempted in order to examine molecular characteristics of each PBP. For this purpose, we took advantage of utilizing the specially constructed strains as a source of PBPs. In these strains, the cellular content of required PBP was amplified ten times or more by the introduction of a ColEl plasmid carrying the relevant PBP-gene and the unwanted PBPs were genetically eliminated as far as possible by the combination of mutations leading to the loss of these PBPs. The constructed strains were JE7434 (*pon*B1085 *fts*I730 *dac*A1191 *dac*B12 pLC29–47), JE5770 (*pon*A980 *fts*I730 *dac*B12 *dac*A1191 pLC19–19) and JE7430 (*pon*A^{ts}1104 *pon*B704 *dac*A1191 *dac*B12 pLC26–6). Membrane fractions prepared by Ribi-cell-disintegrator were solubilized by Triton X–100 in the presence of LiCl. The solubilized materials were desalted by dialysis and pre-fractionated by DEAE-Sepharose column chromatography, in which PBPs were partially separated each other. The fractions containing the desired PBP but almost free from other PBPs were pooled and subjected to covalent Ampicillin–Sepharose affinity chromatography. The covalently bound PBPs to Ampicillin residue of the resin were eluted by neutralized hydroxylamine, desalted by dialysis and stored at 4°C.

In the last procedure, partial separation of PBPs could also be achieved

by eluting PBPs from the affinity resin after successive incubations for increasing length of time at 37°C with neutralized 1 M hydroxylamine. This step-wise elution method in the affinity chromatography to the material obtained after DEAE-Sepharose chromatography improved the purity of the final preparation of PBP-1a & -1b. About 90% homogeneity of PBP1a, 1b, and 3 were obtained as examined in SDS-acrylamide gel electrophoresis. The preparation of PBP-3 obtained after the affinity chromatography was accompanied with a minor PBP which ran faster than the authentic PBP-3 in electrophoreogram. Genetic experiments showed the close relationship between this minor PBP and the PBP-3, suggesting the possible precursor-product relationship. Neither activities for murein synthesis *in vitro* nor those for D–D carboxypeptidase was detected in the purified PBP-1a, -1b or -3 as long as UDP-linked murein precursors, *i.e.* UDP-MurNAc-L-Ala-D-Glu-*meso*-A_2pm-D-[^{14}C]Ala-D-[^{14}C]-Ala, were employed as substrates.

When a membrane fraction of the PBP-1b defective mutant was employed in the *in vitro* system for murein synthesis, polymerization of murein precursors was greatly suppressed, at the same time considerable accumulation of the lipid intermediate was observed. The addition of purified PBP-1b to this PBP-1b defective system promoted murein synthesis with consumption of the accumulated lipid intermediate. Apparently, the purified PBP-1b functioned to synthesize murein in compensation for the defect of PBP-1b of the membrane in the reaction system. Very little stimulation or practically no stimulation in the murein synthesis *in vitro* was observed by addition of the purified PBP-1a or -3, respectively (Table 1).

Table 1. Murein synthetase activity of PBP-1a and 1b, *in vitro*

Addition	Incorporation (cpm)	
	Murein	Lipid-intermediate
None	319	1611
PBPla	606	1602
PBPlb	2999	228
PBP3	381	1708

Substrates: UDP-MurNAc-L-Ala-D-Glu-*meso*-A_2pm-D-Ala-D-Ala ; ^{14}C-UDP-GlcNAc

Membrane : *ponA*^{ts}1104 *ponB*$^{-}$1085 *dacA*$^{-}$1191 *dacB*$^{-}$12

Further Genetical Study of Flagellar Mutants in *E. coli* K-12

Yoshibumi KOMEDA

Twenty nine flagellar genes in *Escherichia coli* K-12 have previously been assigned to three regions of the genome. Flagellar region I is located between *pyr*C and *pts*G, region II is located between *aro*D and *uvr*C, and region III is located between *uvr*C and *his*. In this study flagellar mutants in *Escherichia coli* K-12 were obtained by selection for resistance to the flagellotropic phage χ. They were analysed in complementation tests using Pl phage-mediated transduction. In addition to the *fla* genes already described, 9 more flagellar genes were identified. This analysis defined 6 more *fla* genes in region I (*fla*U, *fla*W, *fla*X, *fla*Y, *fla*Z and *flb*A), 1 more *fla* gene in region II (*flb*B) and 2 more flagellar genes in region III (*flb*C and *mot*D). Region I was shown to include at least 12 *fla* cistrons. Complementation analysis with polar Mu phage-induced Fla$^-$ mutants and with *λ-fla* phage, defined four transcriptional units in region I. These were: *fla*U, *flb*A-*fla*W-*fla*V-*fla*K-*fla*X-*fla*L-*fla*Y-*fla*M, *fla*Z, and *fla*S-*fla*T, with transcription proceeding from left to right. The *flb*B gene was identified to be a member of an operon: *flb*B-*fla*I in region II. In region III, a previously unidentified gene *flb*C was located between *hag* and *fla*N. The *mot*D gene was located between *fla*A and *fla*R and the operon was reoriented as *fla*A-*mot*D-*fla*R-*fla*Q-*fla*P, with transcription proceeding from left to right. Now we have 39 genes (*che*, *fla*, *flb*, *hag*, *mot*) necessary for the synthesis and function of the flagellar apparatus of *E. coli* K-12. Out of these genes, 28 genes (*fla*, *flb*, *hag*) are responsible for the completion of the flagellar structure.

Expression of the hag Gene in *E. coli* K-12: Analysis of hag-lac Gene Fusion

Yoshibumi KOMEDA

Previous studies have defined 28 genes necessary for the synthesis of the flagellar apparatus of *Escherichia coli* K-12. Most flagellar defects result in a Fla$^-$ phenotype which is not very informative biochemically. Most structural and regulatory components of the organelle have no easily measurable activity and only manifest themselves when integrated into the whole organelle. This makes it difficult to measure the synthesis of a flagellar gene product and to study the regulation of expression of the

flagellar genes. The *in vivo* gene-fusion technique developed by M. Casadaban allows an analysis of the regulation of virtually any gene by coupling the *lac* genes to the gene (operon) in question. We decided to apply this method in the study of *fla* regulation, and have been able to fuse most flagellar genes to the *lac* genes by using novel Mu phage carrying the *lac* genes. This study analysed the influence of the flagellar genes on the expression of the *hag* gene (structural gene for flagellin). To this end, a *hag* : : Mud (Ap^r, *lac*) mutant which had the *lac* genes fused to the promoter of the *hag* gene was constructed. This allowed the measurement of the *hag* gene expression by detection of beta-galactosidase activity. The following observations were made: (I) The *hag* gene was shown to be expressed constitutively in Fla^+ cells. (II) *Hag*-gene-expression was positively regulated by *fla*A, *fla*B, *fla*C, *fla*D, *fla*E, *fla*G, *fla*H, *fla*I, *fla*K, *fla*L, *fla*M, *fla*N, *fla*O, *fla*P, *fla*Q, *fla*R, *fla*V, *fla*W, *fla*X, *fla*Y, *fla*Z, *flb*A and *flb*B genes. *Hag–lac* expression was not observed in strains with these *fla* mutations. (III) The *hag* gene was expressed in mutants with *fla*S, *fla*T, *fla*U and *flb*C defects. Therefore, these genes were not involved in regulation of *hag* gene transcription.

III. BIOCHEMICAL GENETICS AND IMMUNOGENETICS

Electrophoretic and Heat-stability Variations of Serum Esterase (Es-1) Isozymes in Wild mice (Mus)

Tadashi AOTSUKA* and Kazuo MORIWAKI

We have found the heat-stability difference betwee two types of Es-1 isozymes (Es-1^a and Es-1^b) in 21 inbred mouse strains, and demonstrated based on the results of cross experiments that the difference in the heat-stability is due to the genetic alteration on the Es-1 locus (no other factors are responsible for observed heat-stability variations in inbred mice: Aotsuka and Moriwaki; *Ann. Rep. Natl. Inst. Genet. (Japan)* **24**: 32, 1977). In the present paper we report the isozyme variations at the Es-1 locus examined with 8 species of wild mice (*Mus*) collected from Japan and Southeast Asia by using both electrophoretic mobility and heat-stability.

Sera were treated at 60°C for 0, 3 and 6 minutes before applying to acrylamide slab gel electrophoresis, by which we could staisfactorily discriminate the difference of heat-stability between Es-1^a and Es-1^b. Three types of heat-stabilities (stable, moderate and sensitibe) were identified based on the intensity of zymograms. For controls, sera of inbred mice, BlOBR(Es-1^a) and C3H/HeJ(Es-1^b), were examined simulaneously. The results are summarized in Fig. 1. In a survey of 95 individuals of wild mice, a total of 7 electromorphs (differing in electrophoretic mobility, *i.e.*, *af*, a, a^-, b^+, b^+, b, b^- and c) were detected. Additional variations for Es-1 locus were found by heat denaturation tests. The five electrophoretic classes (a, a^-, b^+, b and b^-) can further be divided into two or three classes differed in heat-stability; stable, moderate and sensitive. The heat-stability differences within a electromorph occured at three levels of phylogenetic relationships, within a species, among subspecies and species. A total of 14 variants (a doubling of the number of isozymes observed by electrophoresis) were detected by using both electrophoresis and heat-stability tests. If the moderately heat-stable zymogeams are the heterozygotes of heat stable and sensitive alleles, the number of alleles of Es-1 locus become 12. The present survey of hidden genetic variabilities well supply a fruitful information about the genetic diversity within and between *Mus* species.

* Tokyo Metropolitan University

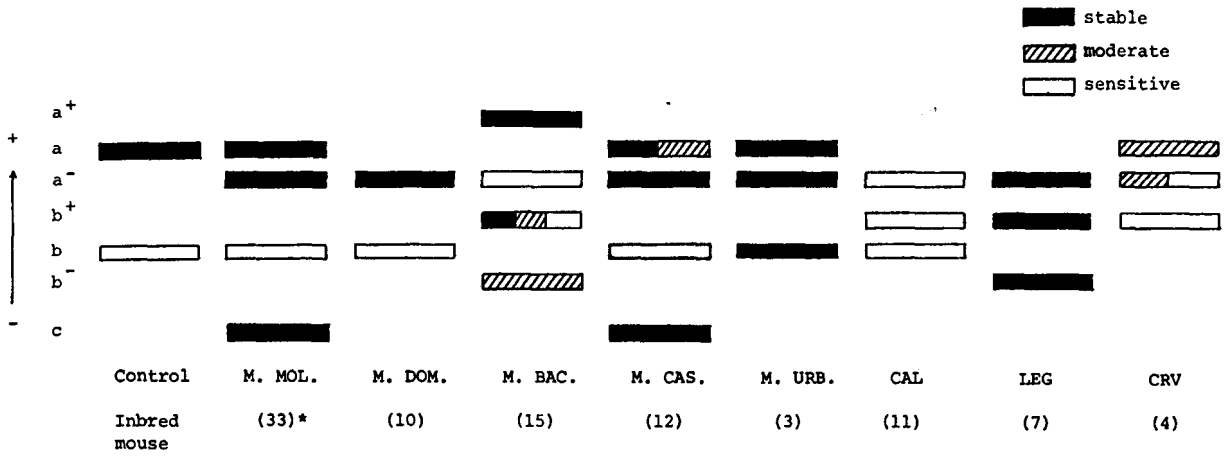


Fig. 1. Electromorphs and their heat-stabilities of serum esterase (Es-1) isozymes of eight wild species of *Mus*. Species abbreviations are; M. MOL.=*Mus musculus molossinus*, M. DOM.=*Mus musculus domesticus*, M. BAC.=*Mus musculus bactrianus*, M. CAS.=*Mus musculus castaneus*, M. URB.=*Mus musculus urbanus*, CAL.=*Mus calori*, LEG.=*Mus legada*, CRV.=*Mus cervicolor*.

* ( ) No. of individuals examined.

Genetic Control of Esterase in Japanese Species of Trillium

Masaaki IHARA and Toru ENDO

We have examined the electrophoretic phenotypes of esterase (Est) of seeds, whole body of the 2nd year juveniles and leaf blade of adult plants in three species as well as their F_1 hybrids. The Est zymogram is shown schematically in Fig. 1.

The result indicates that there are two groups of isozymes preferentially reacting with either 1-naphtyl acetate (α-specific in zone I) or 2-naphtyl acetae (β-specific in zone II) used as a substrate together with fast blue RR salt as a dye coupler. The α-specific bandmorph consists of one cathodally moving isozyme and four anodally migrating ones, whereas the β-specific bandmorph is composed of 1 or 2 anodally and rapidly migrating isozymes. We designate respecitve bandmorphs in the α-specific isozymes as A_1 to A_4 for anodal band and C_1 for cathodal one; those in the β-specific isozymes are referred merely as isozymes in zone II because of no species difference.

The A_1 and A_2 bands recovered in every cross; the A_3 band did not recover in the cross of *tschonoskii*×*smallii* but it recovered in the crosses of *kamtschaticum*×*tschonoskii* and its reciprocal one. Therefore it is necessary to confirm F_2 segregation for the genetic control of A_3 bandmorph.

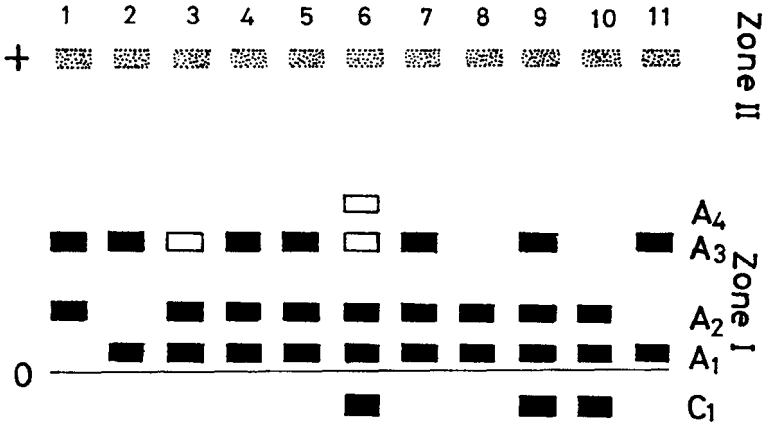


Fig. 1. Esterase zymograms showing the migration rates of the seed isozymes in three species and their F_1 hybrids. (1) *T. kamtschaticum* (K); (2) *T. tschonoskii* (T); (3) T×K; (4) K×T; (5) K; (6) K×S; (7) S×K; (8) *T. smallii* (S); (9) S×T; (10) T×S, (11) T. "O" designates the origin.

The C_1 and A_4 seem to be active in the adult plants and they are probably governed by respective loci.

If the A_1 and A_2 bandmorphs are controlloed by the same locus, it would be explained as follows: F and F+S isozymes appear in *T. kamtschaticum*, and S isozyme does in *T. tschonoskii* and F+S isozyme does in *T. smallii*.

Four plants of *T. Hagae*($2n=15$), which is presumed to be natural hybrid between *T. kamtschaticum*($2n=10$) and *T. tschonoskii*($2n=20$), revealed the same zymogram. The C_1 and A_1 bands of *T. Hagae* are superimposed by those of *T. tschonoskii*; the A_2 and A_3 bands are common with all these species as to their migrating rates. Thus the putative hybrid may have descended from the above two entities.

Maternal Effect Embryo Lethal Mutant in Drosophila melanogaster

Masa-Aki YAMADA and Saburo NAWA

For the investigation of the interaction between egg cytoplasm and zygote nucleus during the development, a X-linked mutant (fs(1) MY-18) was isolated with EMS treatment. The viability of this mutant was almost normal (the ratio of homozygous females to heterozygous females was 0.9). When the heterozygous females of the mutant were crossed to the mutant males, they normally produced their progenies. The homozygous females produced apparently normal eggs in shape and size. However, they were unable to develop into larvae even when fertilized with wild type sperms. The development of the eggs was stopped at various stages from invergination stage to moving stage. In the most of the dead embryos, the abnormalities were observed in the anterior region of embryo.

When small amouts (1–1.5% of egg volume) of the wild type egg cytoplasm were injected into the eggs at cllavage stage laid by homozygous females, 1–2% of the treated embryos could develop into larvae. In the control experiment where the eggs were injected wih the mutant cytoplasm, no larvae were obtained. These results suggest that the fs(1) MY-18 gene affects the production of the specific component of the egg cytoplasm which is essential for the early development of the embryos. When the cytoplasm of the wild type eggs was kept at −20°C for 5 days, the rescue activity was unchanged, showing that the component is considerably stable. The biochemical analyses of the cytoplasmic component are in progress.

IV. DEVELOPMENTAL AND SOMATIC CELL GENETICS

Growth Rate and Cell Cycle Lengths of Hydra

Jun TAKANO[1], Toshitaka FUJISAWA and Tsutomu SUGIYAMA

The epithelial and interstitial cell cycle lengths of 4 strains of *Hydra magnipapillata* were determined by continuous labelling of the cells with tritiated thymidine followed by autoradiography.

It was found that the interstitial cell cycle lengths were nearly constant among the 4 strains examined (about 1 day), although these strains had greatly different growth rates (ranging from the doubling time of 3.1 to 9.3 days). This suggests that hydra may lack mechanism to regulate the rate of interstitial cell proliferation (see Bode, Flick and Smith, 1976).

The epithelial cell cycle length was found to be nearly equal to the doubling time of hydra in 3 out of the 4 strains examined (as previously found in *H. attenuata* by David and Campbell (1972). In one strain (L4), however, the epithelial cell cycle (4.5 days) was much shorter than the doubling time of animals (9.3 days). This appears to suggest that the growth rate of normal hydra depends on the epithelial cell proliferation rate (or *vice versa*), and that the mechanism involved for this is defective in L4.

A Mutant Hydra Strain (sf-1) Containing Temperature Sensitive Interstitial Cells

Beverly A. MARCUM[2], Toshitaka FUJISAWA and Tsutomu SUGIYAMA

Temperature sensitive interstitial cells have been observed in a sexually inbred mutant strain of *Hydra magnipapillata*. This strain, sf-1 (selffeeder-1), contains normal cell numbers when cultured at 18°C. Within 24 hours after increasing the temperature to 23°C, over 99% of the interstitial cells are eliminated. Nevre cells, nematoblasts, nematocytes, and gland cells decrease more gradually, and after one week at 23°C the entire population loses the ability to capture and ingest prey. Cell counts of macerated non-

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feeders (nf-1) indicate that the polyps consist almost entirely of epitheliomuscular cells and digestive cells, with a very small number of gland cells. Chimeric hydra consisting of wild type epithelial cells and sf-1 mutant interstitial cells are also sensitive to the increase in temperature; however, the reciprocal combination of sf-1 mutant epithelial cells and wild type interstitial cells is not temperature sensitie. No wild type strain of *H. magnipapillata* shows this sensitivity to an increase in temperature. Electon microscopic examination of sf-1 tissue indicates that interstitial cells undergo extensive deterioration coincident with the increase to 23°C and that their elimination is not due to phagocytosis by other cell types.

Comparison of Effective Lethal Phases of Embryos from Three Complementation Groups of the *rudimentary* of *Drosophila melanogaster*

Yukiaki KURODA

The *rudimentary* (*r*; 1–54.5) has some complementation groups for a wing abnormality and pyrimidine auxotrophy. In the previous report, it was found that cells obtained from r^{39k} embryos showed a characteristic defect in the maturation of epithelial cells in culture, and that some intermediates in the pyrimidine biosynthetic pathway were effective in repairing the r^{39k} embryoinc cell defect.

