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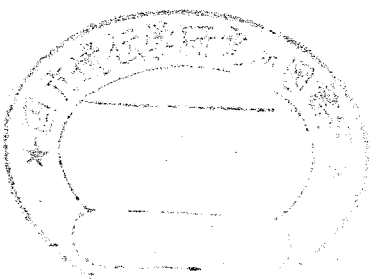

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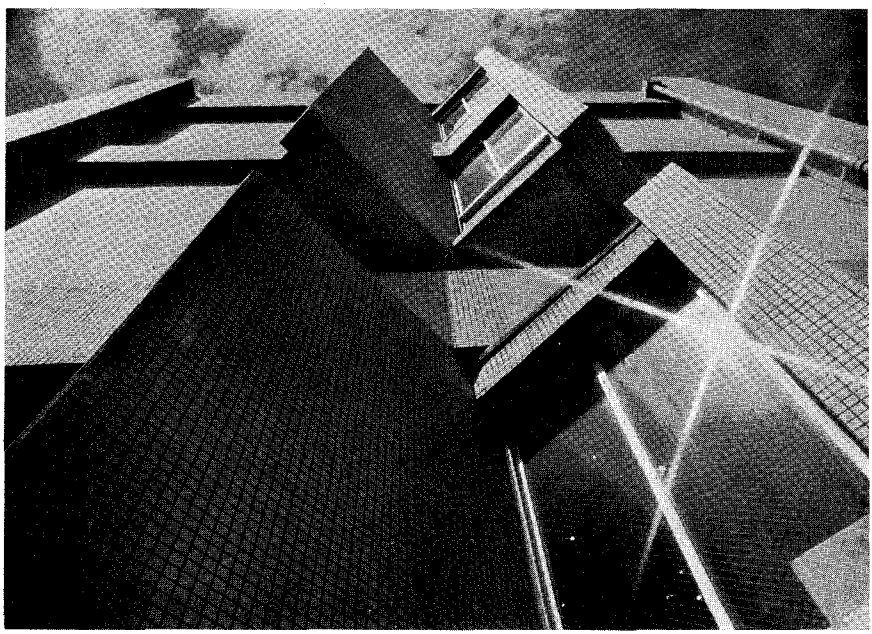

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GENERAL STATEMENT

June 1st of this year was the 30th anniversary of the foundation of the Institute. Wishing to make this occasion the turning point to further development of the Institute, and to thank those who exerted their endeavors for the realization of the Institute as well as those who supported and encouraged us at all times, we planned the following colourful program.

1. Commemoration ceremony and reception
2. Memorial lecture
3. Publication of "Memoir in the Days of the Foundation"
4. Collection of important documents relating to the foundation
5. Memorial planting

The commemoration ceremony was a great success, attended by men of distinction both in and out of the government agencies and institutions: among them attendance of Mr. Keijiro Inai, the Vice-Minister of Education, Science and Culture; Prof. Koji Fushimi, President of the Science Council of Japan; Prof. Seiji Kaya, former President of the University of Tokyo; Prof. Takashi Fujii, member of the Science and Technology Council, honoured us extremely.

Thanks to the contributions from Drs. Seiji Kaya, Kiyoshi Masui, Hitoshi Kihara, Yosito Sinoto, Kiyoshi Okano, Heihachiro Miyayama and many others, the commemorative publication "Memoir in the Days of the Foundation" was full of valuable articles. It would never be possible for such articles to be contributed again from so many authoritative sources concerned. The highlight among them was Prof. Kaya's article, in which Prof. Kaya, the then Director of the Science Education Bureau of the Ministry of Education, Science and Culture, described realistically the progress of the negotiation between the General Headquarters (GHQ) and the Ministry of Education, Science and Culture, in regard to the permission of the foundation of the Institute. In answering the expectation of those farsighted leaders concerned with the establishment of the Institute we renewed our resolution to endeavor all our efforts for further development of our science.

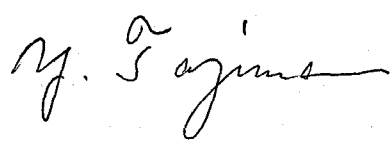
Commemorative lectures were given by Dr. Takashi Sugimura, Director of the National Cancer Center Research Institute, and Dr. James F. Crow, Professor of the University of Wisconsin. Both of them talked to

us enthusiastically and most impressively about their recent works entitled "Mutagenicity and carcinogenicity in our environment," and "Hybrid disgenesis and other strange genetic phenomena," respectively.

We are much indebted and grateful to those persons and corporations who gave us financial support for achieving the above commemoration program.

As to personal change in the staff, Dr. Chozo Oshima, Head of the Department of Physiological Genetics, retired on April 1st. Dr. Takeo Maruyama was appointed the new head. Dr. Oshima affiliated to the Institute in May, 1957, in succession to Dr. Taku Komai. Since then, Dr. Oshima had made his efforts as a leader of *Drosophila* study group to develop population genetics and behavioral genetics of *Drosophila.* By the retirement of Dr. Oshima, all the heads of the three departments which existed at the time of the founding have been brought to the third generation, and the staff members have been quite rejuvenated.

The main building of the Genetic Stocks Center was completed in March and the staff members have all moved to the new building and started their activities there. The construction of the attached laboratories for mice and silkworms were completed in this fiscal year.



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OSHIMA, Chozo, Manager,

PROJECTS OF RESEARCH FOR 1979

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)
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 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* (WATANABE and TAKAMURA)
Evolutionary and ecological genetics of *Drosophila* (WATANABE and KAWANISHI)
Population genetics of *Drosophila* (WATANABE, INOUE, and TSUNO)

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)
Genetic studies in wild populations of Japanese quails (KAWAHARA)
Behavioral genetic studies in mice (FUJISHIMA)
Theoretical studies on breeding techniques (IYAMA)
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)
Molecular mechanisms of unicellular differentiation in *Bacillus subtilis*

(SADAIE)
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 mechanisms 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)
Genetic studies on 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)
Studies on the primary structure of DNA (SOEDA and MIURA)
Studies on structure and function of messenger RNA (MIURA)
Genetical and enzymological studies on *E. coli* polymerase (SUGIURA)
Studies on T_4 RNA ligase (SUGIURA)
Cloning of eukaryotic 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)
Cytogenetic studies of Norway rats and establishment of the chromosomal mutant stocks (YOSIDA)
Experimental breeding of wild rodents for establishment of the new experimental animals (YOSIDA)
Developmental genetic studies on mouse teratomas (NOGUCHI)
Studies on chromosomal polymorphism in Drosophila (INOUE)
Analysis of fibroin genes of silkworm and its relatives (KUSUDA)
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 1979

I. MOLECULAR GENETICS

Relation of the Structure of Cytoplasmic Polyhedrosis Virus and the Synthesis of its Messenger RNA

Kazumori YAZAKI and Kin-ichiro MIURA

Cytoplasmic polyhedrosis virus (CPV) was observed in an electron-microscope using a combination of staining and shadowing methods to see genome nucleic acid and virus core protein simultaneously. With this method projections not only in positions horizontal to the grid, but also vartical were clearly visualized. When the particle was mildly disrupted with EDTA, genome double-stranded (ds)RNA was released from a projection. If the particle was previously fixed with glutaraldehyde and then disrupted, dsRNA was released with a protein particle, which seemed to correspond to the base part of the projection. The protein particle was in most cases at the end of the strand, which sometimes takes a supercoiled structure. When CPV was incubated in medium adequate for mRNA synthesis, swelling or deformation of projections was observed. After CPV was incubated during mRNA synthesis, it was fixed by glutaraldehyde and disrupted by EDTA treatment succesively. Genome dsRNA was released from a virion carrying a protein particle. In this case, protein particles were attached not only to the edge of a strand, but also at various positions along the strands. From these observations, we suggest that transcription in this virus particle proceeds as follows: Genome dsRNA is transcribed by passing through the base part of the projection, where the enzymes for mRNA synthesis are located. A completed mRNA is released from the virion at the projection.

Process of Cap Formation of Messenger RNA by Vaccinia Virus Particles Carrying an Organized Enzyme System

Toshiyuki URUSHIBARA and Kin-ichiro MIURA

Vaccinia virus mRNAs carry the cap structure $m^7G^{5'}pppAm$- or $m^7G^{5'}$-

pppGm- at the 5′-terminus, which is synthesized by a series of RNA polymerase and capping enzymes contained in the virus particle (Annual Rep. No. 25 (1974) p. 14; Urushibara, T., Furuichi, Y., Shimotohno, K. and Miura, K.: FEBS Lett. **49** (1975) 385). The process of the cap formation at the 5′-terminus of mRNA was studied using an *in vitro* system under similar conditions to these of vaccinia virus multiplication in its host cell. After adding a methyl-group donor, [methyl-^{3}H] S-adenosylmethionine, the oligonucleotides, which were the *de novo* synthesized 5′-terminal part of mRNA, were isolated from the RNA-synthesizing virion at appropriate time intervals, and were analyzed. The 5′-5′ confronting nucleotides with 2′-0-methylation, G$^{5'}$pppAm and G$^{5'}$pppGm, were found with the completed cap structure, m^{7}G$^{5'}$pppAm and m^{7}G$^{5'}$pppGm. The confronting nucleotides with only 7-methyl guanine as a methylated component, m^{7}G$^{5'}$pppA and m^{7}G$^{5'}$pppG, were not detected at any incubation time, and it was concluded that methylation at the 2′-position of the 5′-terminal purine nucleoside of mRNA precedes methylation of the 7-position of the blocking guanosine. This result is different from that obtained using the enzymes isolated from vaccinia virus (Moss *et al.*, 1976) and also from the results obtained using other kinds of virus particles, which carry RNA polymerase and capping enzymes. These differences may be due to the specific organization of a series of capping enzymes and RNA polymerase in each virus particle.

Relation of Stability and Presence of the Cap Structure of mRNA

Kazuko YAMAGUCHI and Kin-ichiro MIURA

On incubation of mRNA in a wheat germ extract, the mRNA lacking 5′-cap quickly degraded from the 5′-terminus in an exonucleolytic way, whereas the intact mRNA remained stable. These results show that one of the cap functions is to stabilize the mRNA to prevent its degradation by exonuclease attack (Annual Rep. No. 27 (1976) p. 20. Shimotohno, K., Kodama, Y., Hashimoto, J. and Miura, K. Proc. Nat. Acad. Sci. USA **74**: 2734 (1977)). In addition to an enzyme removing the 5′-blocked structure in RNA, an exonuclease, which degrades mRNA from the 5′-terminus successively, was found in wheat germ extract (Annual Rep. No. 28 (1977) p. p. 14). As the activity of protein synthesis in a cell depends on the state of mRNA, enzyme activity being concerned to stability of mRNA was compared in various kinds of cell.

CP virus mRNA that was labeled uniformly with [^{3}H]UTP was prepared in vitro (Annual Rep. No. 22 (1971) p. 12, Shimotohno, K. and Miura, K.: J. Biochem. **74** (1973) 117). A part of samples was then decapped with tobacco pyrophosphatase (Annual Rep. No. 27 (1976) p. 18, Shinshi, H., Miwa, M., Sugimura, T., Shimotohno, K. and Miura, K.: FEBS Lett. **65** (1976) 254). Either the native mRNA or the decapped mRNA was incubated respectively with extract from various kinds of cell to assay degradation of mRNA. There are two types for kinetics of RNA degradation. In the extracts of wheat germ, baby hamster kidney cell and erythrocyte cell in bone marrow, the decapped mRNA was degraded faster than the native one. However, in the extracts of muscle, liver, and reticulocyte, the native mRNA was degraded at the same rate as the decapped mRNA. Since reticulocyte was differentiated from erythrocyte in bone marrow cell, the state of enzymes relating to the stability of mRNA may change according to differentiation.

Presence of Single-stranded Region Adjacent to the Protein-binding 5′-termini of Adenovirus Genome Double-stranded DNA

Hiroyoshi ARIGA, Hiroto SHIMOJO, So HIDAKA
and Kin-ichiro MIURA

The structural features of a linear duplex DNA of adenovirus are an association of a protein at the 5′-termini of either strand. The replication of adenovirus DNA proceeds through a displacement mechanism. The origin and terminus of replication has been localized near the molecular termini. However, the mechanism of the start of replication at the 5′-termini remains unclear. From these points, the structure of the region near the 5′-termini is required to be clarified.

The [^{3}H] thymidine-labelled Adenovirus 5 (Ad 5) DNA-protein complex was prepared. On the other hand, Ad 5 DNA-[^{125}I] protein complex was prepared from cold Ad 5 DNA-protein complex by Bolton-Hunter reagent of ^{125}I. Both the samples were digested with various kinds of enzyme. The digests were analyzed by the following methods: gel electrophoresis, filter binding assay, Benzoylated-Naphthoylated DEAE (BND)-cellulose column chromatography, and gel filtration using Bio-gel A 1.5 m. With deoxyribonuclease or micrococcal nuclease digestion, DNA was degraded and protein was released. With proteinase K or pronase digestion, protein was

degraded and DNA was released. Since no change was observed in DNA-protein complex with ribonuclease treatment, ribonucleotides do not participate in linking of protein and DNA. The protein was separated from DNA by nuclease S1 under the conditions for single-strand cleavage. Either DNA or protein remains in intact size with this treatment. This suggests that a protein-binding 5′-terminal part of DNA is in a short single-stranded state as written below.

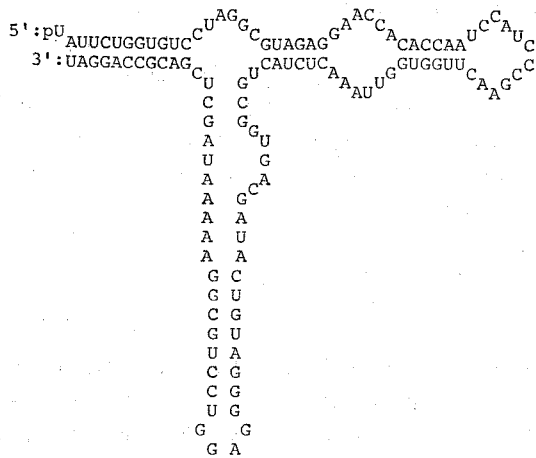
The presence of the similar single-stranded stretch at the 5′-terminus was also observed in some *Bacillus subtilis* phages, which carries a protein at the 5′-terminus of DNA also (Hirokawa, et al). These specific structures may play a common role for the initiation of replication of these DNAs. This work was published in FEBS Lett. **107** (1979) 355–358.

Purification of T4 RNA Ligase and its Application to 5S RNA Sequencing

Masahiro SUGIURA, Mie SUZUKI and Fumio TAKAIWA

A new method is developed for purifying the RNA ligase induced in phage T4-infected *E. coli* cells. It was found that T4 RNA ligase could bind to 2′, 5′-ADP Sepharose in the presence of Mg^{2+} but not in its absence. 2′, 5′-ADP Sepharose chromatography was introduced at the last step of the purification procedure. The purified RNA ligase preparation gave one major band on SDS-acrylamide gel electrophoresis and was estimated to be over 80% pure. The purified enzyme had no detectable RNase activity and was suitable for the joining of short oligoribonucleotides as well as RNA sequencing.

Tobacco chloroplast 5S RNA was purified by two cycles of polyacrylamide gel electrophoresis and labeled at its 3′-end with ^{32}P using the T4 RNA ligase and [5′^{32}P]pCp. The [3′^{32}P] 5S RNA was sequenced from the 3′-end by the chemical method. On the other hand, the 5S RNA was labeled at its 5′-end with ^{32}P using T4 polynucleotide kinase and [γ^{32}P]ATP and then sequenced from the 5′-end by the enzymatic method. The complete nucleotide sequence is shown as a possible secondary structure constructed

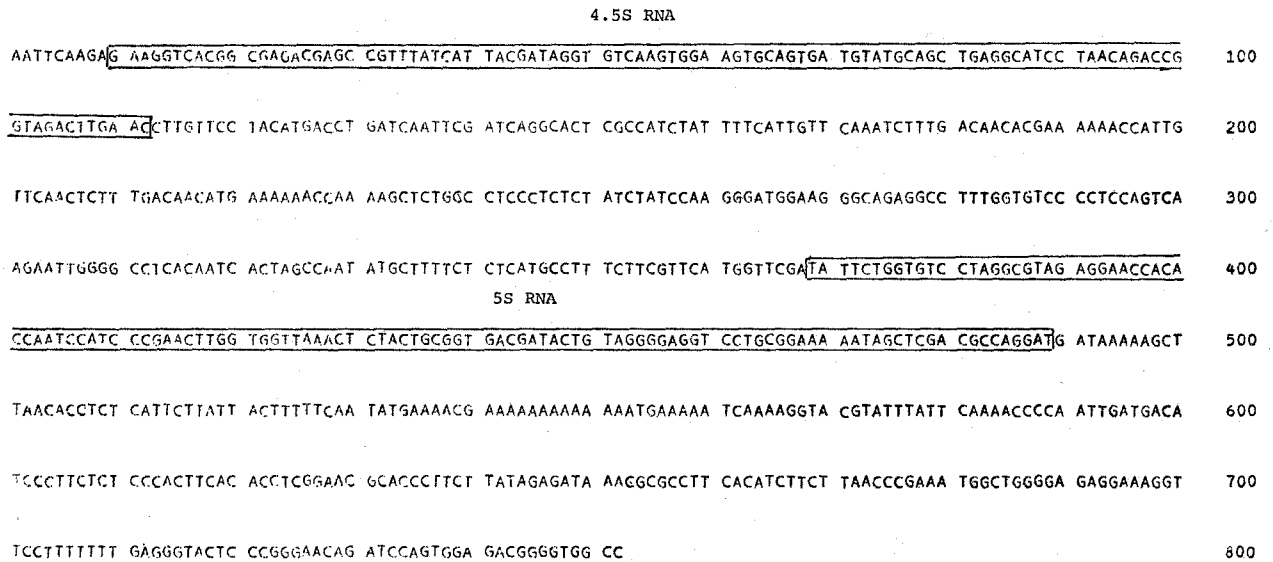


according to Hori. Tobacco chloroplast 5S RNA is 121 nucleotides long and its sequence resembles that of 5S RNA from blue-green algae. T4 RNA ligase purification was carried out with E. Ohtsuka, S. Nishikawa, H. Uemura and M. Ikehara in Osaka University and this work was published in FEBS Letters **97**: 73–76 (1979).

Organization and Expression of the Ribosomal RNA Gene Cluster of Tobacco Chloroplasts

Kazuo SHINOZAKI, Jun KUSUDA, Fumio TAKAIWA,
Naoki TOHDOH and Masahiro SUGIURA

We have cloned tobacco chloroplast rRNA genes using plasmid pMB9 as a vector. Recombinant plasmids pTCl, pTC309, pTC7, pTCP6, and pTCP243 contain most of 16S gene, part of 16S and 23S genes, 4.5S and 5S genes, 16S and 23S genes, and all four genes, respectively. Using these plasmid DNAs, a fine physical map of the chloroplast rDNA was constructed by cleavage with various restriction endonucleases and hybridization of the restriction fragments with ^{32}P-labeled 16S, 23S, 4.5S and 5S RNAs. The order of these genes is 16S-23S-4.5S-5S. The precise location of the 16S and 23S rRNA genes was determined by annealing rRNA to linear pTCP6 DNA and observing the R-loops in an electron microscope. No intervening sequences were detected in these genes. The sizes of coding regions


4.5S RNA
AATTCAAGAG AAGGTCACGG CGAGACGAGC CGTTTATCAT TACGATAGGT GTCAAGTGGA AGTGCAGTGA TGTATGCAGC TGAGGCATCC TAACAGACCG 100
GTAGACTTGA ACCTTGTTCC TACATGACCT GATCAATTCG ATCAGGCACT CGCCATCTAT TTTCATTGTT CAAATCTTTG ACAACACGAA AAAACCATTG 200
TTCAACTCTT TGACAACATG AAAAAACCAA AAGCTCTGGC CTCCCTCTCT ATCTATCCAA GGGATGGAAG GGCAGAGGCC TTTGGTGTCC CCTCCAGTCA 300
AGAATTGGGG CCTCACAATC ACTAGCCAAT ATGCTTTTCT CTCATGCCTT TCTTCGTTCA TGGTTCGATA TTCTGGTGTC CTAGGCGTAG AGGAACCACA 400
5S RNA
CCAATCCATC CCGAACTTGG TGGTTAAACT CTACTGCGGT GACGATACTG TAGGGGAGGT CCTGCGGAAA AATAGCTCGA CGCCAGGATG ATAAAAAGCT 500
TAACACCTCT CATTCTTATT ACTTTTTCAA TATGAAAACG AAAAAAAAAA AAATGAAAAA TCAAAAGGTA CGTATTTATT CAAAACCCCA ATTGATGACA 600
TCCCTTCTCT CCCACTTCAC ACCTCGGAAC GCACCCTTCT TATAGAGATA AACGCGCCTT CACATCTTCT TAACCCGAAA TGGCTGGGGA GAGGAAAGGT 700
TCCTTTTTTT GAGGGTACTC CCGGGAACAG ATCCAGTGGA GACGGGGTGG CC 800

for the 23S and 16S genes are 1.4×10^6 and 0.84×10^6 daltons, respectively. The 16S and 23S genes are separated by a 1.3×10^6 dalton spacer. There is a 0.45×10^6 dalton spacer between the 23S and 4.5S genes. Using plasmid pTC7 DNA, the DNA sequences of the 4.5S and 5S RNA genes and their surrounding regions were determined according to the method of Maxam and Gilbert (see the figure). The coding regions of the mature 4.5S and 5S RNAs are composed of 103 and 121 base pairs, respectively, and the two genes are separated by the 256 base pair spacer.