In the present work, the effective lethal phases were compared for three complementation groups of the *rudimentary* locus, r^9 ($=r^{39k}$), r^{38} ($=r^{56j}$) and r^2 ($=r^{X917}$). Eggs obtained from matings of *r*/*r* females with *r*/Y males were dechorionated and their effective lethal phases were determined by examining the arrested stage of embryos in saline solution.

Among 1,076 embryos from the r^{38} strain, 3.1% died before gastrulation, 47.0% died by abnormal gastrulation, 29.9% died after head and trunk segmentation, 6.7% died after sac-like midgut segmentation, 10.1% died after entrance of air into trachea, 1.8% died after the stage of active movement and 1.4% died immediately after hatching. This indicates that about half of r^{38} embryos died in early phases of embryoinic development.

Embryos obtained from the r^2 strain also showed a similar pattern of the effective lethal phase to that of the r^{38} strain. Among 2,271 r^2 embryos, 58.2% died by abnormal gastrulation. The early lethal phase of these rwo strains of the *r* complementation groups was clearly contrasted with that

of the r^9 strain, in which only 9.3% died by abnormal gastrulation and 40.7% died after entrance of air into trachea.

Embryonic cells from the r^{38} and r^2 strains were cultured to examine their defective cell types. These cells, however, were difficult to be maintained in culture, because their membrane was fragile. This may account for the early death and abnormal gastrulation of embryos in these strains.

Effect of Conditioned Medium on Chondrogenesis of Quail Limb-Bud Mesenchymal Cells in Culture

Yukiaki KURODA and Etsuya MATSUTANI

Mesenchymal cells from the limb-buds of quail embryos can differentiate into cartilage cells in culture. The degree of chondrogenesis depends on the initial number of cells inoculated on the petri dishes. The reason why the chondrogenesis is affected by the cell density may be considered as follows; cells inoculated with high cell density have more mutual cell contacts with each other which enhance the expression of chondrogenesis, and/or the chondrogenesis *in vitro* is stimulated by some factor(s) accumulated in the culture medium during cultivation of cells. In the present experiment the effect of conditioned medium (CM) on the chondrogenesis of mesenchymal cells was examined.

The mesenchymal cells were obtained from the hind limb-buds of quail embryos at stages 22–23. After removal of epithelial layer mesenchymal cells were dissociated into single cells. The CM was obtained by cultivation of 2.5×10^6 cells in 60-mm petri dishes for 2 days at 37°C and stored at −20°C. To assay the activity of CM, 10^6 cells were cultured in 2 ml of medium containing 1.5 ml of CM in 35-mm petri dishes. The cell layer in petri dishes was fixed and stained with toluidine blue. The number of cartilage nodules formed was scored and used as an index of chondrogenesis.

The original CM had no significant effect on chondrogenesis of mesenchymal cells. When the CM was dialyzed against culture medium to compensate for loss of nutrient, it stimulated slightly the chondrogenesis of cells. Next, the CM was condensed to a quater of its original volume by use of a collodion bag, dialyzed against culture medium, and its activity was tested. It was found that a high moleculr weight fraction of CM had a marked effect on chondrogenesis of cells. This culture method needs a relatively much amount of cells and CM. Therefore, a micro-drop culture

method was developed to test the activity of CM. Inocula of 4×10^4 cells were incubated in 10 μl drop of medium containing 7.5 μl of CM in 35-mm petri dishes. Then 2 ml of culture medium were added to the petri dishes after incubation for 2, 4 and 24 hours in micro-drops. By using this culture method, the length of culture time required for manifestation of the CM activity on chondrogenesis was examined. On the fourth day of culture, the number of cartilage nodules was scored. Results indicated that an expression of chondrogenesis was almost completely established even when cells were incubated in CM for only first 2 hours. For details, see *Cell Structure and Function* **3**: 237–248, 1978.

Chondrogenesis of Quail Limb-Bud Mesenchymal Cells in Clonal Culture

Etsuya MATSUTANI and Yukiaki KURODA

The *in vitro* chondrogenesis of mesenchymal cells depends on an initial cell density in culture. It suggests that mesenchymal cells may require their mutual cell contacts as well as some factors released by conditioning the medium. In the present experiment the effect of cell contacts on chondrogenesis was examined by clonal cultures of mesenchymal cells.

Mesenchymal cells were dissociated from the limb-buds of quail embryos at stages 20–21. In the primary clonal cultures, distinct fibroblastic cell colonies were formed, but differentiated cartilage colonies were not found. To obtain the cartilage cell colonies from mesenchymal cells, cells were pre-incubated in the following two ways; 1) Cells were primarily cultured in monolayer, then cells were dissociated again and secondarily cultured in clonal cultures. 2) Cells were incubated in the gyratory shakers to allow the cell contacts in aggregates, then cells were dissociated again and secondarily cultured in clonal cultures.

Cell suspensions containing 10^6 cells were incubated for various lengths of time (4, 8, 12, 16, 24 and 48 hours) in 2 ml of medium in monolayer, or in 3 ml of medium in rotation. In the secondary clonal cultures, various numbers of cells (5×10^3, 2.5×10^3 and 10^3 in 35-mm petri dishes) were cultivated for 2 weeks and then fixed and stained.

Cartilage colonies were found when mesenchymal cells were cultured primarily in rotation, whereas no cartilage colonies were formed in cells primarily cultured in monolayer. The number of cartilage colonies increased

as the pre-incubation time in rotation culture increased. A maximum number of cartilage colonies was obtained when cells were pre-incubated for 24 hours in rotation. The number of cartilage colonies decreased when cells were pre-incubated for 48 hours. Limb-bud mesenchymal cells dissociated from embryos at stages 20–21 fail in the expression of differentiated characters in primary clonal cultures. They, however, can express their potency of chondrogenesis in clonal cultures if they are pre-incubated in their mutual cell contacts for a relatively short period. In embryos at stages 24–25, the condensation of mesenchymal cells proceeds at the center region of the limb-buds, and leads to the chondrogenesis. This suggests that the cellular contacts in mesenchymal cells may be an important step in the chondrogenesis in the limb-buds.

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

Yukiaki KURODA

Sterigmatocystine (STC) is a strong mutagenic mycotoxin which induces chromosome aberrations, DNA strand breaks and 8-azaguanine (8AG) resistant mutations in mouse mammary carcinoma FM3A cells. In the present experiment, the activity of sterigmatocystine to induce 8AG resistant mutations in cultured embryonic human diploid cells was examined.

STC reduced the colony-forming activity of human diploid cells when cells were treated with STC for 4 hours at concentrations of 0.01 to 1.0 μg/ml. The D_0 value calculated from a concentration-survival curve was 0.63 μg/ml.

Cells were treated with STC at various concentrations for 4 hours, cultured in normal medium for 6 days of mutation expression time, treated with trypsin and replated on fresh dishes at a cell density of 5×10^4 cells/dish. After incubation of cells in the presence of 30 μg/ml 8AG for 14 days, the number of 8AG-resistant mutant colonies was scored.

The frequency of 8AG-resistant mutations increased slightly when cells were treated with STC at concentrations of 0.01 and 0.03 μg/ml. At a concentration of 0.1 μg/ml the induced mutation frequency was 10.2 per 10^5 survivors. At higher concentrations more than 0.3 μg/ml of STC, mutant colonies were hardly detected bacause of severe cytotoxicity of STC. This mutagenic activity of STC on human diploid cells is almost similar to

that in mouse FM3A cells previously reported.

Mutagenic Activity of Tryptophan Pyrolysis Products on Embryonic Human Diploid Cells in Culture

Yukiaki KURODA

It has been recently found that typtophan pyrolysis products formed during cooking of foods were strong frame-shift mutagens for *Salmonella typhimurium* with a metabolic activation. These products also induced *in vitro* transformation in embryonic hamster cells. In the present experiment, the activity of tryptophan pyrolisis products, Trp-P-1 and Trp-P-2, to induce 8-azaguanine (8AG) resistant mutations in cultured embryonic human diploid cells was investigated.

Trp-P-1 had a concentration-dependent and severe cytotoxic effect on the human cells. The colony-forming activity of cells decreased as the concentrations of Trp-P-1 increased. The D_0 value calculated from concentration-survival curves for 4 hour- and 15 day-treatments with Trp-P-1 were 0.53 μg/ml and 0.35 μg/ml. Trp-P-2 had a weaker cytotoxic effect than Trp-P-1 on human diploid cells. The D_0 value of Trp-P-2 was calculated to be 3.0 μg/ml.

Cells were treated with Trp-P-1 or Trp-P-2 at various concentrations for 4 hours, cultured in normal medium for 7 to 9 days of mutation expression time, and treated with trypsin solution. Replated cells were selected with 30 μg/ml of 8AG for 14 days, The frequency of 8AG-resistant mutations increased significantly compared with that in untreated control cultures. The induced mutation frequency in cells treated with Trp-P-1 at a concentration of 0.3 μg/ml was 7.0 per 10^5 survivors, whereas that in cells treated with Trp-P-2 at a concentration of 1.0 μg/ml was 2.8 per 10^5 survivors.

These results indicate that tryptophan pyrolysis products had severe cytotoxic effects on hyman diploid cells than AF-2 and phloxine tested in the previous experiments, but the formers were weaker mutagenic for induction of 8AG-resistant mutations than the latters at concentrations giving about fifty percent of surviving fraction.

Genetic Study on Highly Teratocarcinogenic Charactor of 129/ter-Sv Mice

Takehiko NOGUCHI

An inbred subline of mice, 129/ter-Sv, has highest incidence of testicular teratomas (33%) out of 129 sublines. But the incidences observed in siblings vary from mating to mating. The range of variation was from about 80% to less than 10%. If siblings with a low incidence of teratomas were mated, the teratoma incidences of their offsprings nearly always remained low (personal communication with L. C. Stevens and D. S. Varnum). Teratoma incidence of this inbred mice decreased from generation to generation unless selection was made. Another enigmatic point in the teratocarcinogenic charactor of this subline is that incidence of bilateral teratomas is abnormally high. In 129 sublines left testes have two times as high incidence as the right testes. The frequency of bilateral teratomas should be the product of the frequencies of unilateral teratomas if teratocarcinogenesis in the right testes occur independently from that in the left. Theoretically calculated incidence of bilateral teratomas was 0.9%. But the incidence really observed was as high as that of the left testes (L. C. Stevens, *J. Natl. Cancer Inst.* **50**: 235–242, 1973).

Analytical studies on proliferation of primordial germ cells (PGC) of the developing testes of 129/ter-Sv revealed that 129/ter-Sv embryos were heterogenous in regard to the pattern of proliferation and the number of PGC. The embryonic testes of 129/ter-Sv could be divided into three groups. In the testes of group (1), PGC number and their proliferative pattern were normal as compared with the testes of other 129 sublines with much lower incidences of teratomas. In the group (2) testes PGC were present at normal level, but mitotically active stage was prolonged to a little, but a significant extent. The testes of group (3) looked very abnormal; PGC number was reduced extremely and the mitotically active stage was extended considerably. Four out of 44 testes of group (1), 14 out of 22 testes of goup (2) and 9 out of 10 testes of group (3) had teratomas respectively. Group (2) testes appeared in most cases bilaterally. Group (3) testes appeared always bilaterally. These facts explain well the enigma that incidence of bilateral teratomas is very high in this inbred mice.

Bilateral teratomas nearly always result in sterility. If the charactors of groups (2) and (3) are controlled by a genetic factor, mice having the factor

will be removed from the colony due to their high tendency of sterility unless they are retained by selection. This hypothesis coinsides with the regression phenomenon observed in the highly teratocarcinogenic charactor of this sublines.

Even in a litter of embryos, those having groups (1), (2) or (3) sometimes coexisted. The hypothetical genetic factor would, therefore, be a recessive mutation rather than a dominant one. This recessive mutation was tentatively named *ter*. Group (1) (2), and (3) were supposed to be ascribed to genotypes +/+, +/*ter*, *ter*/*ter* respectively. According to this assumption 129/ter-Sv mice could have three different kinds of genotypes. Matings between mice with these genotypes would result in several kinds of combination of the genes. This explains well the large variation in the incidences observed among siblings.

A multipotential Teratocacinoma (STT-2) derived from a Male of 129/ter-Sv Strain

Takehiko NOGUCHI and Yukika WADA

A multipotential teratocarcinoma having near 100% retransplantability was established. This carcinoma named STT-2 was derived from a spontaneous testicular teratocarcinoma found in a male of 129/ter-Sv inbred strain, and has been kept for more than 1 year by subcutaneous or intraperitoneal transplantations. This is the first transplantable teratoma obtained from this inbred strain. Tissues or cell types hitherto identified in STT-2 solid tumors were embryonal carcinoma cells, primitive endoderm cells, embryonic mesodermal and ectodermal tissues, many kinds of epithelia, pigment cells, nervous tissues, striated muscles, adipose tissues, cartilarges, bones with marrows, pancreatic cells. This means that STT-2 is a typical multipotential teratocarcinoma.

In order to get embryoid bodies of this tumor, solid STT-2 was minced and injected intraperitoneally in syngeneic hosts. Tumor tissues induced accumulation of very bloody ascitic fluid. The fluid usually contained relatively a small number of floating bodies (at levels of two figures). The sizes of those floating bodies sometimes reached to several milimeters in diameter. These were identified as cystic form embryoid bodies. They contained a variey of differentiated tissues as well as embryonal carcinoma cells. Tissues or cell types hitherto identified in those embryoid bodies

were proximal endoderm layers which were usually forming the outer layer of the bodies, embryonal carcinoma cells, trophoblastic giant cells, blood islands, primitive mesodermal and ectodermal tissues, cardiac muscles, and so on.

Solid tumors formed at subcutaneous sites usually grew very large. Necrotic parts were relatively small when compared to OTT6050 solid tumors. STT-2 may, therefore, be useful as a source of embryonic substances.

Lethal Hybrid Rescue Gene of *Drosophila simulans*

Takao K. WATANABE

The unisexuality in the hybrid progeny between *Drosophila melanogaster* and *D. simulans* has been known since the discovery of the latter species (Sturtevant, *Genetics* **5**: 488 1920). The hybrid offspring between *melanogaster* females and *simulans* males were females and those in the reciprocal cross were males. In the case of the cross between attached-X *melanogaster* females and *simulans* males the offspring were only males. These abnormal sex-ratios in hybrids are due to selective lethalities exerted during larval stages. According to Sturtevant the interspecific hybrids develop to the adults only if they carry a *simulans* X when the cytoplasm is of *melanogaster*.

A gene, Lethal hybrid rescue (*Lhr* 2–95), which rescues the lethal hybrids between the above species was found on a second chromosome of *D. simulans* from a natural (Kokura) population. The cross, *melanogaster* ♀ × *simulans* ♂, which normally produced only female progeny, resulted in the production of both females and males at an equal frequency when the *simulans* males carried *Lhr*. The reciprocal cross, *simulans* ♀ × *melanogaster* ♂, which ordinarily produces only males, gave rise to about 14% females and 86% males. The cross, $\overline{XX}$/Y *melanogaster* ♀ × *simulans* ♂ carrying *Lhr*, produced a few percent females, although normal *simulans* produced only hybrid males.

The cytoplasmic sex ratio (*SR*) factor and daughterless (*da*) gene killed either hybrid males or hybrid females according to their usual actions, showing no specific interactions with the *Lhr* gene. All hybrids, those rescued by *Lhr* as well as those produced normally, were completely sterile.

V. CYTOGENETICS

The Third Scientific Expedition for the Study of Rodents to Islands and Coastal Area of the Indian Ocean, I. Members, Aims, Schedule and Number of Animals Collected in the Expedition

Tosihide H. YOSIDA

The first and the second scientific expeditions with the financial aid of the Ministry of education, science and culture, Japan, to survey the rodents in Southwest and Central Asia and Oceania were orgainzed in 1968 and 1972. This is the third expedition of the same series with the following aims.

(1) *Members*:

Tosihide H. Yosida (Leader)	
Kazuo Moriwaki	National Institute of Genetics
Hatao Kato	,,
Kimiyuki Tsuchiya	Hokkaido Institute of Public Health

(2) *Aims and route of the expedition*:

In the previous survey, we found that the black rats showed three geographical variants due to the difference of the number and the shape of chromosomes; Asian type ($2n=42$), Oceanian ($2n=38$) and Ceylonese types ($2n=40$). The Asian type distributed in East and Southeast Asia and the northern India and Pakistan, while Oceanian type distributed widely in the world except the above Asian district. The Ceylonese type was only found in Kandy, Sri Lanka, in our second expedition. The main purpose of the third expedition is to know whether the Ceylonese type black rats were distributed widely in Sri Lanka and also to find out which type of black rats, Asian, Oceanian and Ceylonese types, distributes in islands, and coastal area of the Indian Ocean. The other purpose of the expedition is to elucidate the relationship between chromosomal changes and speciation in rodents and other small mammals and to breed them in laboratory, if it is possible, to estabilish new experimental naimals. For the above purpose the expedition was planned to the following areas from November lst to December 14th, 1978; Penang (Malaysia), Sri Lanka, Seychelles, Kenya (Nairobi), Mauritius, and Europe.

(3) *The number of animals collected in the third expedition*:

Species names and the number of animals collected in the third expedition are listed in Table 1.

Table 1. Animal species and number of speciemens collected in the third expedition

Species Rodents	Penang	Sri Lanka	Seychelles	Mauritius	Kanya	Europe	Total
Rattus rattus	10	76	19	20	10		135
R. norvegicus	24	10		4			38
Bandicota	5	3					8
Mus musculus		9	5	17		1	32
M. leggada		13					13
M. minutoides						1	1
Apodemus						14	14
Mastomys					1		1
Clethryonomis						2	2
Insectivora							
Suncus murinus	1	7		2			10
Tenrec			3				3
Primates							
Tupia glis	2						2
Total	42	118	27	43	12	17	259

The Third Scientific Expedition for the Study of Rodents to Islands and Coastal Area of the Indian Ocean, II. Distribution of Ceylonese Type Black Rats

Tosihide H. YOSIDA, Kazuo MORIWAKI, Kimiyuki TSUCHIYA*, Hatao KATO, M. SABARATNAM**, K. D. ARULPRAGASAM** and H. E. FERNANDO***

In our second expedition during 1972, the Ceylonese type was obtained only in Kandy, located in the Central highland of Sri Lanka (This report **23**: 43, 1973). Although the Ceylonese type black rats were obtained in this place, we did not know whether this type of black rats is distributed in the other parts of Sri Landa. In order to know this problem the black rats were collected from the following 9 localities in Sri Lanka during

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Table 1. Karyotypes of black rats Collected in Sri Lanka in the third expedition

Localities	2n 38	39	40	Total	(♀:♂)
Central highland					
Kandy*			15	15	(8:7)
Peradeniya				5	(3:2)
Nuwara Eliya			7	7	(4:3)
Central lowland					
Anuradhapura		4	2	6	(4:2)
Coastal area					
Puttalam	3			3	(1:2)
Colombo	11	2	2	15	(7:8)
Hambantota	10			10	(4:6)
Galle	7			7	(4:3)
Trincomalee	10		1	11	(5:6)
Mannar	11			11	(3:8)
Total	52	6	32	90	(43:47)
(%)	(57.8)	(6.6)	(35.5)		(99.9)

* Collected in 1972.

the third expedition (Table 1); Peradeniya and Nuwara Eliya located in the central highland, Anuradhapura in central lowland, and Mannar, Puttalam, Colombo, Galle, Hambantota and Trincomalee in the surrounding coastal area. A total of 90 black rats was collected in 10 localities including Kandy in 1972. Among them 52 (57.8%) were of Oceaninan type, 34 (27.8%) were Ceylonese type and the remaining 4 (4.4%) were hybrids between the above 2 types. The hybrids had 39 chromosomes just as seen in the F_1 in the laboratory mating.