The 16S, 23S, 4.5S and 5S RNAs are coded for by the same DNA strand and the direction of transcription is from 16S to 5S. *In vitro* Transcription experiments were carried out using the 1.9×10^6 dalton fragment isolated from pTCl and *E. coli* RNA polymerase. Two RNA species with distinct sizes were synthesized and both RNAs hybridized only to the coding strand. This suggests that *E. coli* RNA polymerase can transcibe properly the chloroplast rRNA genes. Parts of this work were presented at the Ozi International Seminar on Genetics and Evolutionary Aspects of Transcriptional and Translational Apparatus held at Tomakomai on September 1979 and were published in Molec. Gen. Genet. **178**: 1–7 (1980), Gene **10**: 95–103 (1980) and Japan. J. Genet. **55**: 121–125 (1980).

Heterogeneity of the Ribosomal RNA Gene Clusters in Rice

Kiyoharu OONO and Masahiro SUGIURA

Rice nuclear DNA was isolated from rice embryos (*Oryza sativa* L. var. Mangetsumochi). The DNA was digested completely with EcoRI and the fragments were separated by 0.7% agarose gel electrophoresis. Two distinct bands could be identified in an ethidium bromide stained gel and the size of these bands was estimated to be 5.2×10^6 and 5.0×10^6 daltons. The DNA fragments in the gel were transferred to nitrocellulose filters and hybridized with rice 25S and 17S rRNAs labeled with ^{32}P. Both intensities of an ethidium bromide stained photograph and of an autoradiograph in the 5.0×10^6 dalton band were higher than those in the 5.2×10^6 dalton band. This indicates that the two fragments are not arranged alternatively and that there is length heterogeneity in rice rDNA populations. From relative intensities of the two bands and the known amounts of λ EcoRI fragments as internal standard, the population of the 5.2×10^6 and 5.0×10^6 dalton bands was estimated to be approximately 0.08% and 0.15% of the total

DNA, respectively. This work was published in Chromosoma (Berl.) **76**: 85–89 (1980).

Structure of Harvey Murine Sarcoma Viral DNA: Nucleotide Sequence of the Terminal Repeats

Eiichi SOEDA and Malcolm MARTIN*

The 5.4 kbp Harvey murine sarcoma virus (Ha-MuSV) RNA genome is composed of a 4.5 kbp insert of rat sequences flanked on the 5′ and 3′ termini by 0.1 kbp and 0.9 kbp of Moloney murine leukemia virus, respectively. The rat sequences contain transforming genes.

The supercoiled DNA intermediates were extracted from NIH 3T3 mouse cells infected with Ha-MuSV and cloned in λgt·WES λB vector system. Three classes of Ha-MuSV DNA inserts were isolated with 6.6, 6.0 and

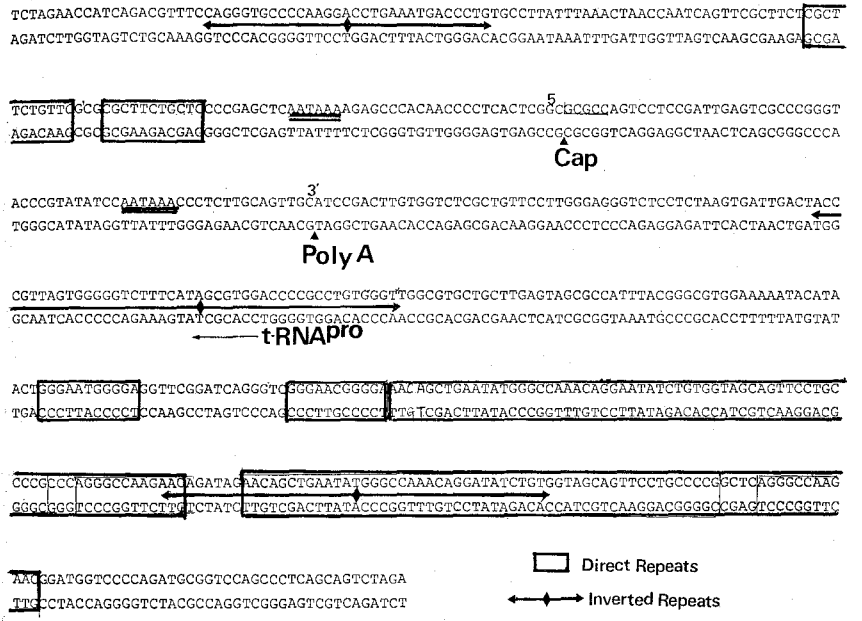


Fig. 1. Nucleotide sequence of the terminal repeats of Ha-MuSV DNA intermediates.

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5.4 kbp long. Restriction enzyme digestion and electromicroscopic examination have indicated that the 5.4, 6.0 and 6.6 kbp Ha-MuSV inserts contain one, two and three copies, respectively, of nucleotide sequences derived from the termini of the RNA genomes.

We have deduced the complete nucleotide sequence of the terminal repeats by the method of Maxam and Gilbert and compared with that of Moloney sarcoma virus DNA integrated into the mink genome (R. Dhar personal comm.).

The terminal repeat sequence is 583 nucleotide long and identified corresponding to minus stop DNA, putative RNA promoter, CAATAAAAG, and signal polyadenylation, AATAAA (Fig. 1). An almost perfect 68 bp duplication is present in the region where the viral DNA is integrated into the host genome as a provirus.

Structural Analysis of Fibroin Genes of Silkworm and its Relative by *in situ* Hybridization

Jun KUSUDA, Kimiji ONIMARU, Sakae SAKATE†, Yoshiaki SUZUKI* and Yataro TAZIMA

Fibroins produced by *Antheraea pernyi* and *Antheraea yamamai* are mainly composed of glycine and alanine as is known for those of *Bombyx mori*. These two amino acids comprise more than 70% of total amino acids in *Antheraea* fibroin, but contents of amino acids other than glycine and alanine vary with species.

Suzuki *et al.* assumed that the fibroin gene of *Bombyx mori* comprises predominantly guanine and cytosine since corresponding codons for glycine and alanine were known to have high G+C contents.

Futher, they were able to comfirm this assumption by sequencing a part of coding region of this gene after cloning to *E. coli*.

From this finding, fibroin genes of *Antheraea* species are considered to be rich in guanine and cytosine and may be homologous, to some extent, to that of *Bombyx mori*. If we could determined the nucleotide sequences of the fibroin genes of related silkworm strains, it seems feasible to estimate, therefore, the phylogenic relationship among the fibroin genes of those silkworm species.

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At first, we attempted to examine the homology of the fibroin gene of *Antheraea pernyi* to that of *Bombyx mori* by *in situ* hybridization using the cloned DNA, as a probe, containing the fibroin gene of *Bombyx mori.*

The DNA extracted from posterior and middle silk glands of the 5th instar larvae of *Antheraea pernyi* was completely digested with EcoRI and separated on 1.5% agarose gel. DNA fragments were transfered to nitrocellulose filters according to Southern's method and hybridized to the cloned DNA (pFb29) which was labelled with ^{32}P by nick translation. Radioactive probe hybridized to single fragment with molecular weight of 2.9×10^6 daltons, but the extent of hybridization was not so completely.

From these results, it was shown that the fibroin gene of *Antheraea pernyi* has some homology to that of *Bombyx mori.*

A New Method for the Construction of a Restriction Map Based on End Assignment

Yutaka TAKEDA, Masao YAMADA and Yukinori HIROTA

Construction of restriction map of DNA is one of the necessary steps in the cloning of a desired gene and in the determination of nucleotide sequence. Many works including restriction map of DNAs have been reported. The authors digested the DNA with many restriction enzymes, measured molecular weight of the restriction fragments, puzzled about arrangement of the fragments and then constructed the restriction map. They sometimes used partial digestion methods, fragment isolation and labeling of ends of larger fragment.

The usual method for construction of a restriction map is mainly based on the precise measurement of molecular weight of restriction fragments. However, there is uncertainty in the measurement of molecular weight by gel electrophoresis especially in the case of the fragments with larger or lower molecular weights, because the standard curve is not linear.

If we can know which restriction enzyme produces the end of each fragment, it will give great benefit for construction of a restriction map. We found that the ends of restriction fragments are easily assigned to restriction enzyme by triple digestion. We report in this communication a systematic procedure for construction of restriction map based on the assignment of end.

Procedure and rule

1) Choose 3 kinds of restriction enzymes, each of which cuts the DNA at several sites. Let us assume A, B and C as restriction enzymes, and n_A, n_B and n_C as numbers of cutting sites of the restriction enzyme in the DNA respectively. The restriction fragments are numbered in the decending order of molecular weight, i.e. A-1, A-2, ... A-n_A in the case of cutting with restriction enzyme A. DNA is digested by all the possible combinations of 3 restriction enzymes, and run in agarose or acylamide gel electrophoresis. For easiness of comparison of restriction fragments, we recommened to use the slab system for gel electrophoresis. This method can be extended so as to use 4 or more restriction enzymes, but the number of digestion markedly increases: i. e. 7 digestions are needed for 3 enzymes, 15 for 4 enzymes and 31 for 5 enzymes. Here we show the case of using 3 kinds of restriction enzymes. The DNA is singly digested with each of A, B and C, doubly digested with A+B, B+C and C+A, triply digested with A+B+C, and then the restriction fragments obtained by the above 7 different digestion mixtures are separated by gel electrophoresis.

2) Correspond the restriction fragment appeared in triple digesion to the fragment in single or double digestion, and then determine which restriction enzyme produces the ends of each restriction fragment taking into consideration of rules described below.

rule 1. When a circular DNA such as a plasmid DNA is digested with a restriction enzyme A, fragments as many as n_A are generated. Fragments as many as n_A+n_B, and $n_A+n_B+n_C$ are generated when a circular DNA is doubly digested with restriction enzymes A and B (A+B), and triply digested with A, B and C (A+B+C), respectively.

rule 2. Fragments obtained by doubly digesting with restriction enzyme A and B are classified into 3 with respect to the nature of the end. One has both ends cut by A (A-A), another has both ends cut by B (B-B), and the other has one A end and one B end (A-B). We designate the fragment with both ends cut by the same restriction enzyme, such as A-A and B-B, as a homofragment, and the fragment with the ends cut by difierent enzymes, such as A-B, as a heterofragment. Homofragments appearing in the double digestion can correspond to the fragment appearing in the single digestion. On the other hand, heterofragments do not correspond to any

fragments appearing in single digestion. It is worthy of saying that the reverse of this proposition is not always true. Any fragments obtained double digestion whose molecular weight is different from that in single digestion must be heterofragments, but the fragment in double digestion whose molecular weight is the same as that in single digestion is not always homofragment except the fragment with lowest molecular weight among that in double digestion.

rule 3. Fragments obtained by triple digestion with A+B+C must appear in at least one of other 6 digestions. This fact is based on the following fact. Fragments obtained by triple digestion with A+B+C are classified into at most 6 with respect to the nature of the ends: i.e. A-A, B-B, C-C, A-B, B-C and C-A. If a homofragment is obtained by triple digesion, the fragment must appear not only in single digestion but also in double digestion. For example, if a A-A fragment appears in triple digestion with A+B+C, the same fragfragment also appears in A, A+B and A+C, because there is neither B nor C site in the A-A fragment. If a heterofragment is obtained by triple digestion (i.e. A-B), it also appears in double digestion with A+B, but not in any other digestions.

rule 4. In each digestion, the sum of the number of ends cutted by a restriction enzyme (i.e. by A) is twice number of site cut by the restriction enzyme i.e.n_A). This rule helps you to find mistakes happened during assignment of end of fragment to enzyme. This rule is also useful to correspond fragments between different digestion when salts in reaction mixture make slight divergence in the mobility of restriction fragments in gel electrophoresis.

3) Determine whether or not there are sites cut by another restriction enzyme on the restriction fragment by single or double digestion.

rule 5. If certain fragment obtained by double digestion (i.e. A+B) is intact after triple digestion, the fragments do not have C site. On the other hand, if the fragment dissappeared by triple digestion, the fragments must have more than one C sites. When the fragment has more than two C sites, at least one C-C fragment appears in triple digestion.

4) Determine the arrangement of each restriction fragment so as to form a circle.

rule 6. Cutting by restriction enzymes produces smaller fragments than

original fragment. In other words, each fragment obtained by triple digestion is derived from larger fragment appeared in single or double digestion than this, except that the fragment is corresponded to some fragment in single or double digestion.

rule 7. The end cut by a restriction enzyme must be connected with the end of other fragment cutted by the same enzyme.

How to arrange the fragment depends on each case. It is easy way to begin to arrange the fragments around the largest fragment in triple digestion. Another easier way is to begin to connect the fragment at the site by the enzyme whose cutting site is few.

Precharacterization of ends of restriction fragments is convenient marker for their arrangement. We applied this method to determine the restriction map of pLC 39–47, and the result will be reported in the journal (Takeda *et al.* Plasmid, in press).

II. MICROBIAL GENETICS

A Model for Distribution of Division Sites in *E. coli*

Masao YAMADA, Takeo MARUYAMA and Yukinori HIROTA

When rod shaped bacterial cells, such as *Escherichia coli*, grow, they elongate and then divide at around the longitudinal center of the bodies. The mechanism reponsible for site determination is one of fundamental issues to be solved.

Mathematical model

We have advanced a mathematical model accounting for the division site, which appears to be consistent with presently available experimental facts. The model is essentially based on Gierer and Meinhardt's pattern formation system. (Gierer, A. and H. Meinhardt. 1972 Kybernetik **12**: 30) We assume two substances, say A and H. Substance A stimulates production of A itself, and H inhibits A. Thus we may call A activator and H inhibitor. In reaction field the two substances differ greatly in their rates of difusion: H is a fast diffusing material and A is slow. We assume the reaction of these two substances is governed by the following pair of differential equations:

$$\frac{\partial A}{\partial t}=\rho+C\frac{A^2}{H}-\mu A+D_a\frac{\partial^2 A}{\partial x^2}$$

$$\frac{\partial H}{\partial t}=CA^2-\nu H+D_h\frac{\partial^2 H}{\partial x^2} \qquad (1)$$

where t and x are time and space variables, D_a and D_h are diffusion coefficients, μ and ν are decaying constants, and ρ is a *de novo* production rate of A. The above equations are nonlinear and difficult for analytic solutions. As in Gierer and Meinhardt, we studied the behavior of the system by numerical method using a computor. It was confirmed that the system leads to formation of high peaks of A in the field and the peaks are separated from each other with a certain distance. Importantly the pattern of the peaks of A is determined entirely by the values of para-

meter appearing in equation (1), but independent of the initial values. In relation to the cell division, the peaks of A can be regarded as division sites.

Experimental result

Cells of temperature sensitive filament former, *fts*, grow and divide normally at nonpermissive temperature as wild type cells. However, at nonpermissive temperature they cannot divide and only form long filaments. Some of the *fts* mutants, such as *fts*A, *fts*E and *fts*I, recovered the ability to divide when cultural temperature was shifted to permissive temperature after 1 or 2 hr preculture at nonpermissive temperature. In a viscous medium, daughter cells could not move apart, so that the site of division could be scored by measuring each cell length in one clone. A typical division site found in long filaments was shown in Fig. 1 A and B. Distance between the septation site and the adjacent cell end is not constant but rather proportional to total length of the clone. We obtained the filaments with various length by varing the cultural period at nonpermissive temperature. Filaments with double length divided at central position only. Filaments with 4-folds length divided into either 1 : 3 or 1 : 2 :1. Most filaments with 8-folds divided into 1 : 7, 1 : 6 : 1, 1 : 2 : 4 : 1 or 1 : 2 : 2 : 2 : 1. Filaments with intermediate length between 4 and 8, divided either 4-folds type or 8-folds type. No other dividing pattern was observed. According the extension of cell length, the division pattern changed discontinuously.

We compared the simulation results of high peaks of A appearing in a field of certain length to the actual division sites formed in bacteria of equivalent length. A typical case of the comparison is given in Fig. 1. Incidentally, when the model was simulated for a small field where at most one peak of A is possible, it always formed at around the middle. On the other hand, a slightly larger field permitted to form two peaks of A and they were at both sites 25% of the field length apart from each adjacent end. According the increase of the length of simulated field, the pattern changed discontinuously. The results obtained by simulation are in agreement with the division pattern of filament cells.

The following two assumptions imposed upon our present model seem to be very critical. One is that at the boundary we assumed A is absorbed, but H is reflected. This assumption is necessary for the pattern of peaks being independent of initial condition. Another critical assumption is $D_a \ll D_h$, which is needed to form sharp and stable peaks of A. To have such peaks,

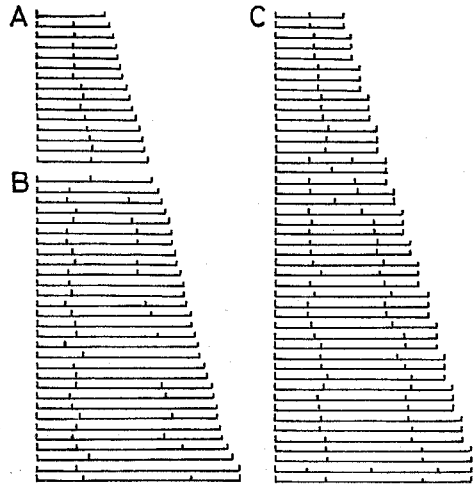


Fig. 1. Comparison of experimental results with simulated results. Left: Division sites observed in filament cells. Cells of PAT84 (*fts*A) were incubated at 42°C for 30 min (A) or 60 min (B), and then cultured at 30°C. Division occurred 20 or 30 min later and the sites were measured under microscope. Each horizontal line represents one clone and dots represent division sites observed. Right: The result of simulation by computor. A set of parameter was substituted into the equation (1) and calculated with computor. Each horizontal line represents the length of the field and dots represent the sites of stable peak of A.

it is necessary to assume the value of D_h is one hundred times of D_a or greater. It is rather difficult to assume such two kinds of molecules to have a great difference in diffusion coefficient in the identical medium. This can however be possible if H diffuses in cytoplasm, while A in membrane.

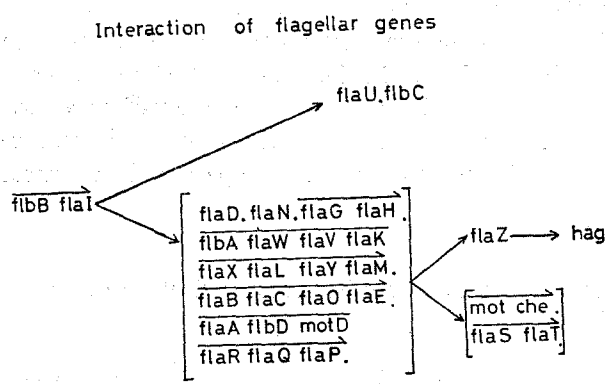
We clearly establish the mechanism in which even fluid material determines specific site in the cell and any rigid apparatus is not necessary essential.

Transcriptional Control of Flagellar Genes in *Escherichia coli* K-12.

Yoshibumi KOMEDA

Previous studies have defined 29 genes necessary for the synthesis of *Escherichia coli* flagellar apparatus. This study analyzed the transcriptional interaction of flagellar genes using the Mu-d (Ap^r, *lac*) phage (Casadaban,

M. J. and S. N. Cohen. 1979 PNAS **76**: 4530) insertion mutants. The flagellar mutants in which the phage were inserted were operon fusion mutants of flagellar genes to the *lac* genes. They allowed the measurement of flagellar operon expression by detection of beta-galactosidase activity. In these mutants, the synthesis of the enzymes occurred constitutively. The promoters whose operons coded for constituent proteins of a flagellum showed higher activity. The lambda transducing phages carrying these chromosomal *fla-lac* fusions were also isolated. They enabled us the extensive examination of Fla$^-$ effect onto expression of each flagellar operon. This result shows that flagellar operons are divided into six classes: (1) *flbB* operon, that controls all of the other flagellar genes. (2) *flaU* and *flbC* operons, that are controlled by the *flbB* operon gene products and are not responsible for other expression. (3) *flbA* operon, *flaG* operon, *flaD* operon, *flaN* operon, *flaB* operon, and *flaA* operon, that are under *flbB* operon control and are responsible for other expression. (4) *flaZ* operon, that is controlled by the gene products of the obove (1) (3) operons and is responsible for *hag* transcription. (5) "mocha" operon, *flaS* operon, that are controlled by the gene products of the above (1) (3) operons. (6) *hag* operon. The Fla$^-$ effects can be converted to a scheme for interaction of flagellar genes using following assumptions. (i) If an "A" gene defect results to failure of "B" operon expression (examined by the expression of *lacZ* on λ"B"-



lac phage), the "A" product is required for transcription of the "B" operon and the "B" operon is situated after "A" in the sequence. (ii) If "C" operon is not expressed on hosts carrying a "D" gene defect and an "E" gene defect and "E" is situated after "D" as shown in (i), the interaction sequence is drawn as D→E→C. The scheme of interaction of flagellar genes was thus drawn as shown below. The assumption of (i) does not specify the interaction mechanism with molecular terms and would be accessible. In second assumption, we currently have no way to descriminate this sequential effect from another multiple effect. This assumption has practically no problem until we can define molecular mechanism. The sequence in the figure has two exceptional genes, *flaE* and *motD*. They were the members of those operons which were controlled by the *flbB* operon genes. Unlike the other members, the *flaE* defect had no effect to expression of group (5) operons and the *motD* defect had no effect for transcription of any of the operons at all. Taking into account of the observations of flagellar precursors by electron microscopy (Suzuki, T. and Y. Komeda, unpublished), the sequence obtained as above was consistent with an assembly pathway.