Interesting finding is that all 27 rats collected from the central highland (Kandy, Peradeniya and Nuwara Eliya) showed only Ceylonese type. In Anuradhapura which is located in the central lowland, however, Ceylonese and hybrid types were found. On the other hand, in the coastal area 57 black rats were collected and among them 52 (91.2%) were of Oceanian type and the other 5 were of Ceylonese (3) and hybrid types(2). The black rats collected in Mannar, Puttalam, Hambantota and Galle located in the northern and southern coast were of only Oceanian type, but the Ceylonese and hybrid types were found in Colombo and Trincomalee located in the west and east side of the island. Finding of these rats in these areas could be

due to the migration from the central highland by some communication.

On the origin of the Ceylonese type black rats we (Yosida *et al.*, *Chromosoma* **45**: 99, 1974) suggested that they could have occurred in southern India from Asian type by the first Robertsonian fusion. From this type the second Robertsonian fusion had been taken, and then the Oceaninan type black rats developed. The latter type with more stronger competitive ability should have expelled the former rats from India to Sri Lanka. By the present survey the Ceylonese type seems to have been driven to the central highland of the island by Oceanian type. Based on these survey it can be said that the Ceylonese type rats are distributed only in the central highland in Sri Lanka and they are isolated just as surrounded by the Oceanian type rats.

The Third Secientific Expedition for the Study of Rodents to Islands and Coastal Area of the Indian Ocean, III. Finding of Mauritius Black Rats with Extra Small Acrocentrics due to Robertsonian Fission

Tosihide H. YOSIDA, Kazuo MORIWAKI, Hatao KATO, Kimiyuki TSUCHIYA, Yuriko OCHIAI and J. MONTY

Depending on chromosome numbers, the black rats, *Rattus rattus*, are classified into three geographical types, such as Asia (42 chromosomes), Ceylon (40 chromosomes) and Oceania (38 chromosomes) (Yoside *et al.*, 1971, 1974). The Asian type black rats are distributed in East and Southeast Asia and northern part of Southwest Asia. The Ceylonese type is found only in Sri Lanka, but the Oceanian type is distributed widely throughout southern part of Southwest Asia, Central Asia, Europe, Africa, Oceania, North and South America. Recently we had an opportunity to observe the chromosomes of black rats inhabiting in Mauritius Island in the Indian Ocean in the third expedition and found that the karyotypes of these animals were markedly different from the other three geographical types described above. All seventeen black rats collected there were characterized by having many extra small acrocentric autosomes. Their basic karyotypes were of Oceania type, because of the presence of the large metacentric M_1 and M_2 pairs, but chromosome numbers in 13 specimens among them were 42, those of 3 specimens 43, and those of the remaining one specimen 44. Although the Oceanian type rat had 2 small acrocentric autosomes

(pair no. 13), 16 Mauritius rats had 10 small acrocentrics, and the remaining one had 8 samll acrocentrics. Comparative karyotype analysis between Oceanian and Mauritius type rats by G-banding staining showed that the extra small acrocentrics found in Mauritius rats occurred due to Robertsonian fission of small metacentric pairs no. 14 and 18 of the original Oceanian type rat. Only one rat with 8 small acrocentrics showed the heteromorphic pair no. 18 consisting of one metacentric and two acrocentrics. The large metacentric M_1 chromosome in 13 of 17 rats examined showed homologous pair, but two of them were heteromophic by involving one metacentric M_1 and two acrocentrics. In the remaining two rats M_1 chromosome was not observed, but acrocentric pairs no. 4 and 7 were included. These acrocentrics were also suggested to be originated from Robertsonian fission of the large metacentric M_1 chromosome. Robertsonian fission seemed to be one of the important mechanism in karyotype evoluton in this animal.

The Third Scientific Expedition for the Study of Rodents to Islands and Coastal Area of the Indian Ocean, IV. Karyotype of Black Rats Collected in Penang (Malaysia), Seychelles, and Nairobi (Kanya)

Tosihide H. YOSIDA, Hatao KATO, Kimiyuki TSUCHIYA, Kazuo MORIWAKI, W. T. MENG*, S. K. CHARLES*, G. GILL** and Hiroyuki HIRUMI***

To know the karyotype the black rats in Penang, Seychelles and Nairobi were examined in our third expedition. In Peanng island (Malaysia) located in the Malacca Straits, 10 rats were collected. All of them were the Asian type with $2n=42$, and they were identified to be Malyan black rats, *Rattus rattus diarddi*, from their external feature. As found in Malayan black rats by us (Yosida *et al.*, *Chromosoma* **33**: 252, 1971) the chromosome polymorphism in pairs no. 1 and 9 were observed in these rats. They were shown in Table 1.

The 19 black rats collected from Seychelles in Indian Ocean showed always a typical Oceanian type with $2n=38$. The black rats in this island were similar to *R. rattus refescens* in India. The Oceanian type occurred in India might have been migrated to this island. In Nairobi, Kenya, 10

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Table. 1. Frequencies of acrocentric and subtelocentric polymorphism in pairs no. 1 and 9 of black rats collected in Pennag, Malaysia

Pairs no.	Chromosome types			Total no. of rats observed
	A/A	A/S	S/S	
1	2	3	3	8
9	1	5	2	

black rats were collected and chromosomes were observed. All these rats were also characterized by the Oceanina type. The karyotype of black rats in South Africa and Somalialnd has been observed to be Oceanian type by Capanna and Civitelli (*Boll. Zool.* **38**: 151, 1971). From these studies distribution of black rats in the coastal area of Indian Ocean in Africa seems to be Oceanian type.

The Third Scientific Expedition for the Study of Rodents to Islands and Coastal Area of the Indian Ocean, V. Hemoglobin Types and Chromosome C-band Patterns of the Mice Collected in Sri Lanka, Seychelles Island and Mauritius Island.

Kazuo MORIWAKI, Kimiyuki TSUCHIYA*, Hatao KATO, Tosihide H. YOSIDA and Mitsuru MINEZAWA**

As an attempt to reveal the differentiation process of mouse species from the view points of biochemical- and cytogenetics, 37 wild mice collected in the present expedition were examined as to the electrophoretic patterns of beta-hemoglobin (Hbb) and the chromosome C-band patterns as well.

Ten mice obtained from Sri Lanka were taxonomically identified as *Mus*

Table 1.

Place of Collection	Name of species	Hbb phenotype					No. of mice observed
		O	OP	P	DS	S	
Sri Lanka	*Mus musculus urbanus*	5	5	1	0	0	10
	Mus leggada	(unique bands)					16
Seychelles Is.	*Mus musculus* subsp.	4	0	0	1	0	5
Mauritius Is.	*Mus musculus* subsp.	0	0	0	2	5	7

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musculus urbanus, tentatively, 16 also from Sri Lanka as *Mus leggada*, 5 from Seychelles island as *Mus musculus* subsp. and 7 Mauritius island as *Mus musculus* subsp. The latter two subspecies were some what similar to *M. m. domesticus*.

Table 1 summarizes the type of beta-hemoglobin found in those mice. Those finding likely support the recent argument assuming wide distribution of Hbb^p allele in the Asian mice (Minezawa *et al., Jap. J. Genet.* **54**: 165, 1979). Chromosome C-band patterns of those mice were mostly similar to those of the laboratory mice, *M. m. domesticus*.

A New Sporadic Robertsonian Fusion of Acrocentric Pairs no. 7 and 10 in the Black Rats from Sapporo

Tosihide H. YOSIDA

The black rats, *Rattus rattus*, have been found to have three geographical types, Asian ($2n=42$), Ceylonese ($2n=40$) and Oceanian ($2n=38$)). The Ceylonese type has been derived from the Asian type by Robertsonian fusion between pairs no. 11 and 12. The Oceanian type was remarkable by having the other Robertsonian fusion between the pairs no. 4 and 7. All about one thousand black rats, so far the chromosomes were observed by the present author in Japan, were characterized by having always 42 chromosomes with the Asian type idiogram. Recently five black rats (*R. rattus tanezumi*) were collected from Sapporo, Japan by courtesy of Dr. Kimiyuki Tsuchiya. Among them four rats were found to possess a normal complement consisting of 42 chromosomes. One female among them, however, was found to have 41 chromosomes in all 15 metaphase cells obtained from the primary cultures of the tail tissue by the author's routin procedure. Among them one large metacentric element was outstanding on account of its consistent occurrence in their metaphase complement. By applying the G- and C-banding techniques it was revealed that the metacentric element was derived from Robertsonian fusion between each one of the acrocentric pairs no. 7 and 10.

Interesting is that the Oceanian type black rat is characetrized by the Robertsonian fusion of pairs no. 4 and 7, and the pair no. 7 is also involved in the chromosome fusion in the present material. This chromosome pair seems to be liable to fuse with the other chromosome. The occurrence of a Sapporo black rat having one large metacentric element

due to the fusion will serve as a matter important for the possibility of the karyotype evolution in the geographical variants of black rats, such as Ceylonese and Oceanian types, through a mechanism of the Robertsonian fusion.

Histological Study on the Hybrid Embryos Developed by Artificial Insemination between Norway Rats and Black Rats

Choji TAYA and Tosihide H. YOSIDA

Histological examination of hybrid embryos developed by artificial insemination between female Norway rats (*Rattus norvegicus*, $2n=42$) and male Asian type black rats (*Rattus rattus flavipectus*, $2n=42$) was carried out with comparison between the hybrid and control embryos which developed by artificial insemination between female and male Norway rats. Although the delay of development of the hybrid embryo was ovserved, the endoedrm and ectoderm differentiated well on the 7th and the 9th day embryos as seen in control ones. The development of the mesoderm, however, was poor in these stages in the hybrid embryos. Until the 10th day after insemination, the hybrid embryos were clearly observed in the decidua although the development has markedly delayed. On the 13th days the embryo had degenerated completely and the rudimental embryonic cells were only observed in the decidua. Lethality of the hybrid embryos seemed to be due to the marked delay of the development and differentiation.

In vivo Sister Chromatid Exchange in Cells of Various Organs of the Mouse.

Naotoshi KANDA and Hatao KATO

In vivo sister chromatid exchange (SCE) in mouse cells derived from various organs was studied by infusing *Brd*U from the tail vein. It was found that at *Brd*U concentration ranging from 2.2–13.5μg/g/h, the SCE frequency in bone marrow cells seemed to stay at a constant level (1.5–2/cell/two cell cycles) whereas it started to rise as the *Brd*U dose exceeded this dose range. When *Brd*U within this dose range was infused continuously from the tail vein for appropriate hours to label chromosomes in various organs, the average SCE frequencies per cell were found to be 1.64 in bone marrow cells, 1.82 in spermatogonia, 1.99 in splenic cells, 2.89 in intestinal

cells and 3.69 in cells from adjuvant stimulated lymph nodes. It was suggested that the spontaneous level of the *in vivo* SCE frequency might be about 1.5–2/cell/two cell cycles in the mouse. In cells derived from intestine and adjuvant stimulated lymph node, some unknown factors might work as a inducer of SCEs resulting in a signifiiant increase in the SCE frequency in these organs.

Analysis of Crossing Over in the Mouse Meiotic Cells by BrdU Labelling Technique.

Naotoshi KANDA and Hatao KATO

Sister chromatid differential staining of male mouse meiotic cells was achieved by the continuous *Brd*U infusion from the tail vein and following fluorescent plus Giemsa staining technique, Analysis of 296 bivalents including XY, and 21 univalents reveals that; 1) The visible crossing over coincided exactly with the chiasmata. 2) No evidence was obtained in support of the chiasma terminalization. 3) Anormalous type of crossing over was found in the monochiasmatic bivalent. 4) Some of the terminal associated bivalents might be achiasmatic, and univalents might originated from such bivalents. 5) Sister chromatid association of XY bivalents may be under genetic control, for the chromatid association of 25 XY bivalents were all between lightly and heavily stained chromatids. 6) The chromosomal site, at whcih crossing over occurred, were unstained, suggesting that this portion may differ structurally from the rest. 7) Sister chromatid exchanges might be rare event during meiosis.

Comparison of Karyotypes between the Japanese Shrew Mole and the Furry Snouted Shrew Mole

Takashi HAMADA and Tosihide H. YOSIDA

Karyotypes of the Japanese sherw mole (*Urotrichus talpoides*) and the furry snouted shrew mole (*Dymecodon polirostris*) belonging to Insectivora were analysed and their evolutional relationship was considered. Both species are similar in external feature, but the latter is slightly smaller than the former and their dental form is different. Chromosome numbers of these species are already reported by Tuschiya and Yosida (This report **21**: 54, 1971), but detail of karyotype is not yet recorded.

Material used in the present study were collected in the foot of Mt. Fuji, Shizuoka. The chromosomes were observed from cultured tail cells by our routine technique. Chromosome number in both species was $2n=34$ and their idiograms were the same as the above report. They are consisted of 11 metacentric pairs (nos. 1 to 11), 2 submetacentirc pairs (nos. 12 to 13) and 3 subtelocentric pairs (nos. 14 to 16), and a large metacentric X and a small minute Y. Although the species had similar karyotype, subtelocentric pair no. 14 is different between them. In the furry snouted shrew mole the length of the longer arm in this pair was longer than that in Japanese shrew mole, and therefore the arm index in both species is very different; in the former species it was 4.5 but in the latter 1.7. Another difference was found in submetacentric pair no. 12. In Japanese shrew mole the short arm of the pair is longer than the furry snouted shrew mole. In the former this chromosome looks metacentric. All other chromosomes in both species are very similar. According to taxonomists the furry snouted shrew mole is more primitive than the Japanese shrew mole. Based on our observations and the taxonomists suggestion, the Japanese shrew mole is suggested to be derived from the furry snouted shrew mole by the following cytogenetical mechanism; the breakage of long arm of pair no. 12 in the furry snouted shrew mole had occurred and the broken end was translocated to the short arm of the pairs no. 14, and then the karyotype of the Japanese shrew mole was established.

Karyotypes of Four Filefishes

Makoto MUROFUSHI* and Tosihide H. YOSIDA

Karyological studies in four species of filefishes belonging to Balistidae (*Stephenolepis cirrhifer, S. japonicus, Paramonacanthus oblongus,* and *Navodon modestus*) were carried out. All these fishes were obtained between June and September, 1978, off the west cosat of Izu Peninsula, Japan, and the chromosomes were observed directly from the kidney or the gill tissues. Diploid chromosome numbers in *Stephenolepis cirrihifer, S. japonicus* and *Paramonacanthus oblongus* were 34, but in *Navodon modestus* it was 40. All chromosomes in these four species were acrocentrics ranging from large to small size. The later one species is identified from the others by different chromosome numbers, but the former three are difficult to be identified by

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the karyotype from each other. These three species, however, were discriminated by the secondary constrictioin in one chromosome pair, which was found in pair no. 9 in the case of *Stephanolepis cirrhifer,* pair no, 6 in *S. japonicus* and pair no. 2 in *Paramonacanthus oblongus.*

Differential Staining of Inactive X Chromosome in 25 Rodent Species

Naotoshi KANDA and Tosihide H. YOSIDA

Twenty-five rodent species, mainly obtained from breeding colonies at the National Institute of Genetics and some were captured in the wild, were used in the present studies. Bone marrow cells and/or cultured cells derived from the tail tip and lung tissues were treated with a 50°C hypotonic solution and stained with Giemsa. With this technique the facultative heterochromatic X chromosome or the facultative portion of large, composite type X chromosomes is stained darker than the other chromosomes, allowing it distinguished from the homologus euchromatic X chromosme in female metaphase cells. Intense staining of the single X chromosome was not observed in male metaphase cells. It is suggested that this differential staining of one of the two X chromosomes might be due to qualitative differences in chromosomal proteins rather than to difference in the degree of chromosomal condensatin or in DNA base sequence.

Comparative Analysis of Japanese Wood Mice from Oki Islands and Mainland from View Points of Biochemical- and Cytogenetics

Hirohisa HIRAI,[1] Teru Aki UCHIDA[2] and Kazuo MORIWAKI

Oki Islands are located about 100 km far from Simane Peninsula, consisting of Dozen and Dogo. Aoki (*Zool. Soc. Tokyo*:31–40, 1915) and Tokuda (*Bio-geog. Soc. Japan* **4**:79 and 127, 1941) termed Japanese wood mice of Mainland as *Apodemus speciosus speciosus* and those of Oki Is. as *A. s. navigator* by their morphological features. Thereafter, Hiraiwa *et al.* (*Sci. Bull. Fac. Agr. Kyushu Univ.* **16**:547, 1958) and Miyao *et al.* (*Mamma. Sci.* **16**:37, 1968) claimed that the animal from Dozen could be distinguished from *A. s. navigator* of Dogo at the level of subspecies, com-

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paring either the tail ratios and the dead centre of their skull specimens. On the other hand, Imaizumi (*Aniaml-life* **1**:79, 1971) denominated the animal from Oki Is. as a new species, *A. navigator navigator.*

We examined those two problems by estimating the genetic distance and by the comparison of chromosome C-band patterns.

Fifty three individuals were collected rom two localities in Oki Is. (Dozen and Dogo) and three in Mainland (Daisen, Tottri and Toyama). Electrophoresis of plasma, hemoysate and liver extract by using thin layer acrylamide gel (Hirai *et al.*, This peport **28**:36, 1978) and cellulose acetate membrane was achieved in order to estimate the gene freuqencies of the following 15 loci, Alb, α_1MG, α_2P, Es-1, Es-2, Es-3, Es-4, Es-10, Gpi, Hb, Id-1, Mod-1, Pgm, Pre-Alb, and Tf. Genetic distance was calculated using those data by Nei's formula (*Am. Nat.* **106**:283, 1972). C-banding patterns in chromosome were observed by BSG method (Sumner, *Exptl. Cell. Res.* **75**:304, 1972).

Genetic distance in the mice between Dozen and Dogo was 0.026 and that between Oki Is. and Mainland, 0.064. Taking the data in rodents by Nei (Molecular Population Genetics and Evoultion, 175–209, 1975) and Britton and Thaler (*Biochem. Genet.* **16**:213, 1978) into consideration, we assumed that genetic distance between Dozen and Dogo was involved in local race and that between Oki Is. and Mainland was at subspecies level, although it might not be concluded based only on genetic distance.

No reports have ever referred of the C-banding patterns in chromosomes of Japanese wood mice hitherto. In the present study, we failed to show any significant difference in the patterns among the mice of various geographic origins.

An Attempt on the Cytological Identification of Chromosome in Some Linkage Groups of the Silkworm

Akio MURAKAMI, Akio OHNUMA and Hirotami IMAI

In the previous report [This report **28**: 68, 1977], it was communicated the result of cytological identificaion of the Z-chromosome in the silkworm with the hypotriploid (3A+ZZ) male and indicated that the Z-chromosome is one of the member of the large chromosome group or the 3rd longer. This method is applicable to other linkage group chromosomes, but it takes time to construct such the hypotriploid individuals for the specific linkage

group chromosome. Whereas, in this insect, it is fairly easy to make the translocated chromosome marked with a small piece of the chromosome in a specific linkage group. Accordingly, it is expected that, with the use of the translocated chromosomes and the highly improved chromosome observation technique (*e.g.*, Imai *et al.*, 1977), the specific chromosome would be possible to cytologically identify and correspond to the linkage group. The present communication will report a result of preliminary chromosome observations to cytologically confirm the genetically well analyed translocated or fused chromosome complexes.