III. BIOCHEMICAL GENETICS AND IMMUNOGENETICS

Geographical Distribution of Two Groups in Japanese Wild Population of Medaka, *Oryzias latipes*

Mitsuru SAKAIZUMI, Nobuo EGAMI[1] and Kazuo MORIWAKI

The medaka, *Oryzias latipes*, is a freshwater fish native to Japan and adjacent region. In the previous report it was suggested that the Japanese wild population of medaka is divided into two large groups by allozyme study (Sakaizumi *et al.* 1980). After that samples from several localities were appended, and more detailed distribution of these two groups was found.

Adult fish were collected from 31 different localities in Japan as follows (number of samples examined in parenthesis): Aomori (8), Hirosaki (5), Niigata (3), Ryotsu (3), Joetsu (3), Kaga (5), Ichinoseki (1), Fujisawa, Iwate-ken (1), Kesen-numa (1), Iwaki (1), Urizura, Ibaraki-ken (5), Mito (3), Tsunezumi, Iabaraki-ken (3), Hokota (3), Shimodate (3), Sakura (5), Odawara (2), Mishima (5), Shizuoka (1), Iwata (1), Toyohashi (5), Ueno (5), Tanabe (5), Okayama (5), Tottori (1), Matsue (1), Hamada (1), Tsuma, Oki Is. (1), Tokushima (1), Kazusa, Nagasaki-ken (5), Reihoku, Amakusa Is. (5). Enzymes in each individuals were analyzed by acrylamide slab and agarose gel electrophoresis.

Geographical distribution and allele frequency of enzymes are shown in Table 1. This result indicates that Japanese wild population of medaka is divided into two groups, one is the fish collected from 6 localities (Aomori, Hirosaki, Niigata, Ryotsu, Joetsu and Kaga) in northern coast of the Japan Sea (Northern population), and the other is the fish from the rest part of Japan (Southern population). In addition, the heterozygosity of northern population is extremely low compared with that of southern population, as shown in esterases, octanol dehydrogenase (*Odh*) and acid phosphatase (*Acp*). These findings suggest that these two populations were isolated each other in fairly ancient times.

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Table 1. Observed allele frequencies at the polymorphic loci of two large populations of Japanese wild medaka

Locus	Allele	Northern population[1)]	Southern population[2)]
Acid phosphatase	a	1.0	.703
(*Acp*)	b	—	.293[3)]
Alcohol dehydrogenase	a	—	.957
(*Adh*)	b	1.0	.043[4)]
Esterase-1	a	1.0	.200
(*Es-1*)	b	—	.645
	c	—	.155
Esterase-2	a	—	.071
(*Es-2*)	b	—	.179
	c	1.0	.750
Esterase-3	a	1.0	.278
(*Es-3*)	b	—	.622
	c	—	.100
Octanol dehydrogenase	a	—	.064
(*Odh*)	b	.759	.786
	c	—	.150
	d	.241[5)]	—
Phosphoglucomutase	a	1.0	.007
(*Pgm*)	b	—	.993
Solbitol dehydrogenase	a	.056	1.0
(*Sdh*)	b	.944	—
Superoxide	a	1.0	—
dismutase (*Sod*)	b	—	1.0

1) Twenty-seven individuals from 6 localities.
2) Seventy individuals from 25 localities.
3) All of the fish from Tanabe and Okayama were Acp-b.
4) Adh-b was found only in the fish from Toyohashi in southern population.
5) Odh-d was found only in the fish from Aomori.

The Purification and Characterization of H-2 Antigens of Japanese Wild Mouse

Toshihiko SHIROISHI, Kazuo MORIWAKI and Takuji TAKEUCHI[1)]

Hitherto, many investigators have tried to caracterize the molecular structures of H-2K, D gene products. Furthermore, comparison of primary structures of H-2 antigens between two independent haplotypes have been

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made by trypsin-digested peptide mapping and amino acid sequence analysis, considering extraordinary H-2 porymorphism. In those studies, however, H-2 antigens were isolated only from the established laboratory mouse and European wild mouse, Mus musculus domesticus, genetically related to the laboratory mouse. In the present study, we attempted to analyze the molecular structures of H-2 antigens of Japanese wild mouse, Mus musculus molossinus, genetically remote from the laboratory mouse, for elucidating the difference of H-2 molecular structures from that of the laboratory mouse. As the sources of H-2 antigens, we used spleen cells of the following strains; B10.BR, B10. D2, B10.MOL.Sg and B10.MOL.YkB. The latter two strains are congenic lines in which H-2 region on chromosome 17 of Japanese wild mouse was introduced into B10 genetic background. Alloantisera utilized for immunoprecipitation are listed in Table 1. The procedure used for the

Table 1. Alloantisera used for immunoprecipitation

Serum designation	Reciepient	Donor	Antigens detected
D-23	(B10×LP.RIII)	B10.A(2R)	H-2K.23
D-4	(B10.AKM×129)	B10.A	H-2D.4
MS-1	(B10.D2×C3H.NB)	B10.MOL.Sg	undefined
MS-2	(B10.D2×C3H.NB)	B10.MOL.YkB	undefined

isolation and purification of H-2 antigens pricipally followed Cillen and Schwartz (1976) with a minor modification.

SDS-polyacrylamide gel electrophoresis of the immunoprecipitates from B10.BR, B10.D2 and B10.MOL. Sg exhibited H-2-like major peak (Fig. 1; a, b, c). Though both B10.D2 and B10.MOL. Sg showed a single peak, it was not demonstrable on B10.MOL. YkB precipitate, instead broad peaks corresponding to molecular weight from 25,000 to 45,000 were observed (Fig. 1d). The major peak demonstrated on B10.BR and B10.D2 precitates were similarly 45,000 dalton, being consistent with many other reports. The peak of B10.MOL. Sg precipitate was approximately 50,000 dalton, indicating the same position as marker protein (heavy chain of human IgG) on gel pattern. This peak is apparently H-2 gene product of B10.MOL.Sg strain, because alloantiserum used for the immunoprecipitation was produced by immunization in which both donor and reciepient mouse have common B10 background with except of H-2 region. It is, therefore, not likely that any surface molecules contaminated the precipitate through puri-

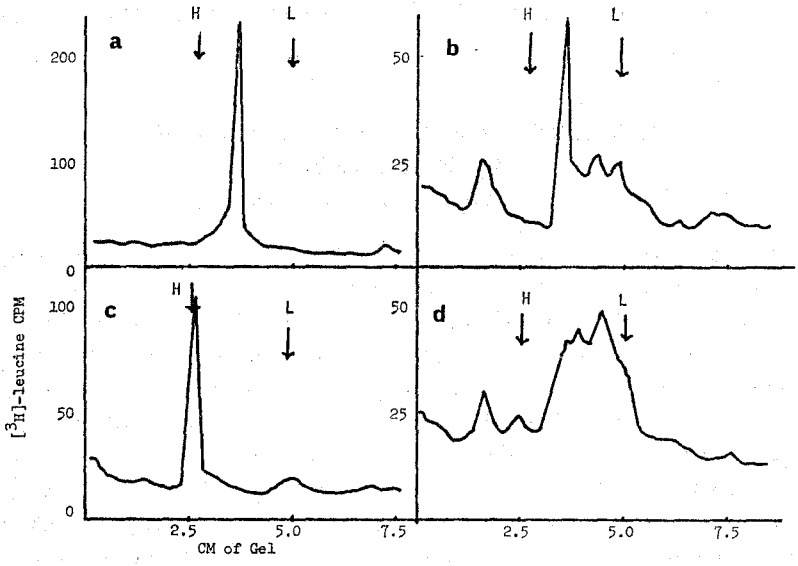

Fig. 1. SDS-polyacrylamide gel electrophoresis patterns of immunopresipitates from lysates of [^{3}H-]-leucine labelled spleen cells. H and L represent the positions of heavy and light chains of human IgG electrophoresed on the same gels. a B10.D2, b B10.BR; c B10.MOL.Sg, d B10. MOL. YkB.

fication procedure. These results led us to conclusion that the molecular size of H-2 antigen of B10.MOL. Sg is about 50.000 dalton larger than that of B10.BR and B10.D2 and, probably, many other laboratory mice. Since the intact H-2 antigen solubilized from cell menbrane is glycoprotein, there are two possibilities for the difference of molecular size between two kinds of H-2 antigens mentioned above. First, peptide part is responsible for the difference. And second, sugar part of the antigens contributes the disparity of the molecular size. To examine which possibility is the case remains unsolubled.

The rason that H-2 peak was not demonstrated in B10.MOL.YkB precipitate could not be clarified in the present study. The electrophoresis of the precipitate showed the peaks of 35,000 and 25,000 dalton, which seem to be two subunits of Ia antigen encoded by H-2 complex region. Interestingly, cytotoxicity test against the B10.MOL. YkB lymphonode cells indicated that this strain does not carry most of the Ia antigenic specificities observed in

many other strains (unpublished data). It seems to be concerned with the present result.

H-2 Antigenic Specificities in B10·MOL H-2 congenic Mouse Strains

Kazuo MORIWAKI, Toshihiko SHIROISHI and Tomoko SAGAI

Since 1975 we have developed several B10·MOL H-2 congenic strains by repeated backcrosses of the mice carrying No. 17 chromosome of Japanese wild mouse (*Mus musculus molossinus*) to B10·BR and B10·D2 strains. As some of them has come to congenic or semicongenic condition, we tried to determine their H-2 specificities using alloantisera produced in the laboratory inbred strains. Table 1 summarizes the reactivity of B10·MOL H-2 congenic strains in hemagglutination test against H-2 antisera produced in inbred strains. As already reported by us (Moriwaki *et al.*, J. Immunogenet. **6**: 97, 1979), H-2.5 showed relatively higher activity. H-2.8 was quite strong in these congenic strains differing from the previous population survey. B10·MOL Okb exhibited no activities at all. In all of those strains, we could rarely detected higher activity of private specificities.

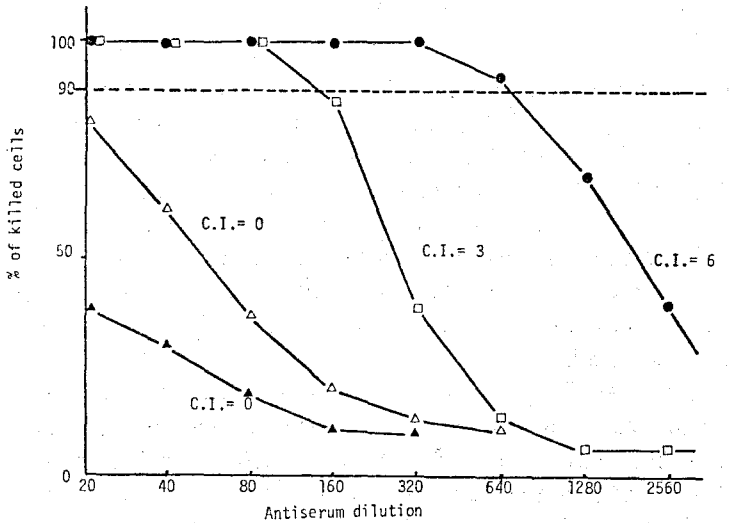

Fig. 1.