In this experiment, T(Z:$\widehat{W^{pe+}}$), T(Z:$\widehat{W^{zebra}}$), T($\widehat{W^{zebra}}$) or known as the translocated zebea and a spontaneously fused chromosome complex, F(VI:XIV), were chosen as sample materials. The T(Z:$\widehat{W^{pe+}}$) line has a complicated chromosome complex between the Z chromosome and W chromosome with a piece of the 5th chromosome including pe^+ gene locus, the T(Z:$\widehat{W^{zebra}}$) line has a translocation between the Z and W chromosome with a piece of the 3rd chromosome including the zebra locus, the T($\widehat{W^{zebra}}$) line has a simple transolcation between the W chromosome and 3rd chromosome marked with the zebra locus. The F(VI:XIV) line is generously supplied from Dr. M. Tsujita. The translocated zebra, which had been constructed by the late Dr. H. Hashimoto (1948). is maintained in our laboratory. The others were recently constructed with the radiation-treatment method and all of them are maintained in our laboratory. The chromosome observation was subjected to meiotic spermatocytes, except for the translocated zebra. For this line, the chromosome observation was made with premeiotic larval oocytes.

In the T(Z:$\widehat{W^{pe+}}$) line, some of preparations were shown to be normal figures at the first meiotic mataphase in spermatocytes with n=28, however, in the others the complicated chromosomeal configuration wth n=27 were clearly observed. In a certain case, it was observed a minute chromosome in meiotic spermatocytes with n=28. Occasionaly, it was observed a more complicated chromosome figure at the first meiotic metaphase in spermatocytes with n=29: a minute chromosome links the chromosome complex having a part of the Z chromosome translocated with a part of W chromosome to the 5th chromosome. There were often obesrved differential chromosome configurations from preparation to preparation, suggesting that this might be resulted from sampling procedures.

In the T(Z:$\widehat{W^{zebra}}$) line, at the first meiotic metaphase in spermatocytes, a translocated chromosome configuration between the Z and W chromosome marked with a piece of the 3rd chromosome was clearly observed.

In the F(VI:XIV) line, the fused chromosome configuration between the VI and XIV chromosomes was frequently detected at metaphase and diakinesis in the first meitoic spermatocytes.

It should be noted, however, that in the translocated zebra, it was not detected the translocated chromosome configuration in the premeiotic oocytes by the air drying preparation method, indicating that chromosomes in the meiotic oocytes are not competent for cytological observations.

Mechanisms on the Sterility in Triploid Silkworms

Akio MURAKAMI, Akio OHNUMA and Hirotami IMAI

It is well known that, in the silkworm, triploid individuals are highly sterile regardless of highly viable as in other organisms. Knowledge on the mechanisms of the sterility in the insect seems to be meagre, although its description on such the phenomenon has been rather extensively reported. The present report aimes at obtaining more information from cytological and genetical viewpoints on the relationship between the sterility and triplidy in the silkworm.

The triploid silkworms used for the present experiments were obtained by the X-ray-indiced method (Murakami, 1976) for females ($3n=81A+ZZW$, where "A" means autosomes and Z or W are sex-chromosomes), and by CO_2-nondisjunction method (Murakami *et al.*, 1978) for hypotriploid males ($3n-1=81A+ZZ$).

In the hypotriploid males, twenty-seven trivalents (3A) and one bivalent (ZZ) were observed at zygoten and pacyten in spermatocytes, but one or two chromosomes began to detach from some trivalent chromosome sets at diploten and diakinesis. Consequently, a number of univalents and bivalents as well as trivalents were appeared at the first meiotic metaphase. As is well established, univalent chromosomes move to either pole by chance and the separation of trivalents was also irregular. Even chromosome segregation is quite unbalanced at the first meiotic division, the division was completed apparently without any abnormal behaviours. In contrast, the second division is remarkably delayed as compared with the normal diploid cells depending on the formation of "twin-spermatids" connected by the chromosome

bridge. This situation remained to the mid-pupal stage, whereas the normal spermiogenesis completes by the last larval instar. It is of interest to note that some of the twin-spermatids transforms finally into spermatozoa, but most of them are non-functional or apyren. The formation of the apyren might be partly due to the spermiogenesis under abnormal physiological or hormonal conditions as compared with the normal ones. It is a matter of course, the sterility in the triploid males is mainly due to the genetical unbalance in chromosomal contsitutions by the abnormal chromosomal segregation in meiosis.

In the triploid females, there were laid two different types of eggs, apparently normal shape eggs varied with their size and flaccid eggs with a small amount of yolk in a ratio of one to one. These normal eggs or oocytes are fertilized normally with haploid sperm, but development of their zygotes is arrested in embryonic stages being due to the unbalanced chromosome constitution as the abnormal chromosome segragation in meiosis. While, the flaccid eggs are unable to fertilize with haploid sperm. In this connection, it is of worthwhile to note here that, in the silkworm, the egg-formation—vitellogenesis and chorion formation—is completed prior to the commencement of the first meiotic division. Consequently, it can be imagined that the occurrence of the flaccid eggs seems to have relation to some defeat at the chromosome (or gene) level in the process of egg-formation. This defeat may be reseuted from the differential sex-chromosome pair in the triploid (ZZW): the chromosome set of a (ZW)Z pair would be able to form the normal eggs, while that of a (ZZ)W would form the flaccid eggs.

In summary, it can be said that the sterility of the troploid silkworms may be caused by the abnormal chromosome (gene) function in oogenesis or the abnormal environmental condition in spermiogenesis as well as the abnormal chromosome segregation on the both gametogenesis.

VI. MUTATION AND MUTAGENESIS IN ANIMALS

Studies on Differential Mutation Response between Bombyx and Drosophila to Some Indirectly Acting Chemical Carcinogens: (2) In vitro Metabolic Activity Test of Drosophila Microsomes for N-Dimethylnitrosamine (DMN) and N-Acetyl-2-Aminofluorene (AAF)

A. R. KASTURI BAI, Michiko, GOTO and Yataro TAZIMA

Bombyx and *Drosophila* respond differentially to some indirect carcinogens/mutagens with regard to mutation production. DMN is clearly effective in producing recessive lethal mutations in *Drosophila*, but it is not so in *Bombyx* when tested by specific loci method. In contrast, AAF is incapable of producing recessive lethal mutations in *Drosophila*, whereas positive mutagenicity is reported for *Bombyx*.

In order to elucidate the mechanism(s) underlying the discordance, we attempted to compare the metabolic activation potential of microsomes between those two insects using back mutation system of *Salmonella*.

Procedures for preparation of microsome fraction, mutagenicity assay with *Salmonella*, etc. were reported previously together with experimental results obtained for *Bombyx*. The work was extended to *Drosophila* using Oregon-R strain and same procedure. Microsome fractions were prepared from homogenates of larva, adult whole body, adult abdomen only and adult whole body that fed on 0.005% DMN for two days.

Experimental results: (1) Microsome fractions extracted from *Drosophila* were incapable, as observed for *Bombyx*, to activate DMN to substances mutagenically positive to *Salmonella* suggesting that both insects lack, or are very weak in, the capacity of activating DMN. (2) *Drosophila* microsome fractions were active in converting AAF to mutagenically positive substance to *Salmonella*, which showed also a good accord with those obtained for *Bombyx*.

Above results do not support our previous expectation that discrepancies in mutation responses between two insects could be explained by the differential metabolic capacities which are detectable by *Salmonella* back mutation system.

Since mechanism(s) concerned with mutation production in insects are different from those known for *Salmonella*, metabolic intermediates that can produce mutations in *Salmonella* might not have been effective on mutation production in insects.

Mutagenicity of Dimethylbenzanthracene in the Silkworm Germ-Cells

Akio MURAKAMI, Michiko GOTO and Yosoji FUKASE

The mutagenicity test in grem-cells of the silkworm with several heterocyclic hydrocarbons, benz(a)pyrene, benzanthracene and so on, has been carried out for several years, the results obtained so far indicated that the hydrocarbons are not mutagenic. Then, the mutagenicity test of 7,12-dimethylbenzanthracene (DMBA), one of known strong mutacarcinogens, has been done with the silkworm egg-colour specific locus mutation method. DMBA was dissolved in 0.025 ml of 0.5% carboxyl methyl cellulose (CMC) solution immediately before use. Solution of CMC was prepared in 0.85% NaCl or saline solution for the silkworm. The mutagenicity of DMBA was tested in (*C108*×*Aojuku*) F_1 hybrid female or male silkworm pupae. A single dose of 5, 10, 20 or 40 μg per capita of DMBA was injected into the ventral body-cavity of seven day-old pupae. The control pupae received 0.5% CMC solution without DMBA. The weight of the hybrid pupa was *ca.* 1 to 1.5 g for female and *ca.* 0.5 to 1.2 g for males. After emergence, treated pupae were mated with untreated marker moths homozygous for egg colour genes at *pe* and *re* on the chromosome 5. Oviposited F_1 eggs were scored on the visible phenotype with whole-body or mosaic mutations.

The result of the experiments indicated, however, that no significant increase of any type of the mutations at both loci in pupal silkworm oocytes and spermatozoa was observed. This finding and others suggest that silkworm pupae are unable to biotransform the heterocyclic hydrocarbons including DMBA into their active forms. It is very possible to expect that this defect would be cover up if the mutation test method were coupled with a metabolic activation system which allow detection of mutagenicity of such the promutagenic compounds are reported previously for the detection of mutagenicity in pupal spermatozoa of the silkworm with 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide [This report **26**: 45, 1975]. DMBA was in-

cubated with the incubation mixture including the rat-liver microsome fractions and the relevant cofactor systems at 37°C for 20 min. The resultant reaction mixture was immediately administered by injection corresponding to a dose of *ca.* 10–50 μg per capita of DMBA at the time of incubation in the body-cavity of the mid-stage female pupae. They were then mated to the marker males to check the occurrence of egg-colour specific locus mutations. The result of the experiments indicated that the incidence of mosaic mutations was almost linearly increased with the treated dose of DMBA, while no significant increase in the whole-body mutations was observed regardless of the loci tested. Frequencies of DMBA-induced mosaic mutations in oocytes were higher at the *pe* locus than *re* locus as observed with the known chemical mutagens for the silkworm.

This finding suggests that the silkworm/rat-liver microsome system—the silkworm egg-colour specific locurs mutation test method coupled with the *in vitro* rat-liver metabolic activation system—is available to the mutagenicity test for promutagenic heterocyclic hydrocarbons.

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The Mutagenic Activity of Mycotoxins in the Silkworm

Akio MURAKAMI, Toshiko OZAWA and Yosoji FUKASE

Aflatoxin B_1(AFTB_1), a metabolic product of *Aspergillus flavus* and its closely related species, iswell known to be the most potent hepatocarcinogenic substance in experimental animals and it is frequently found in a variety of agricultural products subject to spoilage by the mould. Such being the case, this mycotoxin has often been the subject of environmental mutagen studies with a various type of biological materials. Consequently, AFTB_1 is also shown to be highly mutagenic in many biological test systems. Most of the studies tend to indicated that AFTB_1 is not genetically active *per se*, but do require metabolic activation. In view of the general lack on mutagenic effects of AFTB_1 on germ-cells of the silkworm, we carried out to study the potentials of this mycotoxin and Sterigmatocystine (STC), which is a metabolic product of *A. versicolor*, in causing recessive visible mutations in the insect. It should be emphasized that *A. versicolor* grows on rice as well as other cereals.

In the experiments reported here, both sex wild-type F_1 (*C108*$\times$*Aojuku*) hybrid pupae were injected intra-abdominally with 0.025 ml of various concentrations (0.1–10 μg/capita) of the $AFTB_1$ or STC in 0.5% carboxyl methyl cellulose (CMC) solution immediately before use. The germ-cell stage at this stage of pupae are prophase I oocytes and spermatozoa for female and male, respectively. Control pupae received 0.025 ml CMC solution without the mycotoxin. Recessive visible mutations were detected by the *pe:re* egg-colour specific locus method. The treated pupae were mated to the marker stock moths. All F_1 individuals (or eggs) were scored on the visible phenotype with whole-body or mosaic mutations.

It was found that both mycotoxins are highly mutagenic in silkworm pupal oocytes and spermatozoa and that, as the properties of chemical mutagenesis, the frequency of induced mosaic mutations is much higher than that of whole-body mutations. The incidence of whole-body mutations seemed to be somewhat higher than that of he corresponding control. The dose-response relations for the mosaic mutation at both loci of pupal oocytes or spermatozoa were a nearly linear fashion over the dose-range applied from 1 to 10 μg/capita. It should be noted that the dose-response curves of mosaic mutations in oocytes by STC was linear down to a dose at 0.12 μg/pupa or *ca.* 0.1 μg/g body weight and suggesting whether a threshold dose is existent at the very low dose-range or not. The mutagenicity of $AFTB_1$ was higher in spermatozoa than oocytes, whereas that of STC was somewhat less in sperm than oocytes. The mutagenic activity of $AFTB_1$ was about 10 times higher than that of STC in spermatozoa, while it was observed $AFTB_1$ to be as active as STC in oocytes. In any case, the mutagenic activity of these two mycotoxins in the germ-cells of this insect is regarded as one of the most potent chemicals among indirect mutagens tested and that is somewhat weak as compared with those of *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine, Ethyl methansulphonate or Mytomycin C.

The result of the present experiments also showed that in the silkworm germ-cells the mutagenic effect of STC incubated in the presence of rat-liver microsome fraction appeares to be significantly reduced as compared with that in the absence of the liver microsome fraction, which suggesting that the silkworm is physiologically competent in the metabolic activation of the mycotoxins. Accordingly, it seemed that the silkworm egg-colour specific locus mutation test system may be useful as a prescreening for such the mycotoxins that require metabolic activation.

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Genetic Effects of Tranquilizers on Silkworm Germ-Cells

Akio MURAKAMI, Toshiko OZAWA and Yataro TAZIMA

Some ataractic drugs have been tested for their carcinogenicity and cytotoxicity in several biological systems on account of their chemical structure and common usage in our daily life. However, an obvious carcinogenicity of such the drugs has not been reported in distinction to their cytotoxic effects on sme test systems. In order to determine whether or not genetic toxicity were observed in germ-cells of the silkworm, the mid-stage wild-type pupae of F_1 (*C108*×*Aojuku*) hybrids were abdominally injected Chlorodiazepoxide (7-Chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepin-4-oxide) HCl dissolved at a concentration of 50, 100, 150 or 200 μg/pupa in 0.025 ml of 0.5% Carboxyl methyl cellulose (CMC) solution. They were then mated to marker stocks homozygous for egg-colour genes, *pe* and *re* loci, to check the occurrence of recessive visible mutatons. Chlorodiazepoxide HCl used in this study was supplied from the National Institute of Hygienic Sciences, Tokyo as a test sample (#78–20) for the Cooperative Programme on Short-Term Assays for Carcinogenicity in Japan. For the control, pupae were injected with 0.025 ml of 0.5% CMC solution alone.

The results obtained indicate that the lower doses, 50 and 100 μg/pupa of Chlorodiazepoxide did not induce a significant increase in the mumber of both complete and mosaic mutational events in females (or oocytes), while the 150 and 200 μg dose of the drug induced a significant increase in the number of both complete and mosaic mutational events. It is well established that, in the silkworm, the majority of mutants induced in oocytes after treatment with typical mutagenic chemicals is mosaic types, but the incidence of complete ones is very rare. Consequently, it can be said that this high incidence of complete mutations seemed to be a characteristic feature of this drug mutagenesis. Neither dose of Chlorodiazepoxide increased the mutational frequency above control level in the treated male pupae. The cellular difference may be interpreted due to either differential DNA conformation or physiological condition in the germ-cells. Data obtained in the present experiment together with results obtained previously

indicate, however, that Chlorodiazepoxide is certainly not the sort of strong chemical mutagens. At the higher concentrations the sterility was more greatly incrased in the treated female pupae than in the male ones. In any case, there is a possibility that an ataractic drug Chlorodiazepoxide HCl is capable to induce germ-cell mutations in eukaryotes.

Diazepam, 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one, is a structurally related compound to Chlorodiazepoxide and has also been used as a tranquilizer. This drug (#77–22) has also been selected as a test chemical for the evaluation of its mutagenicity and carcinogenicity by the Ministry of Health and Walfare, Japan. Diazepam was found to induce an increase in the number of egg-colour specific locus mutations in the treated pupal oocytes, indicating that it seems to have a positive mutagenic action for grem-cells of the silkworm female. In the Diazepam-treated male pupae with a drug concentration of 100 μg per capita, there was no apparent increase in the frequency of the specific locus mutations over the frequency in control experiments. The mutagenic activity of Diazepam seemed to be about the same as that of Chlorodizepoxide HCl. The marked sterility was also observed at the higher concentration doses in either female or male.

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Induction of Mutations in Hamster Embryonic Cells from Mothers Received Total Body Irradiation of γ-Ray

Tsuneo KADA, Taiji ASANO, Akiko YOKOIYAMA and Naomichi INUI[1)]

Induction of mutations resistant to 8-azaguanine (8AG) has been successfully observed in embryonic cells that had been isolated and cultured in *in vitro* from pregnant hamster exposed to different chemical mutagens (N. Inui *et al.*, *Mutation Res.* **41**: 351, 1976). This method was applied in radiation studies. Syrian golden hamsters on the 11th or 12th day of pregnancy were exposed to γ-ray of different doses from a 6000 Ci source of ^{137}Cs. Subsequently the animals were given standard laboratory chew and water *add libitum* for 24 hours. Their fetuses were then excised, finely minced and dijested with 0.25 per cent trypsin. Primary cultures of the

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trypsinized cells were initiated by seeding $1-5 \times 10^6$ cells into 10 ml of culture medium in 72 cm^2 Falcon plastic tissue culture flasks. Cells were grown in MEM supplemented with 10 per cent FCS (GIBCO N.Y.) at 37°C under 5 per cent CO_2 in air for 72–120 hours. To induce 8AG-resistant mutations, cells were transferred into dishes containing medium with 8AG (10–20 μg/ml). The medium containing 8AG was changed every day for the first 3 days and every 3 days for further 12 to 17 days. The cells in the dishes were fixed and stained with Giemsa and the number of mutant colonies was scored. Induced frequencies increased linearly from 17 to 250 rads (from 10 to 220 mutations per 10^7 viable cells) then a plateau was fond for both 10 and 20 μg/ml AG resistance. Killing effects were expecially pronounced at a dose of 1000 rads, otherwise the present data on mutation induction were obtained under moderate surviving rates.