For the cytotoxicity test, we employed a newly developed flat plate method (Shiroishi *et al*, In Methods of Immunological Experiment, in press, in Japanese). The value of cytotoxicity activity of H-2 antisera is given by the following cytotoxicity index (*C.I.*):

$$C.I. = \mathrm{Log}_2 N/10$$

Where N is the highest antiserum dilution that killed more than 90% of cells. This index is 0 at N less than 20. Fig. 1 demonstrates an example.

Cytotoxic activity against B10·MOL H-2 congenic mice is summarized in Table 2. Again, several public specificities as H-2.1, 3, 5, 35 and 8 were quite strong, but not many private ones were detected. It is noteworthy that each *molossinus* H-2 chromosome showed positive reaction in either one of K or D end as to the known private specificities.

Moreover, B10·MOL Okb exhibited definitely several H-2 specificies in spite of their complete absence in hemagglutination test. This suggests that type I and type II on the erythrocytic H-2 expression could be controlled by MHC.

Table 1.

B10, MOL-H-2 congenic lines	H-2 antigenic specificities Public								Private				
	K, D					K			$H\text{-}2^b$ K	$H\text{-}2^d$ K	$H\text{-}2^s$ D	$H\text{-}2^r$ K/D	$H\text{-}2^f$ D
	1	3	5	28	35	8	11	25	33	31	12	18	9
B10.MOL. TeA	0	0	3	Nt	Nt	3	8	0	1	0	1	1	0
B10, MOL. TeB	0	0	2	Nt	1	8	0	Nt	1	0	1	0	0
B10. MOL. Sg	1	5	4	0	0	0	8	0	0	0	0	0	0
B10. MOL. Okb	0	0	0	0	6	0	0	Nt	0	0	0	0	0
B10. MOL. YkB	0	0	4	Nt	0	8	0	Nt	1	0	0	0	6
B10 congenic lines carrying H-2 haplotypes of inbred strains	4[1]	8[1]	6[2]	5[1]	3[1]	8[3]	2[1]	3[1]	2[4]	Nt	4[5]	4[6]	6[3]

The value of reactivity is given by following hemagglutination index (*H.I.*). *H.I.*=Logt *N*/10, where *N* is the highest antiserum dilution that hemagglutination is observed. Nt=not tested. 1)=B10.A, 2)=B10.BR, 3)=B10.M, 4)=B10, 5)=A.SW, 6)=B10.RIII.

Table 2.

B10. MOL-H-2 congenic lines / congenic lines	H-2 antigenic specificities: Public K, D					Public K			Public D	Private H-2^b K	H-2^b D	Private H-2^d K	H-2^d D	Private H-2^s K	H-2^s D	Private H-2^q K	H-2^q D	Private H-2^k K	H-2^k D	Private H-2^r K/D	Private H-2^f D
	1	3	5	28	35	8	11	25	13	33	2	31	4	19	12	17	30	23	32	18	9
B10.MOL.TeA	1	0	2	0	5	6	1	0	0	3	0	0	0	0	0	0	0	0	0	0	0
B10.MOL.TeB	2	0	2	0	6	6	1	0	0	3	0	0	0	0	0	0	0	0	0	1	0
B10.MOL.Sg	4	5	5	1	0	5	2	3	0	0	0	0	0	0	1	0	0	0	0	2	0
B10.MOL.Okb	0	3	0	2	4	3	0	0	0	1	0	5	0	0	0	0	0	0	1	1	0
B10.MOL.YkB	0	0	1	0	0	6	0	0	0	1	0	0	0	0	0	0	0	0	0	0	5
B10 congenic lines carrying H-2 haplotypes of inbred strains	4	6	5	3	7	4	0	3	5	6	4	7	5	2	5	4	4	3	6	5	5
					B10.A	(H-2^a)				(B10)		(B10.D2)		(A.SW)		(DBA/1)		(B10.BR)		(B10.RIII) (B10.M)	

Relationship between Laboratory Mice and the Subspecies *Mus musculus domesticus* Based on Restriction Endonuclease Cleavage Patterns of Mitochondrial DNA

Hiromichi YONEKAWA*, Kazuo MORIWAKI, Osamu GOTOH*, Junko WATANABE*, Jun-Ichi HAYASHI*, Nobumoto MIYASHITA, Michel L. PETRAS** and Yusaku TAGASHIRA*

The mitochondrial DNAs of house mouse, *Mus musculus* in twenty-five different laboratory strains showed identical cleavage patterns on digestion with ten different restriction endonucleases (Table 1). These cleavage patterns were identical to those of Canadian wild mice, *M. m. domesticus* but clearly different from the patterns of three Asian subspecies, *M. m. bactrianus*, *M. m. castaneus* and *M. m. molossinus* (Fig. 1). Based on comparison of the cleavage maps of the four subspecies, the time of devergence between *M. m. domesticus* and three Asian subspecies was estimated to be

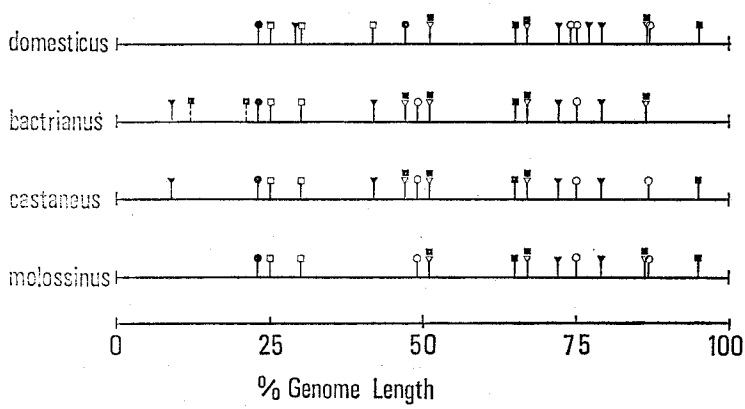

Fig. 1. Clevage maps for mtDNAs from four subspecies of mice Cleavage sites for individual restriction enzymes are identified by vertical lines topped with the following symbols ▼ BamHI, ○ EcoRI, ■ HindII, □ HindIII, ● PstI and ▽ HpaI. Positions of one of HindII sites are not certain in the maps of *M. m. bactrianus*. Possible sites in this case are represented by vertical dashed lines topped with the appropriate symbols. The linear map is arranged by assuming the 0 position to the origin of DNA reprication; length is given in percent of total genome. The direction of DNA replication is to the right.

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Table 1. The laboratory mouse strains used, their source and their restriction enzyme cleavage patterns

Strains	Source	Restriction endonuclease used									
		EcoRI	BamHI	HindII	HindIII	HpaI	HpaII	HaeII	HaeIII	PstI	SmaI
Inbred											
AKR/Ms	Stm	*	*	*	*	*	*	*	*	*	*
BALB/c	CRJ	*	*	*	*	ND	*	ND	*	*	*
BALB/c	Yok	*	*	*	*	*	*	*	*	*	*
C57BL/6J	Stm	*	*	*	*	*	*	*	*	*	*
C57BL/6N	CRJ	*	*	*	*	*	*	*	*	*	*
C57BL/10Sn	Ms	*	*	*	*	*	*	*	*	*	*
C3H/HeN	CRJ	*	*	*	*	ND	*	ND	*	*	*
C3Hf/Bi	Stm	*	*	*	*	*	*	*	*	*	*
CBA/H	Stm	*	*	*	*	*	*	*	*	*	*
DBA/1J	Ms	*	*	*	*	ND	*	ND	ND	*	ND
DBA/2	Yok	*	*	*	*	*	*	*	*	*	*
RIII/2J	Ms	*	*	*	*	*	*	*	*	*	*
129/Sv-Cl-C-P	Ms	*	*	*	*	*	*	*	*	*	*
FRG	Csk	*	*	*	*	*	*	*	ND	*	ND
DDD	Jms	*	*	*	*	*	ND	ND	ND	*	ND
DDO	Osb	*	*	*	*	*	ND	ND	ND	*	ND
DDY	Yok	*	*	*	*	*	*	*	*	*	*
NC	Crk	*	*	*	*	*	*	*	*	*	*
KK	Wak	*	*	*	*	*	*	*	*	*	*
Mutant											
Beige (C57BL/6J)[1)]	Stm	*	*	*	*	*	*	*	*	*	*
T1ALD(AKR)	Stm	*	*	*	*	ND	ND	ND	ND	*	ND
Closed colonies											
ddN	JCL	*	*	*	*	ND	*	ND	*	*	ND
ICR	JCL	*	*	*	*	*	*	*	*	*	*
Swiss/Ms	Stm	*	*	*	*	ND	*	ND	*	*	ND
SL	Stm[2)]	*	*	*	*	ND	ND	ND	ND	*	ND

* The heterogeneities were not observed in the cleavage patterns.

[1] Original strain is shown in parenthesis.

ND; Not done
CRJ; Charls River Japan
Crk; Dr. S. Sakai, Cancer Res. Inst., Kanazawa Univ.
Csk; Drs S. Suzuki & K. Tsuji, Central Res. Lab., Chugai Pharmaceutical Co., LTD
JCL; CLEA JAPAN
Jms; Dr. K. Suzuki, Animal Breed. Unit, Inst. Med. Sci., Univ. Tokyo.
Ms; Natl. Inst. Genet.
Osb; Dr. J. Kawamata, Res. Inst. Microbial Dis., Osaka Univ.
Stm; Dr. H. Shisa, Dept. Pathol., Saitama Cancer Center Res. Inst.
Stm[2]; Dr. Y. Honma, Dept. Chemotherapy, Stm
Yok; Dr. M. Nakagawa, Dept. Vet. Sci., NIH (Japan)
Wak; Dr. M. Nishimura, Wakayama Medical College

1–2.5$\times 10^6$ years. These results suggest that the genetic background of laboratory mice is mainly derived from *M. m. domesticus*.

Evolutionary Relationships among Five Subspecies of *Mus musculus* Based on Restriction Enzyme Cleavage Patterns

Hiromichi YONEKAWA*, Kazuo MORIWAKI, Osamu GOTOH*, Jin-Ichi HAYASHI*, Junko WATANABE*, Nobumoto MIYASHITA, Michel L. PETRAS** and Yusaku TAGASHIRA*

The intra- and intersubspecific genetic distances between five subspecies in the species *Mus musculus* were estimated from comparison of restriction enzyme cleavage patterns or maps of mitochondrial DNA (mtDNA). The subspecies used are an European subspecies, *M. m. domesticus* and Asian subspecies, *M. m. bactrianus*, *M. m. castaneus*, *M. m. molossinus* and *M. m. urbanus*. Although nomenclature of *M. m. urbanus* has not been standardized, the nomenclature of the others are widely accepted. For each subspecies except *M. m. urbanus*, at least two local races trapped in distantly separated localities were examined. Intrasubspecific heterogeneity was found in the cleavage patterns of mtDNAs obtained from two races of *M. m. bactrianus* and *M. m. castaneus*. In *M. m. molossinus* and *M. m. domesticus*, however, no intrasubspecific heterogeneity was detected. From the cleavage patterns of the mtDNAs, the four subspecies except *M. m. urbanus* could be clearly distinguished from each other but *M. m. urbanus* had identical cleavage patterns to those of *M. m. castaneus* with several enzymes (Table 1).

Quantitative estimates of genetic distances between these local races or subspecies were obtained by comparing cleavage maps of the mtDNA with various restriction enzymes. Nucleotide divergence of mtDNA and time of divergence between two local races were extimated to be less than 0.4% and less than 0.15$\times 10^6$ years in *M. m. bactrianus* and less than 0.3% and 0.1$\times 10^6$ years in *M. m. castaneus*, respectively. On the other hand, nucleotide divergences between European subspecies *M. m. domesticus* and Asian subspecies *M. m. bactrianus* and *M. m. castaneus* were 7.1% and 6.4%, respectively. The time of divergence were calculated from these values to be 2.5 and 2.3$\times 10^6$ years, respectively. It was also shown that the nucleo-

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Table 1. *Mus musculus* subspecies used, their collection localities and their set of restriction enzyme cleavage patterns of mtDNAs

Subspecies		Condition	Sex (No. used) M.	Sex (No. used) F.	Collection localities	The set of restriction enzyme cleavage patterns[a] Bm	Ec	H2	H3	He2	Hp1	Ps	Bg	Hp2	He3
M. m. domesticus		wild populations	(25)	(3)	Windsor,Canada	A	A	A	A	A	A	A	A	A	A
		SK/Cam (Inbred strain)		(1)	Skokholm Island[d] England	A	A	A	A	A	A	A	A	A	—
M. m. bactrianus		under inbreeding[b] in Ms	(13)	(7)	Kabul, Afghanistan	C	C	B	B	B	B	B	B	B	B
		under inbreeding in Ms	( )	( )	Lahole, Pakistan	C	C	B	B	B	B	B	B	C	C
M. m. castaneus		under inbreeding in Ms	(97)	(71)	Quezon City Philippines	C	B	C	B	C	C	B	A	D	D
		under inbreeding in Ms	(2)	(1)	Taichung, Taiwan	C	B	C	B	C	C	B	A	D	E
M. m. molossinus		wild populations	(12)	(11)	Mishima, Japan	D	B	A	B	C	A	B	A	A	F
		wild populations	(4)	(3)	Hakozaki, Japan	D	B	A	B	C	A	B	A	A	F
		under inbreeding[c] in Ngo (Mot)	(3)	(14)	Tsushima,Japan	D	B	A	B	C	A	B	A	A	F
		wild populations	( )	( )	Yonaguni Island Japan	D	B	A	B	C	A	B	A	A	F
M. m. urbanus		under inbreeding	(1)		Banderuwela, Sri Lanka	C	B	C	B	—	—	B	A	—	—
M. musculus subsp.	A	wild population		(1)	Kota Kinabal[e] Indonesea	C	C	B	B	B	B	B	B	—	—
	B	wild population		(1)	Kota Kinabal[e]	C	D	C	—	—	—	—	A	—	—
	C	under inbreeding in Ms	(5)	(4)	Mauritius	A	A	A	B	—	A	A	A	—	—
	D	under inbreeding in Ms	(7)	(3)	Seychelles	A	A	A	B	—	A	A	A	—	—

[a] Abbreviation used are Bm; BamHI, Ec; EcoRI, H2; HindII, H3; HindIII, He2; HaeII, Hp1; HpaI, Ps; PstI, Bg; BfII, Hp2; HpaII and He3; HaeIII. Letters refer to the types of cleavage patterns in order of discovery in each enzymes.

[b] Ms; National Institute of Genetics.

[c] Ngo; Dr. K. Kondo, Nagoya University.

[d] Gift from Dr. M. Nishimura of Wakayama Medical College.

[e] Gift from Mr. M. Harada of Osaka City Univeristy Medical School.

tide divergence and time of divergence between *M. m. molossinus* and other two Asian subspecies were comparable with those between *M. m. molossinus* and *M. m. domesticus* (about 3.5% and 1×10^6 years, respectively). These results suggest that *M. m. molossinus* is situated at a unique evolutionary position in Asian subspecies. The values for intraspecific divergence of *Mus musculus* mtDNA were comparable to those of *Rattus* mtDNAs but smaller than *Peromyscus* mtDNA.

In vitro hybridization of endosperm ADH in the Japanese and the American species of *Trillium*.

Masaaki IHARA and Toru ENDO

As shown in our earlier report (Ihara & Endo 1978), at least 5 loci are responsible for the genetic control of the endosperm ADH of the Japanese species. They are $Adh^{F(K)}$, $Adh^{S1(T)}$, $Adh^{S2(T)}$, $Adh^{S(U)}$ and $Adh^{F(S)}$.

Electrophoretic patterns are explained as follows: only one homodimer (K^fK^f) in *T. kamtschaticum* ($2n=10$); two homodimers ($T^{s1}T^{s1}$, $T^{s2}T^{s2}$)

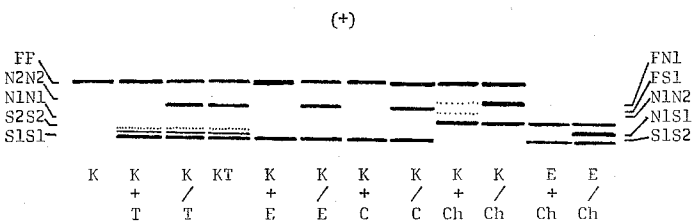

Fig. 1. Zymograms showing endosperm ADH isozymes hybridized *in vitro* with a fast moving isozyme from *T. kamtschaticum* and slow moving isozymes. Only major bands are drawn in some zymograms for the sake of clarity. Enzyme extracts were made from 100 g of seeds in each sample, ground separately in a mortar and pestle with 0.4 ml of a phosphate buffer (pH=6.8) containing 0.1 M 2-mercaptoethaNol, 0.1 M NaCl and 0.4 M sucrose; then the slurry was centrifuged at 12,000 rpm for 60 min at 0°C. The supernatant thus obtained was subjected to *in vitro* hybridization at-30°C for more than 12 hr, followed by a 2–4 hr dialysis against the extracting solution except NaCl at 4°C. A starch gel electrophoresis was conducted at 250 V for 4 hr at 8°C in a refrigerator. Abbreviation: K (*kamtschaticum*) T (*tschonoskii*), E (*erectum*), C (*cernuum*), Ch (*chloroperatlum*), KT (*kamt.*×*tsch.*), K+T etc. (two enzyme extracts mixed after dissociation-reassociation had taken place), K/T etc. (a mixture of two enzymes subjected to be the dissociation-reassociation process).

and a heterodimer ($T^{s1}T^{s2}$) in *T. tschonoskii* (2n=2o); two homodimers (U^sU^s, S^fS^f) and a heterodimer (U^sS^f) in *T. smallii* (2*n*=20), respectively. Meanwhile three American species were examined by a starch gel electrophoresis. *Trillium erectum* (2n=10) and *T. cernuum* (2*n*=10) revealed only an anodally moving slow band, similar to the Japanese slow one (e.g., $T^{s1}T^{s1}$ and U^sU^s). In *T. chloropetalum* (2n=10) three bands were detected, each at positions between U^sU^s and U^sU^f isozyme bands, between U^sU^f and S^fS^f isozyme bands and near the position of U^sU^f isozyme band of *T. smallii*. Since no segregation of the pattern was seen in seeds of each fruit, the ADH isozymes are probably controlled by a compound locus. Endosperm ADH isozymes may be dimers in the Japanese as well as the American species of *Trillium*, which is demonstrable by means of *in vitro* hybridization techniques. Our results are shown in Figure 1, proving the dimer hypothesis. The slow migrating isozymes were common in their mobility with the Japanese and the American species and even with the species of *Paris* (Ihara 1979), which may provide another dimension to be worked out from molecular evolutionary point of views.

IV. DEVELOPMENTAL GENETICS AND SOMATIC CELL GENETICS

In Vitro Cultivation of Gonads from a Female Sterile Mutant *fs231* of *Drosophila melanogaster*

Yukiaki KURODA

Developmental studies on gonads including germ cells may provide some important informations for analyzing the maternal effects of sex-linked recessive lethal genes and the cytoplasmic factors affecting the differentiation of cleavage cells in early embryogenesis. In the present work, an attempt has been made to culture the pupal gonads from a female sterile mutant *fs231* to investigate the expression of abnormality in gonads which may cause the female sterility in their development. The mutant *fs231* (1-22.7) was kindly provided by Dr. R. C. King, Northwestern University and maintained in a stock balanced by FM3.

Dechorionated eggs were sterilized by 70% ethyl alcohol and transferred to autoclaved foods. Pupae grown under sterile conditions were dissociated in physiological salt solution to isolate gonads. Ovaries and testes thus obtained were cultured in T-5 flasks with a coverslip in their bottom in Medium K-17 supplemented with 15% fetal calf serum and 0.1 μg/ml fetuin.

Cells migrated from the peritoneal sheath of ovaries and testes proliferated actively and formed cell sheets around the original explants. In single cysts of primary spermatocytes liberated from the intact testes, each process of meiosis of spermatocytes, the development of spermatids and the differentiation of mature sperm were normally observed. In isolated ovaries, however, no marked differentiation of oogonia and oocytes was observed.

Since it has been reported by King (1978) that *fs231* ovaries contained numerous tumor cells, the establishment of cell lines from these tumor cells are under investigation.

Effect of Cell Association on *In Vitro* Chondrogenesis of Mesenchyme Cells from Quail Limb-buds

Yukiaki KURODA and Etsuya MATSUTANI

Mesenchyme cells obtained from limb-buds of chick embryos can differentiate into cartilage cells *in vitro*. These cells from chick embryos at stage 25 or later can form cartilage colonies, whereas those from younger embryos can not. This developmental stage coincides with the stage at which limb-bud cells acquired the stabilization of chondrogenic ability *in situ*.

We studied the cartilage-forming ability of mesenchyme cells obtained from the hind limb-buds of quail embryos at stages 21–22. In the primary clonal cultures, only fibroblastic cell colonies were formed, but no differentiated cartilage colonies were found. To obtain the cartilage colonies from these cells, we examined the effect of cell association on stabilization of the cartilage-forming ability of these cells.

The mesenchyme cells were pre-incubated as monolayers, as cultures with gyratory shaking, as precipitated pellets or as cultures with reciprocate shaking for 4–48 hours, then the cells were dissociated and cultured for 14 days at low cell density (10^3, 2.4×10^3, 5×10^3 cells/35 mm dish) to test their ability to form cartilage cell colonies.

In mass monolayer cultures, the cells did not form multilayers during this pre-incubation time. When the pre-incubated cells were cultured as low cell density for 14 days, the colonies formed consisted entirely of fibroblastic cells. In contrast, when cells had been pre-incubated for 5 days, they had formed multilayers, then formed cartilage cell colonies in cultures at low cell density. These results suggest that the two-dimensional cell association in monolayer cultures may not stabilize the chondrogenic ability of mesenchyme cells.