Studies on the Natural Antimutagens in the Environment

Tsuneo KADA, Yoshiko OHTA, Masako HARA, Akiko YOKOIYAMA and Tadashi INOUE

We already reported tha certain bio-metalic compounds such as cobaltous chloride possess potent antimutagenic capacities in bacteria (*E. coli* WP2 and *B. subtilis mut*-1) against spontaneous an radiation (UV and γ-ray) -induced mutabilities (*Ann. Rept.* NIG No. 28: 77, 1977; *Proc. Japan Acad.* **54B**: 234, 1978). Recent observations showed that similar antimutagenic effect exists also in cultured mammalian cells such as the strain V79 (Chinese hamster) for mutations producing 8-azaguanine resistant cells by γ-irradiation. Two types of hypothesis are under examination of mechanisms involved. In the first, the cobaltous ions may serve as a error-proofing cofactor in radiation-induced SOS enzyme(s) involved in error-prone DNA repair and replication. In an alternative one, we assume that the radiations might affect primarily genes of mutator function and the induced constitutive mutator clones are sensitive to the metal compound.

We are now studying extensively on antimutagenic activities in the natural environment including food materials. Our first survey on vegetables and fruits showed that Shiitake mushrooms contain a potent antimutagen which has difusible low molecular weight. An another example of plant antimutagens was found in the family of lily.

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

Induction of Mutation and Phage by Synchrotron Orbital Radiation in *Bacillus subtilis* Spores

Tsuneo KADA and Yoshiko OHTA

It is interesting, from radiation-biological and photobiological standpoints, to know how synchrotron orbital radiation (SOR) interacts with cellular DNA. Our present studies showed clearly that the SOR induces gene mutation as well as SPO2 phage development in *Bacillus subtilis* by exposing their spores.

For experiments of mutation induction, spores of *B. subtilis* NIG 1121 (*his met*) were prepared on Schaeffer's medium, treated with lysozyme then with SDS and washed well with distilled water. They were placed on Millipore filter round disks (pore-size; 0.22 μm; diameter 25 mm), put in the SOR chamber (Nuclear Research Center, University of Tokyo) dried in vacuum and irradiated in high vacuum. The irradiated spores were resuspended in distilled water, and plated after appropriate dilutions on broth-enriched minimal agar (MB plates) to determine the survival as well as induced inductions ($His^- \rightarrow His^+$). At doses 10–40 mA·min, the frequency of induced mutations increased linearly (from 13 to 150 His^+ per 10^8 survivals.

For experiments of phage induction, a *B. subtilis* strain H17 was lysogenized with phage SPO2 and spores of lysogenic bacteria were prepared. The spores were placed on Millipore filter and exposed to SOR of different doses in vacuum. Each filter carrying irradiated spores (about 1.8×10^7) was immersed in distilled water (0.3 ml) by shaking for 2 hours at room temperature, heated at 75°C for 20 minutes, combined with 3.5 ml of NY medium and divided into 0.5 ml portions. They were incubated at 37°C by shaking for different periods. After adding one drop of chloroform, each tube was incubated at 37°C for 15 minutes and free phages were titrated. The number of free phages increased during incubation of the irradiated spores and this effect was much greater for the SOR light-exposed spores, indicating clearly a inductive effect of SOR irradiation.

A Desmutagenic Factor from Plant (*Brassica oleracea*) for Mutagenic Principle of Tryptophan Pyrolysate has Peroxidase and NADPH-Oxidase Activities

Tadashi INOUE and Tsuneo KADA

A desmutagenic factor for Trp-p-2 which is a potent mutagen discovered by Sugimura and his collegues in pyrolysis products of tryptophan, was purified to near homogeniety from cabbage (*Brassica oleracea*) leaves. The factor had a molecular weight of 43,000 and had 45.2 μg of sugar per mg of protein as determined by phenol/sulfonic acid method. The purified factor exhibited a hemo-protein-like absorption spectrum with Soret band at 404 nm, and α and β bands at 640 nm and 497 nm, respectively. When reduced with sodium hydrosulfite, the Soret and α bands shifted to 437 nm and 555 nm, respectively, and β band disappeared. Treatment with cyanide resulted in shift of the Soret band to 421 nm. These spectral properties are very similar to those of peroxidase of other plants especially of horseradish which also has NADPH-oxidase activity.

We then examined whether the desmutagenic factor had peroxidase and NADPH-oxidase activity. The peroxidase activity was determined using hydrogen peroxide and *o*-dianisidine as substrates; and NADPH-oxidase activity was determined by a decrease in absorbancy at 340 nm owing to the oxidation of NADPH. The experiments yielded the results that the factor had both peroxidase and NADPH-oxidase activities. The former activity was fully dependent on both resorsinol and Mn, and the latter activity was inhibited by catalase. These properties are also very similar to those of horseradish peroxidase, but commercial horseradish peroxidase had no desmutagenic activity for Try-P-2.

Further Characterization of a *Bacillus subtilis* Endonuclease Specific for Apurinic Sites in DNA

Tadashi INOUE and Tsuneo KADA

We have identified several kinds of enzymes which may function in the repair of gamma-irradiated DNA in extracts of *Bacillus subtilis* cells using a new assay system in which a capacity of cellular extracts to enhance the priming activity of gamma-irradiated DNA for purified DNA polymerase was measured. Preliminary experiments revealed that one of them was

an endonuclease specific for apurinic sites in DNA (T. Noguti and T. Kada, *Biochim. Biophys. Acta* **395**: 294, 1975) and the other was 'cleaning' exonuclease (T. Inoue and T. Kada, *Bicohim. Biophys. Acta* **478**: 234, 1977). The former enzyme was purified to near homogeniety and had properties described below.

The endonuclease was a monomeric protein and had a molecular weight of around 56,000. The enzyme was specific for apurinic sites in double-stranded DNA and had a pH optimum at 8.0. It was slightly stimulated with 50 mM NaCl but completely inhibited with 500 mM NaCl. It required no divalent cations and was insensitive to EDTA; it had no associated exonuclease. These properties are very similar to those of *Escherichia coli* endonuclease IV, which is also insensitive to EDTA and has no exonuclease activity, and very different from those of the main endonuclease for apurinic sites (endonuclease VI) of the same bacterium. Detailed properties of the *Bacillus* enzyme has been described (T. Inoue and T. Kada, *J. Biol. Chem.* **253**: 8559, 1978).

Phenotypic Expression of Waxy Starch Mutants in Cereals

Etsuo AMANO

In waxy starch mutants, amylose fraction of starch is reduced. Some of the mutant lines showed intermediate phenotype between waxy (*wx*) and normal (*Wx*). Intending to show that they are really intermediate mutants and probably due to missense mutations, quantitative measurements were made. The method was modification of the blue value method. Each starch solution sample was made up from a single kernel by autoclaving. After staining by iodine, the sample soultion was diluted in a colorimetric measuring cell. Transmission value of the sample solution was measured in two wave lengths. 430 nm blue light was used to monitor the dilution by measuring the common and major faction of starch, amylopectine, which was stained reddish purple. When the solution was diluted to 50% transmission in 430 nm, an electric signal was generated to command a digital printer to register the transmission value of 660 nm red light. This value would reflect the content of amylose fraction which was stained blue by iodine.

In this year, 27 waxy mutant lines of maize and 18 EMS induced waxy mutants of rice were analyzed. In maize, three EMS induced mutants were

confirmed to be intermediate waxy. Other mutants, including two UV induced and eight EMS induced mutants were very close to standard waxy (wx^C) or deletion mutant (wx^R). Among the EMS induced waxy mutants in maize, 27.3% were intermediate.

In rice, 18 waxy mutants induced by EMS in Norin No. 8 were analyzed and nine mutants (50%) were considered to be the intermediate types. They distributed rather continuously from complete waxy to nearly normal phenotype. Higher ratio of intermediate type in rice and its relation to dosage effect of waxy gene found in this Japonica rice variety are under inverstigation.

Dose Response of Maize Strains to Chronic Gamma-Ray Irradiation*

Etsuo AMANO and Yasuo UKAI**

To compare the dose response of mutation to chronic gamma-rays, 19 strains of waxy maize (*wx*, *Ae*) were planted in Gamma Field of Institute of Radiation Breeding, NIAS, Ohmiya, Ibaraki. The plants were transplanted into the field after three week's growth and irradiated for 20 hours a day during whole growing stage. Plant height and leaf lenght-width ratio at maturity were also recorded as a measure of the effects of radiations on plant growth.

Previous experiments indicated that if amylose in starch were suppressed by waxy (*wx*) gene, forward mutation from normal (*Ae*) to amylose extender (*ae*) could be detected in pollen stained by iodine (Nelson's I_2-KI solution). Although the frequency of mutation was at the order of 10^{-4} at the highest, examinations of 10^5 pollen grains per plant were not so laborious and this genetic analysis seemed to be a very sensitive system. The mutant pollen grain *wx ae* stains darker by iodine than *wx Ae* pollens. The darker stain was favorable to distinguish mutant pollens from physiologically damaged pollens and to detect the mutant pollen out of a large number of the light colored pollens of original strains. This darkly stained pollen might include both mutation from *Ae* to *ae* and reverse mutation from *wx* to *Wx*. In the present experiments, various *wx* strains being used, reverse mutation might occur in some strains. However, as the radiation

* Cooperative Research Project sponsored by the Agriculutre, Forestry and Fishery Research Council, MAFF.

** Institute of Radiation Breeding, NIAS.

might not induce point mutation, reverse mutation of *wx* to *Wx* seemed to be very infrequent or unlikely. The *wx* strains being all homozygous for each *wx*, normal recombinant could not be expected. The colorations of the mutant pollen were intermediate, *i.e.*, lighter than *Wx* pollen, so that wind blown and oter contamination could be discriminated.

Plant height of the most sensitive strain decreased above 150R/day but in the most resistant strain the height decreased above 230R/day. Frequencies of mutant pollen were examined under a low power binocular microscope using large cover and slide glasses. Total number of pollen examined was estimated from counts of 15 positions on the slide. Then the entire area (50×75 mm^2) was scanned to score dark mutant pollen grains. Fluctuation of the freuqency of the dark pollen among the slides was large probably due to mutation sectoring, but pooled data showed linear increase from 3.8R/day up to 11R/day. In the most resistant strain, the linear increase continued up to 23R/day. Other strains showed saturation above 16R/day. Correlation between leaf width-length ratio and pollen mutation was examined. The strains tested evenly distributed showing no correlation as a general trend. However the results could be interpreted as showing that the most sensitive strain in genetic damage was also sensitive to growth inhibition. The same correlation was seen in the most resistant strain. Among the intermediates, some showed higher sensitivity in mutation but were resistant in growth inhibition, other strains showed the reverse reactions. Overall difference in sensitivity between extreme cases was not large. Leaf width–length ratio differed by a factor of 10. Other indices differed by factors of two to four.

Induction of Waxy Mutants in Rice by Reactor Radiations

Etsuo Amano

In maize, the frequencies of viable waxy (*wx*) mutants in radiaton treated progeny were as low as 1/10 or less of the powerful chemical mutagen, EMS, treatment. To compare with maize, and to examine the characteristics of radiation induced waxy mutants, seeds of rice, *Oryza sativa*, var. Norin No. 8. were irradiated in the thermal neutron facility of Kyoto University Reactor (KUR)*. Dry seeds were sealed in polyethylene bags and irradiated in the D_2O Irradiator Tube for 3,4, or 5 hours. Reactor output during

* Research Reactor Institute Kyoto University

the treatments was 5 MW. The biological effects of the reactor radiation were compared to those of ^{137}Cs gamma-rays by two weeks seedling height. Suppressions of seedling heights which were about 60% of the control were comparable from 30KR to 40KR of the gamma-ray. To avoid confusion on the origin of mutants obtained due to chimeric sectoring, it is favorable to limit the number of panicles developed from a single seed. For this reason, X_1 plants were kept in the seedling flats in greenhouse until harvest, so that only a few panicles developed from a single seed. After harvest and drying each panicle was threshed and hulled separately. Opaque or other waxy like grains were tested by iodine, after scraping off small portion of endosperm surface using a small grind stone. Among 1306 panicles pooled, in which more than ten grains could be examined, six panicles (0.459%) segregated *wx* mutant grains. These six mutations were independent in their origins. It was noticed that some of them showed intermediate phenotype, or in other words, they were leaky *wx* mutants. In the present experiment, frequency of *wx* mutants induced was as high as EMS treatment. This high frequency and the occurence of leaky *wx* mutation became a new problems to be investigated.

Test of Environmental Mutagens with a Soybean Strain, T-219

Taro FUJII

Strain T-219 of *Glycine max*, made available through the kindness of Dr. B. K. Vig of the University of Nevada, possesses gene Y_{11}. This gene imparts green color to $Y_{11}Y_{11}$ plants while $Y_{11}y_{11}$ plants are light green and $y_{11}y_{11}$ plants are yellow. When somatic mutation occurred in $Y_{11}y_{11}$ seeds, the first two simple leaves show yellow spots by forward mutation, green spots by back mutations, and twin spots consisting of green and yellow ones by somatic crossingover (B. K. Vig *Mutation Res.* **31**: 49, 1975).

The mutagenicity of AF-2, β-propiolactone, DAPA and ICR-170, which had been known as mutagens through microbial testing systems, were tested by the use of this soybean strain. The concentrations of these chemicals appllied to the seeds were decided on the basis of those applied in microbial tests and those of EMS through the kindness of Drs. Kada and Inoue. The results showed 0.0002 μg/ml of AF-2 as well as 1 μg/ml of β-propiolactone produced 1,000 revertants per plate or *ca.* 10^7 cells, and a similar mutation frequency was observed with 20 μg/ml of EMS. On the

other hand, an appreciable increment in mutation frequency was observed with 2 μg/ml of EMS in the soybean system. Accordingly, about 1/10 of concentrations used in the microbial system were used in the present experiment.

About 130 seeds (25 g in dry weight) of T-219 strain were soaked in 30 ml solution with given concentrations of a chemical for 24 hours at 20°C. The solution was almost completely absorbed by the soaked seeds and their weight increased to 53 g. Treated seeds were sown in wooden boxes filled with field soil and kept in a greenhouse (20–30°C). Mutant spots appearing on the first two simple leaves were scored, and the mutagenicity of each chemical compound was measured by the increase of spots per leaf. Significant increase in mutation frequency were observed with 0.00008 μg/ml of AF-2 or 0.2 μg/ml of β-propiolactone, and the frequency increased almost linearly with incraesing concentration. An increment in mutation frequency was also observed with 0.125 μg/ml of DAPA and with 0.0025 μg/ml of ICR-170.

This preliminary experiment indicates that the soybean system is highly sensitive to mutagene and is useful for testing the mutagenicity of various environmental agents. Furthermore, this system can be used for checking the mutational effect of long-term seed storage.

VIII. POPULATION GENETICS (THEORETICAL)

Change of Gene Frequencies by Natural Selection under Population Number Regulation

Motoo KIMURA

By incorporating a population number regulating mechanism into the formulation of genic selection involving a pair of alleles (A_1 and A_2) with respective frequencies x and $1-x$, it is shown that the change of x in one generation is given by $\Delta x=sx(1-x)/\overline{W}$, in wihch $\overline{W}$ is the mean absolute selective value (in Wright's sense). It is also shown that, in the process in which advantageous allele (say A_1) increases from a low frequency to a high frequency, quasi-equilibrium is rapidly attained where $\Delta\overline{W}\approx 0$. In this state we have $\overline{W}\approx 1+(s^2/\bar{c})x(1-x)$ in the case of logarithmic population number regulation, and $\overline{W}\approx 1+s^2x(1-x)/(\bar{c}N)$ in the case of logistic regulation. In these expressions, s is the selective advantage of A_1 over A_2, and $\bar{c}$ is a coefficient relating to the total population number regulation. It is pointed out that the approximation formula $\Delta x=sx(1-x)$ is valid under wider circumstances than usually suggested by the conventional treatment of genic selection. For details, see *Proc. Natl. Acad. Sci. USA* **75**: 1934, 1978.

Stepwise Mutation Model and Distribution of Allelic Frequencies in a Finite Population

Motoo KIMURA and Tomoko OHTA

A mathematical theory is developed that enables us to derive a formula for the equilibrium distribution of allelic frequencies in a finite population when selectively neutral alleles are produced in stepwise fashion (stepwise mutation model). It is shown that the stepwise mutation model has a remarkable property that distinguishes it from the conventional infinite-allele model (Kimura-Crow model): as the population size increases indefinitely while the product of the effective population size and the mutation rate is kept at a fixed value, the mean number of different alleles contained in the population rapidly reaches a plateau which is not

much larger than the effective number of alleles (reciprocal of homozygosity). For details, see *Proc. Natl. Acad. Sci. USA* **75**: 2868, 1978.

Effect of Overall Phenotypic Selection on Genetic Change at Individual Loci

Motoo KIMURA and James F. CROW

The selective advantage of an allele G_i (relative to the mean of alleles at this locus) is given by

$$s_i = -A_i \int_{-\infty}^{\infty} W(X)F'(X)\mathrm{d}X/\overline{W}$$
$$= A_i \int_{-\infty}^{\infty} W'(X)F(X)\mathrm{d}X/\overline{W},$$

in which A_i is the average excess of the allele on the character, X; $W(X)$ is the fitness fuction; $F(X)$ is the frequency function; $\overline{W}$ is the mean fitness; and the prime denotes differentiation. With truncation selection $s_i = A_i F(C)/\bar{w}$, in which $F(C)$ is the ordinate at the culling level and $\bar{w}$ is the proportion saved; this does not depend on any assumption about the distribution of $F(X)$. If the character is normally distributed, $s_i = A_i I/\sigma^2$, in which I is the selection differential and σ^2 is the variance of the character distribution. Finally, if the logarithm of the fitness is proportional to the squared deviation from the optimum and the character is distributed normally, $s_i = A_i K(X_{op} - m)$, in which X_{op} is the optimum value of the character, m is the mean value, and K is a constant determined by the variances of the fitness function and the frequency function. Truncation is the most efficient form of directional selection in the sense of producing the maximum gene frequency change for a given effect of the gene on the character, but fitness functions can depart considerably from sharp truncation without greatly reducing the efficiency. For details, see *Proc. Natl. Acad. Sci. USA* **75**: 6168, 1978.

Theoretical Study of Genetic Variability, Assuming Stepwise Production of Neutral and Very Slightly Deleterious Mutations

Takeo MARUYAMA and Motoo KIMURA

A mathematical theory is developed that enable us to compute the amount

of genetic variability maintained in a finite population, assuming that mutations occur in stepwise fashion ("Stepwise Mutation" or "Ohta–Kimura" Model) and that both selectively neutral and slightly deleterious alleles are involved. Two numerical examples show that, if very slightly deleterious mutations are prevalent, the amount of genetic variability increases much more slowly as the population number increases than is the case when all the mutations are strictly neutral. For details, see *Proc. Natl. Acad. Sci. USA* **75**: 919, 1978.

Sequence Variability of Immunoglobulins Considered from the Standpoint of Population Genetics

Tomoko OHTA

The gene family of variable region of immunoglobulins is an interesting example to apply the theory of gene diversity of multigene families. The variable region contains about 110 amino acid sites and these sites are classified into the hypervariable and the framework regions. The former is known to constitute antigen binding sites. The theoretical results on the equilibrium and transient behavior of the identity coefficient were applied to the analyses of sequence variability in the variable regions of immunoglobulins. The probability of amino acid identity between corresponding residue sites at different but homologous immunoglobulin sequences was computed both within and between species from data compiled by Kabat and his associates. The average identity coefficients of amino acid was obtained for the hypervariable regions and also for the framework regions. From statistical analysis based on population genetics theory it is concluded that somatic mutations cannot be the major cause of hypervariability and that the results can readily be explained by the germ line theory. For details, see *Proc. Natl, Acad. Sci. USA* **75**: 5108, 1978.

Theoretical Study on Genetic Variation in Multigene Families

Tomoko OHTA

Genetic variation contained in a multigene family was theoretically investigated from the standpoint of population genetics. Unequal crossover is assumed to be responsible for the coincidental evolution of mutant genes

in a chromosome. When the allowed latitude of the duplicated or deleted number of gene units at unequal crossover is 10–15% of the total gene number in one family, the arrangement of gene lineage in a chromosome is shown to be roughly random. The equilibrium properties of genetic variation or the probability of identity of two genes within a family (identity coefficient) were studied under mutation, unequal crossover, interchromosomal crossover and sampling of gametes. The identity coefficient of a multigene family within *a chromosome* is shown to be approximately

$$C_0=\frac{\alpha}{\alpha+2v+\frac{\beta}{3}\frac{4N_e v}{1+4N_e v}},$$

in which $\alpha=2k/n^2$ with k=effective number of cycles of unequal crossover and with n=number of gene units in a family, v is the mutation rate per gene unit, β is the rate of interchromosomal crossover per family and N_e is the effective size of the population, all measured by the rate per generation. The identity coefficient of a gene family between *two different chromosomes* becomes approximately $C_1=C_0/(1+4N_e v)$.

The equilibrium properties of the means, the variances and the covariance of the two measures of identity coefficient are investigated by using the diffusion equation method under the assumption of constant number of gene units in the multigene family. Some models of natural selection based on identity coefficient are considered. The possible significance of the variance and covariance of identity coefficient among the chromosomes on the adaptive differentiation of gene families such as those producing antibodies is discussed. For details see *Genet. Res.* **31**: 13, 1978 and *Genetics* **88**: 845, 1978.

Polymorphism and Loss of Duplicate Gene Expression: A Theoretical Study with Application to Tetraploid Fish

Naoyuki TAKAHATA and Takeo MARUYAMA

We studied the fixation of null alleles at independent duplicate loci, assuming that wild-type active alleles mutate irreversibly to nonfunctional null alleles and that the population is finite and panmictic. Solving the two-dimensional Kolmogorov backward equation numerically, we obtained the rate at which one of the active genes is lost and the amount of

heterozygosity at specified times. Previously harmful genes, including recessive lethals, can be fixed at one of the duplicate loci, which would not happen with a single locus. Examination of data from several fish families showed that the rate of fixation of null alleles is too slow and the amount of heterozygosity too small to be compatible with complete recessivity at all loci. Our conclusion differs in this regard from that of Bailey *et al.*, *Proc. Natl. Acad. Sci. USA* **75**: 5575, 1978. They also reported that the time taken for 50% of the loci to be fixed for null alleles is approximately $15N+v^{-3/4}$ where N and v are the effective population size and mutation rate when the alleles are completely recessive and the double homozygote is lethal. We found that the fixation rate depends not only on N, but also on Nv. For details see Proc. Natl. Acad. Sci. USA *76*: 4521, 1979.

IX. POPULATION GENETICS (EXPERIMENTAL)

Seasonal Change of Inversion Frequencies in a Mishima Population of Drosophila melanogaster

Yutaka INOUE

Drosophila flies were monthly collected from a population of Mishima. Several banana traps were placed for a week at a shrine backyard prior to collection. *D. melanogaster* as well as other domestic species were collected from June to November with a temporal decrease of population size in mid-summer. The average frequency of polymorphic inversions was higher in summer and lower in spring and fall; 8.0% (June), 9.8% (July), 15.5% (Aug.), 14.4% (Sept.), 9.1% (Oct.), 5.8% (Nov.). Four common cosmopolitan inversions (*2Lt*, *2RNS*, *3LP*, *3RP*) showed the same seasonal change in frequency. Two inversions (*In*(2L)*W* and *In*(3L)*Y*), endemic to the Eastern Japan and the frequencies were very low, appeared only in summer and early fall. The seasonal change suggests that the polymorphic inversions prefer hot to cool. This phenomenon is very consistent with the high frequency of inversions in the Ishigaki-jima (Okinawa) population and the low frequency in the northern (Sapporo) population.

Further Genetic Studies of the Katsunuma Population of Drosophila melanogaster

Takao K. WATANABE and Yutaka INOUE

Genetic variations in the second chromosone of *D. melanogaster* have been studied for 16 years in the Katsunuma population. The frequency changes of deleterious genes and chromosome inversions are shown in Fig. 1. Lethal and male-sterile chromosomes increased in frequency drastically in the early seventies. After a while their frequencies reduced a little and became a constant level as about 25% and 10% respectively. Female-sterile chromosomes, on the contrary, increased in frequency gradually after 1975 which overwhelmed male-sterile freauency in 1978.

Polymorphic inversion chromosomes such as *In*(2L)*t* and *In*(2R)*NS* had

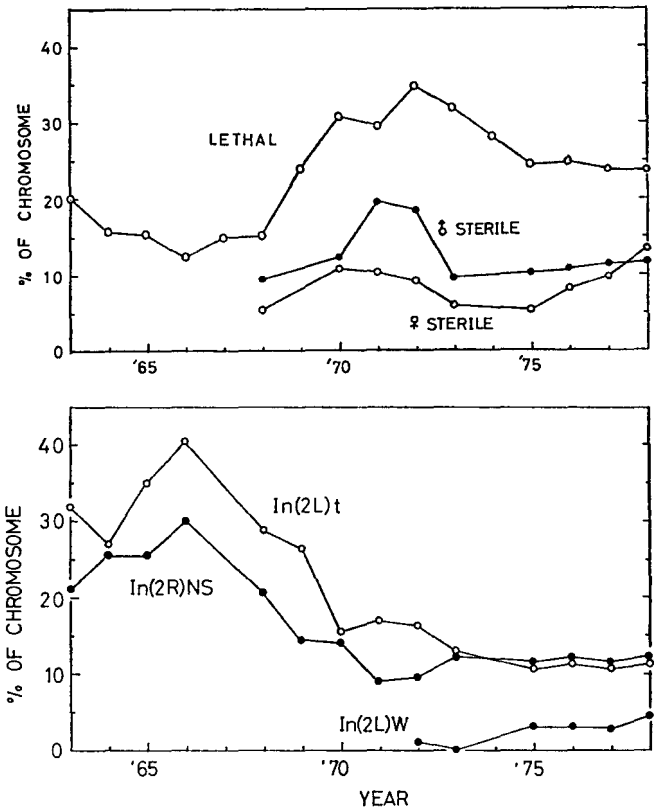

Fig. 1. Frequency changes of deleterious genes and chromosome inversions in the Katsunuma population.

been highly maintained in the Katsunuma during the sixties. However, their frequencies became a third or a half after the early seventies. A new inversion, *In(2L)W* (28C–32C), appeared in the Katsunuma in 1972 and thereafter it became a member of polymorphic inversions though the frequency was about 3–4%.

X. EVOLUTIONARY GENETICS

Phylogeny of *Drosophila* Based on the Mating Preference

Takao K. WATANABE and Masaoki KAWANISHI

One-side mating preference among sibling species of *Drosophila* has been interpreted as follows: Females of a derived (new) species do not mate well with males of the ancestral (old) species whereas females of the ancestral species readily mate with males of the derived species. This hypothesis has come from the assumption that isolation between the females of new species and the males of old species allow a favorable situation for an incipient species to maintain its population, and that the species specific mating behaviors have been conservatively maintained by each species.

The evolutionary sequence drawn by the asymmetrical mating preferences only suggests the 'relative age of each species', which resulted in a unidirectional phylogeny of each sibling species group of *Drosophila*. Then, the phylogeny is revised by seeking the immediate ancestor of each species from the degree of genetic closeness. For example, the evolutionary sequence of the *virilis* group is (1) *virilis*, (2) *littoralis*, (3) *novamexicana*, (4) *americana*, (5) *texana*. But the most genetically close species of *novamexicana* is *virilis* instead of *littoralis* among the older species than *novamexicana*. Therefore the phylogeny of the group is branched as *virilis*→*littoralis*, and *virilis*→*novamexiacna*→*americana*→*texana*. This phylogeny is very consistent with those of chromosomal (Stone, *UTP* 6205: 507, 1962) and electrophoretical (Nei, *Am. Nat.* 105: 385, 1971) evolution.

This method is also useful for understanding of phylogenetic relations of other sibling species group of *Drosophila* whenever data on the reciprocal mating preferences are available. The evolutionary sequence of the *melanogaster* subgroup is determined by the asymmetrical matings as follows; (1) *melanogaster*, (2) *simulans*, (3) *yakuba*, (4) *erecta*, (5) *teissieri*, (6) *mauritiana*. The phylogenetic relation revised by seeking the immediate ancestor is as follows; *melanogaster*→*simulans*→*yakuba*→*erecta*, *simulans*→*teissieri*, and *simulans*→*mauritiana*. *D. melanogaster* is the ancestral species and *D. simulans* evolved from it. From *D. simulans* three African endemics evolved: *D. yakuba* first, *D. teissieri* second and *D. mauritiana* third.

Again, this phylogeny is very consistent with those of species distribution (Bock and Wheeler, *UTP* 7213: 1, 1972) and chromosomal (Lemeunier and Ashburner, *Proc. Roy. Soc. B. 193*: 275 (1976) evolution.

XI. HUMAN GENETICS

Host Resistance to the Gene for Retinoblastoma

Ei MATSUNAGA

Retinoblastoma is a malignant neoplasm of the eye affecting one per *ca.* 20,000 infants. There are two forms, hereditary and nonhereditary. The nonhereditary form, which comprises most sporadic unilateral cases, represents about 60% of all retinoblastomas. The remaining 40% are hereditary and due to an autosomal dominant gene with incomplete penetrance and expressivity. While all bilateral cases, whether sporadic or familial, are to be regarded as hereditary, a gene carrier can be unilaterally affected or remain unaffected. By analyzing data from 261 pedigrees with two or more cases of retinoblastoma, we arrived at a simple conclusion: the three distinct phenotypes of the gene carriers, *i.e.*, unaffected, unilaterally and bilaterally affected, are determined largely by inherited host resistance to the retinoblastoma gene.

Segregation analysis of the data revealed that penetrance and expressivity in children who received the gene from a carrier parent declined consistently with decreasing degree of parental expressivity. For example, if the parent was bilaterally affected, the proportion of affected children was 0.49 and the disease was bilateral in 90% of the cases; if the parent was an unaffected carrier, the proportion decreased to 0.31 and 54% were affected bilaterally. The possibility of multiple alleles with different penetrance at the retinoblastoma locus could be ruled out by the variability in penetrance in different sibships in the same family. The observed distribution of the three phenotypes among the gene carriers was consistent with a multifactorial model with two thresholds for host resistance, but not with a Poisson distribution of tumors as assumed by Knudson (*Proc. Natl. Acad. Sci.* **68**: 820, 1971). Furthermore, with the host resistance model there was no need to postulate delayed mutation in order to account for the reported pedigrees with two or more collateral relatives affected with retinoblastoma. The heritability of host resistance was estimated at approximately 90%. For details, see *Am. J. Hum. Genet.* **30**: 406, 1978.

Recurrence Risks to Relatives of Patients with Retinoblastoma

Ei MATSUNAGA

Prior to modern ophthalmologic care, retinoblastoma had been a lethal condition, with rare exceptions of spontaneous regression. Owing to increasing success in the medical treatments, however, cases inherited from those who survived retinoblastoma are gradually increasing in many countries. Appropriate genetic counseling is therefore of great importance to the patients and their family members.

Estimates for recurrence risks of retinoblastoma have been given usually on the assumption that the hereditary form is due to a dominant gene with 80% penetrance. However, our studies demonstrated that penetrance and expressivity of the gene in children declined with decreasing expressivity in the carrier parent. Accordingly, using the segregation ratios of 0.49, 0.42 and 0.31 in children from bilaterally affected, unilaterally affected and unaffected carrier parents respectively, and the proportion of sporadic bilateral and unilateral cases that were inherited from an unaffected carrier parent estimated at 0.10 and 0.07 respectively, recurrence risks to first- and second-degree relatives of patients with retinoblastoma were calculated. The risk to a first child of an unaffected person having a bilaterally affected parent is as low as about 1%, in contrast to the usual estimate of 6.7% derived from the assumption of constant penetrance of 80% of the retinoblastoma gene. For details, see *Jpn. J. Ophthalmol.* **22**: 313, 1978.

Quantitative Analysis of C-Positive Variants in Man

Jun-ichi AZUMI, Yasuo NAKAGOME and Ei MATSUNAGA

In the Paris Conference (Suppl., 1975), the use of numerical expression, 1 through 5, was recommended for the purpose of describing the size of a variant. However, no specific difinitions were given for the individual classes (numerals). As to the qh regions of nos. 1, 9 and 16, the use of the size relative to either a long arm of chromosome 21 (21 q) (Müller *et al.*, *Cytogenet. Cell Genet.* **15**: 239, 1975) or a short arm of chromosome 16 (16p) (Patil and Lubs, *Human Genet.* **38**: 35, 1977) was proposed. The ratios thus obtained were assigned more or less arbitrarily into five classes.

In the present report, area of C-positive qh regions of chromosomes 1, 9

and 16 was measured using a Nikon-Vickers M85 microdensitometer. A few different methods including those of Müller *et al.* (1975), Patil and Lubs (1977) and a new system were compared.

The mean area of C-positive 1qh, 9qh and 16qh segments were 1.98 ± 0.31 ($\mu m^2 \pm SD$), 1.39 ± 0.28 and 0.83 ± 0.17, respectively. It appeared that all 16qh segments were smaller than both a 21q and a 16q, while almost all 1qh segments were larger than either of them. When results were expressed in the numerical system, the mean scores for 1qh segments were 3.8 and 3.5, while those for 16qh were 2.3 and 1.9 respectively, by Müller *et al.* and Patil & Lubs' system.

A new system ranks a qh segment into one of 5 classes based on how different they are from an average of each 1qh, 9qh and 16qh region in terms of standard deviation (SD). It is proposed that a variant with in $\pm$ 1SD of an average be assigned to the intermediate class (class 3) in the Paris Conference system (Suppl., 1975), one within $\pm$ 2SD to either small or large class (calss 2 or 4) and all other to either very small or very large (class 1 or 5). The present system compares favorably with any other methods so far tested as to the detection of a qh variant. For details, see *Jpn. J. Human Genet.* **24**: 99, 1979.

XII. BEHAVIORAL GENETICS

Genetical Analysis of Noise Sensitivities of Homozygous Strains of *Drosophila melanogaster*

Chozo OSHIMA and Tsuguhiko TAKAMURA

Many homozygous strains for different second chromosomes, have been made by Cy/Pm method using many male flies, collected in a Katsunuma natural population in 1975. Seven homozygous strains among them were used in the experiment. Locomotor activities of individual fly in a small glass cell ($4 \times 10 \times 50$ mm) under LD 12 : 12 with dusk and dawn of three days and continued DD of three days under constant 25°C were recorded by the electronic actograph. Locomotor activities of flies of each strain showed two high peaks at dusk and dawn and continued at low level during the subjective daytime with individual circadian rhythm under DD environment. The pure sound (2000 Hz, 100 phon) was projected for 5 hours (0.00–5.00 AM) on resting flies and the grade of sensitivity was determined by the activity occured for the period.

Five flies of each seven strains were examined and a strain numbered 59 was sensitive and a strain 83 was unsensitive. Other five strains were intermediate between them, but their sensitibities were close to unsensitive strain 83. Then, sensitive strain 59 was crossed with other six strains reciprocally and locomtor activities of each hybrid strain under noise environment were recorded as the same method mentioned above. From the result, the gene(s) manifesting the noise sensitivity of strain 59 was recessive to gene(s) of strain 83, and incomplete recessive to gene(s) of other strains. This result was very similar with the result ontained in the previous year and then, those results were put together. Only two of three strains among twelve strains had sensitive nervous center, bioclock controlling the locomotor activity.

Actual Activity at Dusk and Dawn is not Needed for Entrainment of Circadian Activity Rhythm of *Drosophila melanogaster* under Following Continuous Darkness.

Tsuguhiko TAKAMURA and Chozo OSHIMA

Under LD 12 : 12 with dusk and dawn environment for three days, wild typed flies of *D. malanogaster* became most active at dusk and dawn and they were less active during day and night. When they were continuously put into darkness (DD) for following three days, they moved continuously at their subjective daytime and it was showed that they were diurnal insects, and that they could memorize the time of dusk and dawn period in the previous three days.

To memorize the time, that is, to entrain the bioclock, is the actual movement under LD daily cycle needed? We kept flies under the environment of LD 12 : 12 with dusk and dawn and of 12°C for three days and some flies did not move at dusk and dawn becasue of the low temperature. At five hours after the onset of continuous darkness, the temperature shifted from 12°C to 25°C and the locamoter activity of flies was recorded by actograph. Some flies, having no experience of actual movement at dusk and dawn for previous three days, moved continuously during the sunjective daytime for following three DD days. This fact indicates that the actual locomotor activity at dusk and dawn of daily LD cycle is not needed to entrain the circadian rhythm of bioclock under following DD environment.

Visual System Mutants and Light-Dark Cycle Entrainment in Activity Rhythm of *Drosophila melanogaster*.

Tsuguhiko TAKAMURA and Chozo OSHIMA

Ability of mutant flies to adjust locomotor activity rhythm to daily light-dark (LD) cycle was examined, which had such various defects in the visual system as *w, cn bw* (no eye pigment), *eym, ey*N (no compound eye), *so* (no compound eye and no ocelli), *e, t, non*A (no on-off transients in electroretinogram (ERG)), *rdg*A, *rdg*B, *norp*A (no receptor potensial in ERG), *sev* (seventh rhabdomeres absent), and *ora* (outer rhabdomeres absent).

One double mutant, *sev non*A, and one triple mutant, *sev non*A;*ora*, among these mutants could not entrain the daily rhythm of locomotor activity to LD cycle and showed free running circadian rhythm. Considering our

results of mutants in ERG and rabdomeres in the light of Pak's review on mutants affecting visual systems (in Handnook of Genetics. Vol. 3, ed. R. C. King, New York, Plenum, 1976), it is concluded that the input of LD information to medulla either through peripheral retinula cells (R_{1-6}) or central retinula cells ($R_{7,8}$) may be enough to synchronize the locomotor activity with outer LD cycle.

All morphological mutants have normal entrainment ability of LD daily cycle and it is confirmed that compound eye and ocelli are not necessary to catch the change of light and dark environment. However, there are some difficulties in explaining the results of ERG mutants and the morphological mutants at the same time.

Double mutant *sev non*A showed normal eclosion rhythm under daily LD cycle, and it was assumed that photoreceptors for locomotor activity rhythm and for eclosion rhythm were different. Our data also suggests that the photoreceptor for locomotor activity rhythm itself is not the circadian oscillator because *sev non*A flies showed normal free running period.

Oviposition Behavior of *Drosophila melanogaster*: Ethogram and Hybrid Analysis.

Tsuguhiko TAKAMURA and Chozo OSHIMA

Two strains of *D. melanogaster*, which differ as to the preference of oviposition site were set up by artificial selection. One prefers paper placed on the medium (P line), the other lays eggs on the medium avoiding the paper (M line). To analyze these behavioral difference, the ethogram was made by observing directly the egg-laying behavior. A cycle of oviposition of the species is a chain of four behavioral components. Oviposition (*D. melanogaster* lays one egg at a time), intensive extension of abdomen and its contraction soon after, walking and food intaking, searching with egg-guide bending abdomen downward, then again oviposition. The searching behavior seems to be induced by the stimuli through tarsi because there is difference in the frequency of the induction of searching behavior between on the paper and on the medium. Actual judgment, if the place is appropriate for oviposition, is made with egg-guide. This was demonstrated by the fact that flies, whose tips of egg-guides were cauterized, lost discriminating ability of oviposition sites.

The difference between P and M lines was explained by the difference in

egg-inserting tendency. M line flies could insert eggs into the soft medium but they could not insert eggs into the paper. On the other hand, P line flies could lay eggs both on the paper and on the medium because they dropped eggs. A new method to investigate only egg-inserting tendency was established. after counting the number of eggs laid on the medium, the medium surface was treated with 2M NaOH solution for 20 minutes, then the surface was washed with water current. With this treatment eggs dropped and glued to the surface of the medium were washed away and eggs inserted into the medium were left. Percentage of eggs left on the surface is the measure of egg-inserting tendency. Using this method, both P and M lines and their F_1 hybrids were examined individually. The distribution pattern of F_1 flies was almost between the parents and indicated that the charactor was controlled by polygenes.

Different Photo-preferences in Drosophila simulans and D. melanogaster

Masaoki KAWANISHI and Takao K. WATANABE

Photo-preferences of the sibling species, *Drosophila simulans* and *D. melanogaster*, were studied using an apparatus and population cage having a gradient of light intensities. *D. simulans* preferred to stay and to lay eggs at light places whereas *D. melanogaster* did not show such a strong light preference. Selections of eggs in the light gradient cages made it possible to segragate a mixed species population into different species populations; selection for photopositive flies soon eliminated *D. melanogaster* and selection for photo-negative or photo-neutral flies finally eliminated *D. simulans*. The difference of the photo-preference seems to be a cause of coexistence of the sibling species in nature. For ditails, see *Japan. J. Genetics* **53**: 209, 1978.

XIII. ECOLOGICAL GENETICS

An Observation of Wild Rice Species in Tropical Australia

Hiko-Ichi OKA

Two wild rice species, the Oceanian type of *Oryza perennis* complex and *O. australiensis*, are distributed in tropical Australia. The germ plasms of these taxa so far made available for us were quite limited and were not enough for variation studies. Also, there was no publication on the conditions of their natural habitats. I had a chance to visit Darwin and Kununurra in the early April of 1978, and observed wild rice populations at 11 sites. Of them, one was of *O. australiensis*, eight were of *O. perennis*, and the remaining two had both species growing sympatrically.

Both of the two species were found in marshes and river flood plains, which are inundated (50 cm or less) in the rainy season but are dry in the dry season. The Oceanian type of *O. perennis* is an annual grass. It formed big populations in river flood plains. A hill or a small clump consisted of many plants each with one or a few tillers. Some plants standing in water showed floating habit. The naturally shed seeds were found sticking in the mud with their strong awns. At three sites, a part of the population was in the shade of trees. The plants under trees were small in size and late-maturing. Four populations were in pasture or in roadside ditch, while others were in the natural state. Plants growing in a rice field as a weed were also found.

O. australiensis had short rhizomes,and appeared to be of perennial habit. But the populations consisted of scattered small clumps each with several panicles, suggesting that reproduction was mainly by seed. When this species was sympatric with *O. perennis*, it occurred in a somewhat elevated place as compared with the latter in lower places. On the basis of these observations and those from our previous study-tours in other parts of the world, the conditions of the habitats of different wild species were summarized in a table.

Niche Differentiation between Two Wild Rice Speices Observed in Nigeria

Hiroko MORISHIMA and Hiko-Ichi OKA

Two wild rice species, *Oryza breviligulata* and *O. perennis* subsp. *barthii* (=*O. longistaminata*) are commonly found in marshes and depressions in the inland of Nigeria. The former is an annual species while the latter is perennial and propagates itself mainly by rhizomes. From our field observation in 1977 (Report: Oka *et al.*, 1978) and 1978 experiments at Misima, we found the following points.

Both *O. breviligulata* and *O. longistaminata* are distributed in varying water conditions and under varying degrees of habitat disturbance. In 23 percent of the sites observed, these two species coexisted side by side. However, examining populations of one species separated from the other, we found that *O. breviligulata* tended to be associated with annual herbs and *O. longistaminata* with perennial herbs. Further, the former species was distributed in the area where the dry season continued longer and rainfall was more unpredictable than was the latter species. Although the two species can be sympatric in the same habitat, these differences indicate that they are differentiated in niche requirement.

Most of sympatric populations of the two species were found in strongly disturbed sites like fields under cultivation or roadside depressions. The populations of *O. longistaminata* sympatric with *O. breviligulata* or with its domesticated type, *O. glaberrima*, when tested at Misima, produced many plants with a high seed productivity as shown by large number of seeds per plant, heavy seed weight, and high seed fertility. The reproductive effort evaluated in terms of percent seed weight to total plant weight was 6.3 percent on the average (ranging 0 to 25%) in sympatric populations, but it was 0.5 percent on the average (0 to 7%) in populations of this species separated from those of other species. *O. breviligulata* did not show such a difference between sympatric and separated populations. The data for acid phosphatase isozymes suggested the possibility of gene flow to occur from *O. breviligulata* to *O. longistaminata* in sympatric populations.

Genetic Diversity in Rice Populations of Nigeria: Influence of Community Structure

Hiroko MORISHIMA and Hiko-Ichi OKA

Two rice species, *Oryza glaberrima* and *O. sativa*, are often mix-planted in west Africa, and their populations, particularly those of *O. glaberrima*, are highly polymorphic in seed type. It was found that the seed-type diversity within *glaberrima* population as shown by the "amount of information," $H=-\sum p_i \log_e p_i$, was correlated with species diversity in the field (also shown by H), and gave a curvilinear regression on the proportion of *glaberrima* plants to the total number of rice plants. The highest diversity occurred when the proportion was 50–60 percent. A similar trend was also found for *sativa* populations. These relationships suggest that the complexity of the biotic environment as shown by species diversity is related to the genotypic diversity in rice population. (*Agro-Ecosystems* **5**: 263–269)

Variation Studies in African Wild and Cultivated Rice Species

Yoshio SANO and Hiroko MORISHIMA

A total of 122 accessions of rice species were collected from natural populations or from farmers fields in our visit to Nigeria in 1977 (*Ann. Rep.* **28**: 101). An accession consisted of seeds sampled at random from 20 to 30 plants of a population, either on a single plant basis or in bulk. Out of them, 572 plants from 46 populations were tested in concrete beds with automatic shortday control. The results are summarized as follows:

a) *Cultivated species.* In a majority of rice fields observed in Nigeria, the two species, *Oryza sativa* and *O. glaberrima* were mix-planted in varying proportions. Records on a single plant basis were taken for 242 plants from 11 populations regarding culm length, numbers of tillers and panicles per plant, ligule length, degree of seed shedding, numbers of primary and secondary panicle branches, seed fertility, reproductive effort or harvest index, and other 13 characters. The two species showed distinct differences in ligule length and some other characters, and most of the plants were clearly distinguishable into the two species. However, the populations from Kaduna (K-2) and Danbatta (D-3) contained a few plants with intermediate characters which were not classifiable into the two species. The selfed progeny of such intermediate plants segregated for ligule length

and showed a low seed fertility (55–78%). In fact, a few plants which appeared to be F_1 hybrids were found in the field at Kaduna (K-2). These data suggest that introgression occurs between the two rice species when they are mix-planted in a certain condition. This was also recognized from an observation of acid phosphatase isozymes.

b) *Wild species.* Nine populations of *O. perennis* subsp. *barthii* (=*O. longistaminata*), 11 populations of *O. breviligulata*, and 5 populations of *O. punctata* (330 plants in total) were tested. Records were taken for culm length, tiller number, panicle number, seed weight, awn length, seed fertility, reproductive effort, etc. *O. breviligulata*, an annual species, showed a high reproductive effort. The strains from eastern Nigeria tended to have higher reproductive effort than those from western Nigeria (65–35%). Strains of *O. glaberrima* were also scattered on the same regression line of reproductive effort on longitude. The other wild species showed lower values of reproductive effort (1–25%). *O. perennis* subsp. *barthii* appeared to allocate photosynthetic product mainly into vegetative organs including rhizomes.

In *O. punctata*, diploid (2n=24) and tetraploid (2n=48) forms were found. Seven strains collected in the Sahel zone near Lake Chad were diploids, while another strain obtained in a humid forest near Ibadan was tetraploid. All diploid strains were annual while the tetraploid strain was perennial. The diploid strains had short anthers, long awns, and a high reproductive effort which characterized predominantly self-pollinated annuals, as compared with the perennial strain. This suggests that the mode of differentiation in *O. punctata* is similar to that in *O. perennis* in which the occurrence of a perennial-annual continuum represents the main direction of differentiation.

Competition and Copper Tolerance in Two Sedges, *Cyperus difformis* and *Fimbristylis miliacea*

Hiroko MORISHIMA

Among major weed species of rice fields, *Cyperus difformis* (abbrevaited C) is most tolerant to copper while *Fimbristylis miliacea* (F) is least tolerant so far as their vegetative growth is concerned. Examinations of buried seed populations showed, however, that soil samples from copper-polluted fields produced more F plants than those from control fields (Morishima and Oka, Agro-Ecosystems **3**: 131, 1977). To see how their competitive relation in reproduction is affected by copper pollution, an experiment was carried out

as follows:

The seeds of C and F strains obtained from copper-polluted (P) and control (N) fields were mix-sown (1 : 1) to make the following four combinations, C(P)+F(P), C(N)+F(N), C(P)+F(N), and C(N)+F(P). They were sown on normal and toxic (200 ppm copper) soils filled in plastic trays with two replications, in 1977 spring. At maturity, plant number per tray and total dry weight were recorded for respective species. The seeds naturally sown on the same trays germinated in the next season (1978). The number and total dry weight of the second generation plants were recorded again at maturity.

On normal soil, F generally showed in different strain-combinations higher survivorship than C in the first generation. In the second generation, F exclusively dominated over C, its frequency ranging from 83 to 100 percent.

On toxic soil, however, C dominated in all strain-combinations in the first generation. In the second generation, C was dominant in C(P)+F(P) as well as in C(N)+F(N). F was dominant in C(P)+F(N) and C(N)+F(P). The data thus suggested that F was a strong competitor with C on normal soil and could replace C even on toxic soil in certain conditions.

Neighborhood Effects between Rice and Barnyard Grass Strains, I. Performance in Mixture and Aggressiveness as Influenced by Planting Density

Louis ASSÉMAT* and Hiko-Ichi OKA

In order to look into the effect of neighboring plants on growth performance at varying planting densities, two strains of rice (*Oryza sativa* L.) and two of barnyard grass (*Echinochloa crus-galli* Beauv.) were tested in a mixture-diallel experiment with three replications. Seedlings of the four strains were transplanted in rows radiating from a center in pure and mixed (alternate planting) stands in all possible combinations so as to test them under changing densities. In both pure and mixed stands, the effect of density on single plant dry weight and panicle weight could be represented by linear regressions of performance on density when the data were transformed into logarithms. A regression equation derived from Kira's equation, $w_{ij(d)}=\bar{w}_{ij}-b_{ij}d$, was used, where w_{ij} stood for single plant performance of strain i neigh-

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bored by j and d was the deviation of a given density from mean density (plant number per unit area), both in logarithms. The model used for estimating neighborhood effect was: $w_{ij}=\mu+g_i+h_{ij}$, where g_i stood for genotypic effect in pure stand and h_{ij} for the increment or decrement in i due to the aggression of j ($h_{ij}=0$). As the regression coefficient was modified by neighbors, it was shown by b_i+q_{ij}, where b_i stood for the coefficient in pure stand and q_{ij} for its change in mixture. When these models were combined, the neighborhood effect at given density was shown by $\bar{h}_{ij}-q_{ij}d$, where $\bar{h}_{ij}$ was the mean for all densities and $-q_{ij}d$ represented its density-dependent portion.

Analysis of variance of the data proved that the neighborhood effects estimated were in a greater part significant. The data showed that h_{ij} and q_{ij} were correlated, resulting in a tendency of neighborhood effects to decrease with increasing density. But there were a few exceptions to this trend. In many strain-combinations, h_{ij} and h_{ji} had inverse signs, but they were not always inversely correlated and the data indicated that mode of interference could change according to the combination of strains and planting density. Generally, the aggressiveness of barnyard grass was high at low densities at which the resistance of rice was lowered. The competition effect between barnyard grass strains more sharply decreased with increasing density than that between rice strains. Cooperative association effect was detected in a few strain-combinations at high densities.

Nieghborhood Effects between Rice and Barnyard Grass Strains, II. Experiments on the Mechanisms of Interaction between Plants

Louis ASSÉMAT and Hiroko MORISHIMA

Two strains of rice (A and B) and two of barnyard grass (C and D) were tested in pure and mixed stands in all possible combinations, with and without plastic pipes placed in the ground to keep the roots of each plant separate. After the data were transformed into logarithms, the neighborhood effects produced by the interaction of shoots and by that of roots were estimated on the basis of an additive model. Both shoot and root interactions appeared to play important roles in producing neighborhood effects. Generally, root interaction was cooperative while shoot interaction was antagonistic, with the exception of a case between rice strains in which root interaction was also antagonistic.

The neighborhood effects estimated from the planting density experiment with the same four strains (foregoing report) showed strong correlations with the percent cover by the leaves measured in pure stands 57 to 71 days after seed-soaking. This suggested that the above-ground growth rate as shown by percent cover was a determinant of neighborhood effects and root growth would be correlated with shoot growth.

A mix-planting experiment with early-sown and late-sown seedlings of rice strains (A and B) proved that the interference between the early and late plants of the same strain was magnificient. This suggested the role of variation in germination time in plant populations.

Increasing Frequency of *Drosophila simulans* in Yamanashi-ken

Takao K. WATANABE and Masaoki KAWANISHI

Since 1975, we have continued the collection of *Drosophila* flies around Mt. Fuji area to know the mode of invasion of *D. simulans* to Katsunuma (Yamanashi-ken) from the Pacific coast. Banana traps were placed for a week in early November to collect domestic or semidomestic species of *Drosophila*. The percents of *D. simulans* among *D. melanogaster* and *D. simulans* are listed in Table 1, since the two species seemed to compete the same niche.

Table 1. Percent of *D. simulans* among *D. melanogaster* and *D. simulans* (N)

Locality	1975		1976		1977		1978	
	%	N	%	N	%	N	%	N
1. Mishima	95.4	486	94.9	275	95.1	122	98.7	231
2. Susono	75.7	74	43.6	55	19.4	108	56.9	174
3. Gotenba	50.0	2	50.0	4	2.3	132	25.0	8
4. Fujiyoshida	0	10	0	6	0	38	17.4	23
5. Otsuki	0	8	0.5	412	0.4	231	2.4	208
6. Katsunuma								
A) Vineyard	0	850	0	1375	0	1332	0.1	2294
B) Shrine	—	—	—	—	0.7	288	7.0	431
7. Kofu	0	25	0	56	3.2	126	1.7	118
8. Nirazaki	0	21	0	163	0	450	0	365
9. Kajikazawa	0	15	0	142	1.3	301	1.7	423
10. Minobu	0	27	0	16	1.1	355	6.9	628
11. Tomizawa	38.5	13	0	9	4.3	137	33.0	176
12. Fujinomiya	98.5	199	98.9	189	91.8	355	97.3	111

In 1975 no *simulans* was collected in the northern and western areas of Mt. Fuji (4–10). But the invasion of *simulans* has occurred in the recent two years from the eastern side of Mt. Fuji from Mishima (1 to 4), the western side from Fujinomiya (12 to 9), and the west-bound route from Tokyo (5 to 7). Only Nirazaki (8) is now free from *simulans*.

An interesting difference in the frequecy was observed in Katsunuma (6). Two places (A and B) are a few hundred meters apart but the ecological niches are remarkably different. Vineyard (A) is surrounded by a winery and human houses with a lot of debris of grapes. Shrine (B) is nearby a highway surrounded by a lot of deciduous trees. *Drosophila melanogaster* was found predominantly in the former place, but *D. simulans* seemed to increase its population in the latter place.

Different Food Preferences in *Drosophila simulans* and *D. melanogaster*

Masaoki KAWANISHI and Won Ho LEE

Three kinds of fruit juice, orange (OR), grape (GR) and apple (AP), were used for collection of *Drosophila* in nature and for culture in laboratory. *D. simulans* was remarkably attracted by apple and grape juice while *D. melanogaster* preferred orange juice. On the other hand, relative number of *simulans* progeny (*simulans*/*melanogaster*) emerged from the mixed species vials containing each fruit juice medium was as AP>GR>OR which suggested that the food preferences of adults and larvae were basically identical in the present species.

Apple juice food was relatively advantageous to *D. simulans* in early days of laboratory cage life of mixed species population as well as in vials. However, *D. simulans* had been mostly eliminated from the mixed cage population within 100 days probably due to other factors than food preference. For details, see *Jap. J. Ecol.* **28**: 231, 1978.

XIV. APPLIED GENETICS

The Sporophytic Pollen Sterility in Rice: Its Genetic Basis and Intervarietal Relationships as Shown by F_2 Sterility

Hiko-Ichi OKA

The partial pollen sterility of true-breeding lines derived from a varietal hybrid of rice (*Oryza sativa* L.) was attributable to duplicate genes causing sporophytic sterility in certain homozygous combinations, in the same manner as set forth by Oka and Doida (1962) for seed sterility. The sporophytic pollen sterility was characterized by an instability of pollen development resulting in a large variance of pollen fertility among spikelets of the same plant. To examine the distributions of gametophytic and sporophytic sterilities in varietal hybrids, the F_1 and F_2 plants from crosses of 38 rice strains with 3 test-strains (an Indica, a Japonica, and an Asian annual strain of *O. perennis*) were observed for pollen fertility. The cytoplasmic male sterility did not seem to be involved in the material. The magnitude of sporophytic F_2 sterility was estimated by subtracting from the mean F_2 sterility an estimate of gametophytic F_1 sterility possibly transmitted to the F_2 population. Both the gametophytic and sporophytic sterilities of hybrids were involved in the Indica-Japonica differentiation of rice varieties, but they were not correlated among individual crosses. The crosses with the test-strain of *O. perennis* showed little sterility in both the F_1 and F_2 generations. This suggests that the wild progenitor—the Asian form of *O. perennis* would have dominant genes at many of duplicated loci and recessive mutations accumulated with domestication might have resulted in the differentiation of varietal groups like the Indica and Japonica types. (*Jpn. J. Genetics* **53**: 397, 1978)

Character Variations in Backcross Derivatives between *Oryza sativa* and *O. glaberrima*: M-V Linkage and Key Characters

Yoshio SANO and Hiko-Ichi OKA

To look into the mode of association of parental characteristics in the hybrid progeny of the two rice species, character variations were observed

in B_2F_1 plants and B_2F_6 (including a few B_1F_6) lines obtained from backcrosses between a *sativa* (Acc. 108, an Indica from Taiwan) and a *glaberrima* (W025, from Guinea) strain. The backcross derivatives had different coefficients of relationship to *glaberrima* (or to *sativa*) parent ranging from 1/8 to 7/8. Ligule length, regenerating ability of excised stem segments, and various other characters were recorded. The measurement of a plant, i, for a character was evaluated by $x_i=(X_i-\mu/d$, where $\mu=(X_s+X_g)/2$, $d=(X_s-X_g)/2$, and X_s and X_g stood for the parental *sativa* and *glaberrima* values, respectively.

The distributions of x values showed appreciable transgressiveness in panicle axis diameter and three other characters, particularly in the B_2F_6 population, as a result of gene recombination. Awn length also served as a measure of gene recombination since both parents were awnless. The standard deviation of x values for different characters was used as an index showing recombination of parental characteristics or "character discordance". These data suggested that parental genes could be recombined almost freely in this species hybrid. In ligule length, secondary branch number, and some other characters, however, B_2F_6 lines showing parental values ($x_s=1$; $x_g=-1$) were larger in number than those showing an intermediate value. It was also found that the lines showing interdemiate values tended to have a low seed productivity. Awn length was inversely correlated with seed productivity. Further between two of three independent marker genes involved in the cross, a tendency to restriction of recombination was detected. These facts were considered as indications of so-called M–V linkage.

Among the characters investigated, the probability of misclassification between *sativa* and *glaberrima* estimated from intra- and inter-specific variations was correlated with transgressiveness in the hybrid. Ligule length and secondary branch number per primary panicle branch, which seemed to be useful as key characters, were practically non-transgressive and were subjected to M–V linkage more obviously than other characters.

Gene Markers Obtained in *O. glaberrima*

Yoshio SANO

Among more than 500 strains of *Oryza glaberrima* maintained in our stock, only a few striking morphological differences useful for genic analysis are found. In order to more gene markers and compare their loci and

effects with those of similar genes known in *O. staiva*, the seeds of a strain of *O. glaberrima* selected for colorless pericarp and insesitivity to photoperiod were treated with EMS (*Ann. Rep.* **27**: 97). From about 7000 M_2 plants, a total of 96 mutants expressing characters like glutinous endosperm, "Daikoku" dwarfism, ligulelessness, long empty glume, and glabrousness were selected (*Ann. Rep.* **27**: 101). These characters were found to be each crossed with a strain of *O. staiva* (T 65) having a corresponding marker gene. The complementation test showed that the mutant strains of *O. glaberrima* carried genes similar to *wx*, d_1, *lg*, *g*, and *gl* known in *O. sativa*, respectively. There are 10 mutants showing "Daikoku" dwarfism. All of them had the same recessive gene, which was symboled d_1. The same was the case with 12 strains showing ligulelessness.

In *O. sativa*, the d_1 mutant is known to have the second internode (from the top) remaining non-elongated. In the *glaberrima* d_1 mutant, however, the second internode elongated. The F_1 plants between *sativa* d_1 and *glaberrima* d_1 strains also showed elongation of the second internode. This suggests either that the d_1 in *sativa* and d_1 in *glaberrima* differ in action, or that their effect is modified by the genetic background.

Mutant characters like brown leaf spots, narrow leaf, small grain, and brittle culm were also found to be controlled each by a recessive gene in the asme manner as found in *O. sativa*.

Sensitivity of Barnyard Grass, *Echinochloa crus-galli* to a Herbicide

Shinya IYAMA

Four lines of barnyard grass were selected out of the material collected from various parts of Japan regarding the sensitivity to the herbicide con-

Table 1. Sensitivity of parent lines and F_2 progenies of barnyard grass to propanil.

Parent line	% Survival	F_2		% Survival	Mid-parent % survival
93–5 (R1)	58.1	R1×S1	1	33.4	38.9
81–27 (R2)	61.8	"	2	37.8	
52–8 (S1)	19.8	"	3	35.3	
51–4 (S2)	38.6	S1×R2		40.8	40.8
		R1×S2	1	55.5	48.4
		"	2	47.1	

taining propanil. Crosses were made between lines showing high and low sensitivities and their F_2 seedlings were examined with regard to sensitivity. In percent survival after a spray of propanil, the F_2 means were almost equal to mid-parent values as shown in Table 1 suggesting that the sensitivity was controlled by genes with additive effects.

The Effect of Noise on Egg Laying Performance of Japanese Quails*

Takatada KAWAHARA

A wild strain of Japanese quail, *Coturnix coturnix japonica*, propagated for 12 generations in a domestic condition after being captured in the field, showed changes in various productive and behavioral characters toward the domesticated type. The wild strain was compared with a domestic strain with regard to their sensitivity to noise. The birds, 208 wild and 19 domestic, were reared under a noise treatment with buzzer, 95 phons, working one hour, and 12 times a day for 20 days. The daily photoperiod applied was 18-hour light and 6-hour darkness. Air temperature (25°±3°C), humidity (about 55%), and other environmental conditions were kept as con-

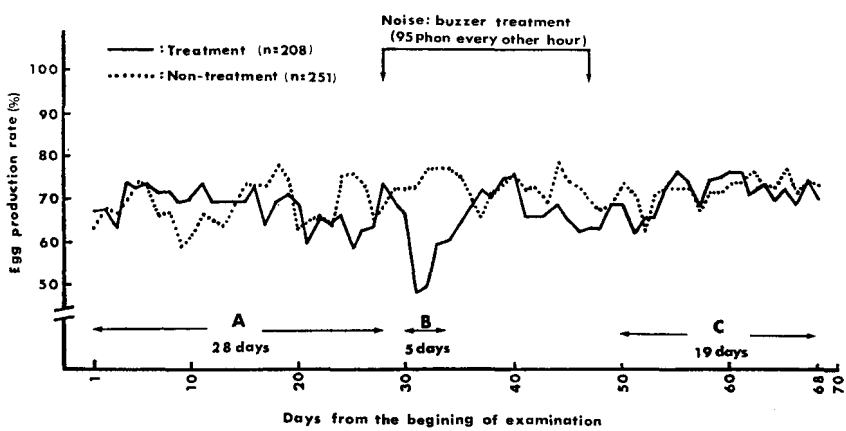

Fig. 1. Egg production curves in noise treated and noiseless control groups of wild strain.
A, egg production in the noiseless period before treatment; B, during noise treatment; C, in the noiseless period after treatment.

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stant as possible. The egg laying performance of the wild strain was reduced under the noise treatment, but the domestic strain showed no depression. The egg production rate of the wild strain decreased by 11.4 percent (66.9% in the noiseless period before treatment, A in Fig. 1 and during noise treatment, B in Fig. 1), while that of the domestic strain did not change (84.8% in the noiseless period befoe treatment and 85.3% during noise treatment).

In the wild strain, the egg production rate was recovered in 9 days even though the noise tratment continued for 20 days. Its egg production rate was recovered by 14.8 percent (55.5% during noise traetment, B in Fig. 1 and 70.3% in the noiseless period after tratment, C in Fig. 1). Heritability of the reactions of wild strain to noise treatment, estimated from the components of variance, was 0.08 in the reduction rate of egg production as shown by the difference between periods A and B; it was 0.11 in the recovery rate of egg production as shown by the difference between periods C and B. In both reactions, genetic variance was much smaller than environmental variance.

A Dark Frayed Feather Nervous Disorder Mutant of Japanese Quail

Takatada KAWAHARA

During the course of propagation of a domestic foundation stock of Japanese quail, *Coturnix coturnix japonica*, which has a plumage pattern and other characters of the wild type. A female variant was found in the early summer of 1975 from the foundation stock. The mutant birds obtained from the F_2 of the female variant had following characteristics. 1) Dark down and adult plumage; 2) frayed flight feathers; 3) a nervous disorder characterized by a tremor of small amplitude. These characters appeared in chicks of one day old, but the condition of nervous disorder was variable among individuals and differed according to growing stages and environmental conditions. The mutation was semi-lethal and viability up to 20 weeks after hatching was about 40%. Mortality was caused by dislocation of the hip joint. The mutant birds had lower values in body growth, fertility, hatchability, age at sexual maturity and egg production rate than the normal birds.

The results of various matings indicate that this mutant is due to a new

autosomal recessive gene, symboled *dn*. Changes in these three characters, *i.e.*, dark plumage, frayed feathers and variable magnitude of nervous disorder indicate some change in the development of organs of ectodermal origin. The recessive gene may be a chromosomal dificiency or translocation although it is not detected yet.

Effects of Noise on the Learning Performance of Mouse Strains with Different Learning Abilities and their Hybrid*

Tohru FUJISHIMA

To examine the effect of noise on the avoidance learning performance in inbred strains and hybrids, crosses were made between two inbred strains, C3H/HeMs and SWM/Ms. The former showed the highest discriminatory ability while the latter showed the highest avoidance avility (*Ann. Rep.* **25**: 82). These strains and their F_1 mice were exposed to one-hour "pink" noise of 100 phons six times at one-hour intervals every night for a period of one or three weeks, and they were tested with an automated Y-maze apparatus in which a buzzer and a lamp were used as the conditioned stimuli and an electric shock as the unconditioned stimulus.

In the first training session after a one-week or three-week exposure to noise, the mice showed no remarkable depression in the avoidance learning performance. However, an additional one-week exposure to noise resulted in an inhibition of consolidation of avoidance learning effects in the parental inbred strains. The hybrid mice showed no such response. The data also showed no acclimatization to noise in the activity and memory effect.

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Nitrogen Fixation in the Rhizosphere of Rice

Taro FUJII, Yoshio SANO, Sinya IYAMA and Yukinori HIROTA

(a) *Nitrogen fixation in rice strains.*

We have proved nitrogen fixation in rice due to association of certain bacteria in the rhizosphere by the use of an improved experimental technique (*Ann. Rep.* **28**: 114). For more reliable evaluation of nitrogen fixing activities, we observed the relation between nitrogen fixing activity and plant age and diurnal variation, and recognized the following points. (1) Nitrogen fixing activity was evident even at an early stage of growth and

increased gradually with aging. The activity reached the maximum at the haeding stage, and after holding a high value for about 2 weeks it decreased with ripening. (2) Nitrogen fixation by algae in the paddy field is often included in that due to rhizosphere bacteria resulting in over-estimation of bacterial nitrogen fixing activity. In our experiment, we covered soil sufrace in the pot with alminum foil to hinder algal growth by shading. Yet, we observed appreciable nitrogen fixing activities. (3) Nitrogen fixing activity showed diurnal variation. Activity was highest in the early morning and evening, and was lowest around noon and mid night. (4) To observe the genetic control of nitrogen fixing ability in rice strains, strain C5444 showing a high activity was crossed with a standard strain T65, which was analyzed for different genes and had a low or moderate nitrogen fixing activity. Eighty F_3 lines from the cross were assayed for acetylene-reducing activity. The lines showed a wide variation in the activity which appeared to be transgressive. The data suggested that the nitrogen fixing ability was controlled by more than one gene and that new strains having higher nitrogen fixing activity would be selected from varietal hybrids.

(b) *Nitrogen fixing bacteria in the rhizosphere of rice*

A 250 g sample of soil in which rice roots extended was stirred in 200 ml of sterilized water, kept for 2 days at 30°C, and 1 ml of supernatant was suspended into 10 ml of nitrogen-free liquid medium containing 10 g/l of glucose and 2 μg/ml of biotin. The medium was incubated at 30°C for 2 days in darkness either in aerobic or in anaeroic condiaion. An appreciable acetylene reducing (nitrogen fixing) activity was observed in both conditions showing the presence of nitrogen fixing bacteria in the medium. In corraboration with Dr. K. Komagata of Institute of Applied Microbiology, Tokyo University, nitrogen fixing bacteria were isolated on a nitrogen-free medium or on a malate medium. By this method, a *Clostridium* strain was obtained previously. This year, several nitrogen fixing bacteria of spiral, filamentous, or rod shapes were isolated and the strans were purified. The experiment indicated that at least six species of nitrogen fixing bacteria were present in the rhizosphere of rice.

An inoculation experiment was carried out with rice plants raized in non-sterilized and germ-free soil conditions in test tubes into which a *Clostridium* strain was added. The non-sterilized plants showed an appreciable increase in acetylene reducing activity after the inculation of *Clostridium*. This indicated the contribution of this bacterium to nitrogen fixation in the rice

rhizosphere. The germ-free plants showed no acetylene reducing activity even of the bacterium was inoculated. This suggested that the soil became unfavorable for the bacterial growth as the results of sterilization at 120°C.

In order to look for bacterial species capable of more efficient nitrogen fixation in the rice rhizosphere, the germ-free plants grown on nitrogen-free agar medium in test tubes were inoculated with bacteria isolated from the rhizosphere. *Clostridium* could grow very well under this condition, so far examined.

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ABSTRACTS OF DIARY FOR 1978

February	14	249th Meeting of Misima Geneticists' Club
	23	250th Meeting of Misima Geneticists' Club
	24	137th Biological Symposium
June	29	251st Meeting of Misima Geneticists' Club
July	10	252nd Meeting of Misima Geneticists' Club
	11	138th Biological Symposium
	24	139th Biological Symposium
August	10	140th Biological Symposium
September	19	141st Biological Symposium
October	4	253rd Meeting of Misima Geneticists' Club
November	21	142nd Biological Symposium
December	6	143rd Biological Symposium

FOREIGN VISITORS IN 1978

January	12	RAJAN, S. S., c/o United Nations, Rangoon, Burma.
	28	JAIN, Ashok, Embassy of India, Tokyo.
	31–Feb. 1	BROCK, R. D., Plant Breeding and Genetics Section, FAO IAEA, Austria.
February	5–March 6	SCHWARZ, Uli, Max-Planck-Institut, Germany.
	28–Mach 6	SHARMA, G. P., Panjab University, India.
March	7	Lee, SE-YONG, Korea Atomic Energy Res. Inst. Korea.
March	13–21	CROW, James F., University of Wisconsin, U.S.A.
	14	LAMSEEJAN, S., Atomic Energy Lab., Kasetsart University, Bangkok, Thailand.
March	24	MANEEPHONG, Chairerg, Kasetsart University, Thailand.
April	27	BALAL, M. S., Field Crops Res. Inst., Agricutural Research Center, Giza, Egypt.
May	11	KLINGBERG, M. A., Israel Inst. for Biol. Res., Ness-Ziona & Tel-Aviv University Medical School, Israel.
	11	KLINGBERG, W., Israel Inst. for Biol. Res., Ness-Ziona & Tel-Aviv University Medical School, Israel.
	12–Feb. 1, '79	KASTURI BAI, A. R., Bangalore University, India.
	18	SHADAKSHARASWAMY, M., Bangalore University, India.
	23	KAO Nan Kuo, Prairie Regional Lab., National Research Council of Canada, Canada.
	23	SORIANO, J. D., University of the Philippines, Philippines.
June	18–Sept. 12	MARCUM, Beverly A., University of California, Irvine, U.S.A.
July	20	MANKAD, P. G., Embassy of India, Tokyo.

August	7–8	SHELDON, Bruce, C.S.I.R.O., Sydney, Australia.
	8	WARID, Warid A., University of Cairo, Egypt.
	10–11	KING, R. C., Northwestern University, U.S.A.
	28–29	JUKES, T. H., University of California, U.S.A.
September	17	KRISHNASWAMI, S., Central Sericulture Research and Training Institute, Mysore, India.
	17	NARASIMHANNA, M. N., Central Tasar Research Station, Ranchi, India.
	18	A representative team of Chinese biologists, Academia Sinica, Peking, China.
	26–27	SHAHI, B. B., Parwanipur Agriculture Station, Nepal.
Ocotober	11	HSUEH, YU-KU & others, Chinese Delegation of Food Science 8 Technology, China.
	19	BAKER, Herbert G., University of California, U.S.A.
November	18–22	STEVENS, Leroy C., Jakson Laboratory, U.S.A.
December	8	BRENNER, S., University Postgraduate Medical School, Cambridge, England.

AUTHOR INDEX

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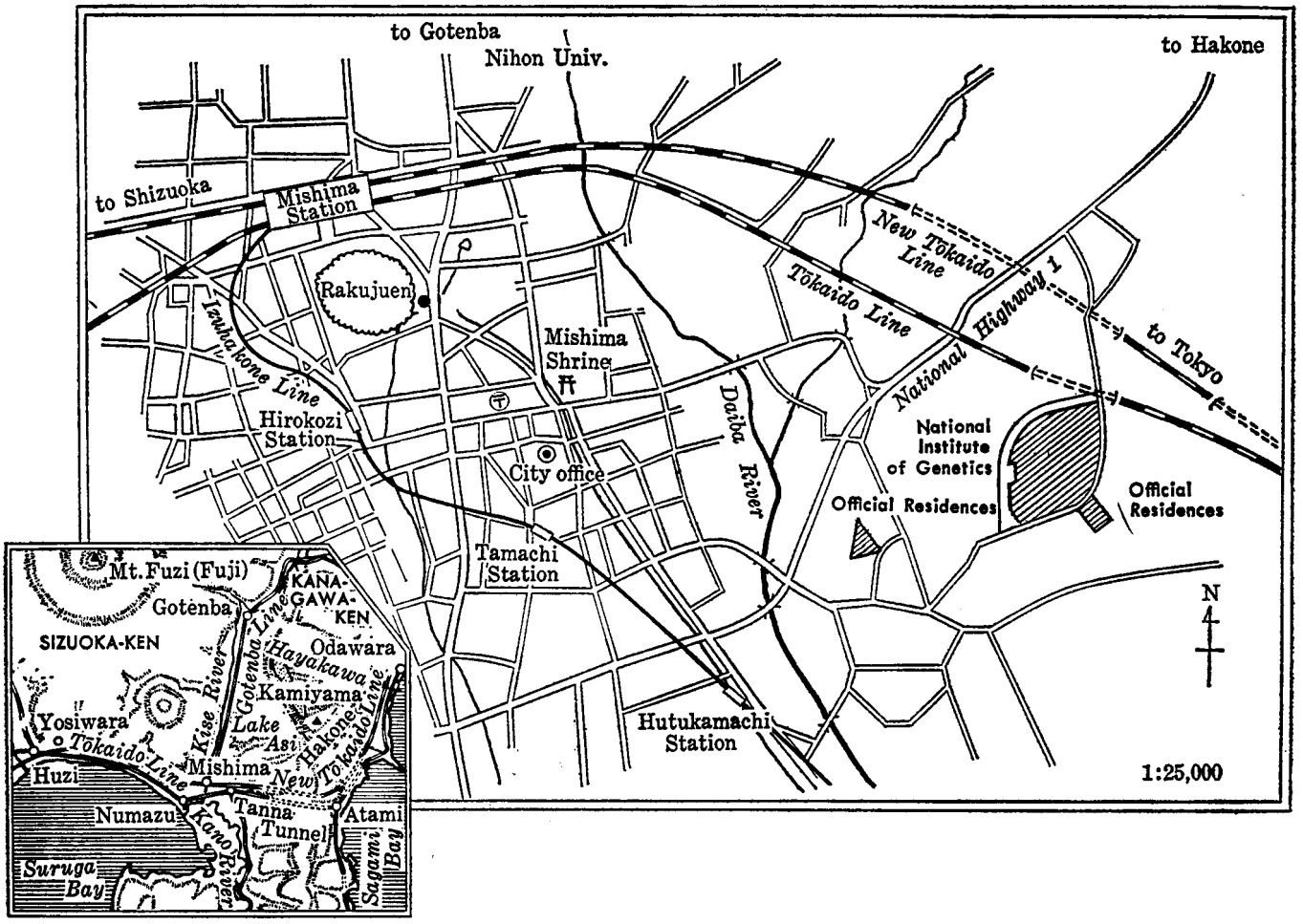
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