When cells were pre-incubated with reciprocate shaking to prevent their cell association, the small number of cell colonies were formed in cultures at low cell density, and a few cartilage cell colonies were found exceptionally in cultures with the initial inoculum of 5×10^3 cells per dish. In gyratory shaking cultures, colonies of cartilage cells appeared after a minimum pre-incubation time of 12 hours, and increased in number with the increase in the pre-incubation time to a maximum (12.8 ± 2.2 cartilage colonies/5×10^3 initial cells) after pre-incubation for 24 hours.

Cell pellets obtained by centrifugation were pre-incubated in centrifuge tubes, then the cells were dissociated and incubated at low cell density. Colonies of cartilage cells appeared after a minimum pre-incubation time of 16 hours and increased to a maximum ($50.4 \pm 4.9/5 \times 10^3$) in cultures of cells pre-incubated for 48 hours.

These results indicated that the formation of cartilage colonies depended upon the type of cell association and duration in pre-incubation period. Gyratory shaking or pellet cultures probably mimics the process of cell condensation in the prospective chondrogenic region in the limb-buds. This suggests that the three-dimensional cell association for a certain period may stabilize the ability of mesenchyme cells to differentiate into cartilage cells.

Dose-rate Effects of Trp-P-1 on Survival and Mutation Induction in Cultured Human Diploid Cells

Yukiaki KURODA

Trp-P-1 is a tryptophan pyrolysis product contained in cooking and processing fish and meat and it has a strong mutagenic activity for *Salmonella typhimurium*. In the present experiment, the dose-rate effects of Trp-P-1 on survival and mutation induciion in cultured human diploid cells were examined.

When cells were treated with Trp-P-1 at various concentrations for 1, 2, 3, 8, 16 and 24 hours, the survival of cells decreased depending on concentrations and exposure times. When the cytotoxic effects of Trp-P-1 were compared at the same product value of concentrations and exposure times, higher concentrations with shorter exposure times were more effective in reducing cell survival than lower concentrations with longer exposure times. The concentrations of Trp-P-1 giving 50% survival which were calculated from concentration-survival curves for exposure times of 1, 2, 3, 8, 16 and 24 hours were 13.6, 6.2, 3.5, 3.1, 1.2 and 1.1 μg/ml, respectively.

Cells treated with Trp-P-1 at various concentrations for 1, 2, 4 and 24 hours were cultured in normal medium for the mutation expression time of 6 days, and replated cells were selected with 30 μg/ml of 8-azaguanine (8AG). The frequency of 8AG-resistant mutations increased as the concentrations of Trp-P-1 employed for each exposure time increased. Trp-P-1 at higher concentrations with shorter exposure times induced 8AG-

resistant mutations at higher frequency than that at low concentrations with longer exposure times.

These results indicate that the effects of Trp-P-1 on survival and mutation induction in cultured human diploid cells were dose-rate dependent. This suggests that some degradation or inactivation may occur in Trp-P-1 during its incubation with human cells or that human diploid cells may have some repair activity for their chemical-induced damages, as shown in the case of radiation-induced damages.

Analysis of the Decreased Hatchability of Eggs Laid by Flies Reared on Excess Yeast in *Drosophila melanogaster*

Kiyoshi MINATO

It is known that flies fed on excess yeast lay eggs at their maximum rate. It was, however, found that the hatchability of these eggs decreased remarkably. The similar phenomenon has also been observed by Robertson and Sang (1946). Some analytical experiments were carried out on this phenomenon.

When twenty newly eclosed flies (Oregon R; 10 females and 10 males) were reared at 25°C on agar-cornmeal-dead yeast-sugar food, the surface of which was partially covered with the thick suspension of live Baker's yeast in 3×10 cm glass tube, with the daily change of food, flies laid eggs at a very high rate (50 eggs/female/day) after a few days. But the hatchability of these eggs decreased gradually, reaching about 50% on the tenth day.

On the fourth day (the average hatchability was 70%), the individual female flies were separated and examined for the hatchability of their eggs for one day. No flies showed zero hatchability of their eggs and all flies showed evenly lowered hatchability, indicating that the decreased hatchability was not due to the presence of unmated female flies in the population.

In addition to the egg hatchability, the larval and pupal mortalities were examined for ten days. It was found that the decrease in the egg hatchability after several days was accompanied with an increased rate of larval lethality, in which many larvae showed little motility immediately after hatching. The above findings suggest that the decrease in the egg hatchability in this case may be due to some physiological abnormality of eggs during development.

A Normal Diploid Teratocarcinoma (OTT10A-5) obtained from a 6-day Embryo of B10.A Mouse

Takehiko NOGUCHI, Choji TAYA and Kazuo MORIWAKI

Transformation of multipotential cells of the mouse embryo can be experimentally induced by transplanting early embryos into the adult testis (Stevens, Develop, Biol. **21**: 364, 1970) or under the kidney capsule (Solter, *et al*, Nature **227**: 503, 1970). When 6-day embryos were transplanted under the kidney capsule, inducibility of teratocarcinomas was several times lower in strain B10.A/SgSn mice than in strain 129 mice. Low inducibility of the experimental teratomas in the mouse has been ascribed to low permissiveness of the host environment to the growth of embryonal carcinoma cells.

A teratocarcinoma (OTT10A-5) was induced from a strain B10.A by transplanting a 6-day embryo into the scrotal testis of a B10.A×C3H F1 hybrid male. After 3 succesive passages in the hybrid males, this teratocarcinoma has been serially transplanted in the syngeneic, B10.A hosts. Transplantabilities of OTT10A-5 in B10.A and the hybrid males were 86% and 56% respectively. This result suggests that a mouse strain having low inducibility of embryo-derived teratocarcinoma by the transplantation method do not necessarily have low permissiveness to transplantable teratocarcinomas.

Chromosome G-band patterns of the stem cells in this teratocarcinoma did not differ significantly from normal diploid cells during 5 serial transplantation generations (for 8 months). The solid tumors contained embryonal carcinoma cells and derivatives from all three germ layers. It has been suggested that euploidy of teratocarcinoma stem cells may be associated with developmental totipotency in the blastocyst environment (Cronmiller and Mintz, Develop. Biol. **67**: 465, 1978). Stem cells of this teratocarcinoma may, therefore, be promissing vehicles for deliberate introduction of specific mutant genes into mice via blastocyst injection method (Mintz, and Illmensee, Proc. Nat. Acad. Sci., 72: 3585, 1975).

The Establishment of Some Clonal Cultures of a Tetratocarcinoma Derived from Strain B10.A Mouse

Yoshitake NISHIMUNE*, J Aizo MATSUSHIRO*, Yuko OGISO*, Choji TAYA, Takehiko NOGUCHI and Kazuo MORIWAKI

Transplantable teratocarcinoma (OTT10A-5) cells from strain B10.A mouse were adapted to growth in tissue culture, and two groups of clones were established after 3 serial cloning. One group (18 clones) was cloned by using feeder layer cells, and the other in the medium containing 10^{-4} M β-mercaptoethanol without feeder layer. They could form solid tumors containing various kinds of tissues, showing the multipotentiality.

The stability of the multipotentialities and the euploidy of the cultured clones during their long-term propagation *in vitro* was examined. All of the observed clones (A211, A425 and A222) which have been established on feeder layer cells have normal diploid G-band patterns and also have the multipotentiality even after continuous culture *in vitro* for over three months (30 generations) or after freezing in liquid nitrogen. However, the clones (Bβ721, Bβ838) established in the medium containing β-mercaptoethanol without feeder layer exhibited a slight change in the G-band patterns, though they have kept multipotentiality.

Thus, the cultivation of the clones of teratocarcinoma on feeder layer cells seems to keep the stability of karyotype, but that in the medium containing β-mercaptoethanol may have the trend to emerge chromosome changes. The multipotentiality seems to be more stable charactor than the karyotype.

Characterization of the Lethal Hybrid Rescue (*Lhr*) Gene of *Drosophila simulans*

Tsuguhiko TAKAMURA and Takao K. WATANABE

Lethal hybrid rescue (*Lhr*) gene (2-95) of *D. simulans* (Watanabe, Jap. J. Genet. **54**: 325, 1979) was studied further by crossing it to several *D. melanogaster* complex chromosome lines. Watanabe (1979) reported *Lhr* rescued 100% of lethal hybrid males from the cross *melanogaster*♀ × *Lhr simulans*♂ but that it could not rescue the female hybrid of the cross $\overline{XX}$/Y *malanogaster*♀ × *Lhr simulans*♂. We repeated the latter cross again using

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two different $\overline{XX}$/Y chromosomes in order to confirm that *Lhr*'s rescue activity was limited to males and that it cannot rescue $\overline{XX}/Y^s$(Y^s: *simulans* Y) hybrids. In the first cross with C(1)DX, which was used by Watanabe, no $\overline{XX}/Y^s$ females was rescued as expected. However, in the cross with C(1)RM, 33.3% of the progenies were $\overline{XX}/Y^s$ females. Further, *Lhr simulans* was crossed to X/X/Y *malanogaster*. In this cross, usual lethal hybrids, X^m(=melanogaster X)Y^s, $X^mX^mY^s$, $X^mY^mY^s$, were all rescued. These data show that *Lhr* does not work male specifically.

C(1)DX is known of lacking bb^+ locus on both arms, and also Y chromosome of *D. simulans* dose not carry bb^+ locus (Ritossa and Atwood, P. N. A. S. 56: 496, 1966), while *melanogaster* Y carry bb^+ locus. Thus, the hybrid, C(1) DX/Y^s, does not carry any bb^+ locus which is known to code ribosomal RNA. Therefore it was natural that the hybrid could not be rescued by *Lhr* because it did not carry r-DNA at all. This was convinced by the cross between Df(1)bb^-/FM4 *melanogaster*♀ and *Lhr simulans*♂. Only FM4/Y^s male was rescued and no Df(1)bb^-/Y^s were recovered. From all the crosses so far done, following two possibilities were extracted. 1) Hybrids carrying *simulans* Y are all lethal and *Lhr* can rescue all such lethals. 2) Hybrids carrying *simulans* X are all viable except female hybrid of *melanogaster*♂×*simulans*♀, and *Lhr* rescues all the hybrids without carrying *simulans* X. It is not yet answered which is the correct choice.

Gonad Development and Gametogenesis in the Opposite Sex of the Silkworm, *Bombyx mori*

HARUO YOSHIDA[1] and Akio MURAKAMI

In the silkworm, *Bombyx mori* L., there have been often detected sexual mosaics or gynandromorphs. These observations suggest that sex-hormon-like substances in this insect do not take part in the expression of the sexual characters. Unfortunately, functions of gametes in the gynadromorph are unable to investigate by mating experiments. It has been well known facts that transplanted rudimentary gonads in the opposite sex silkworm are apparently developed regradless of the sex of host and/or donor. However, biological information on the gametogenesis in the opposite sex host seems to be somewhat meagre. The aim of this experimental series is to

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elucidate the effects of differential physiological condition on the gametogenesis and/or sexual differentiation of gonads in the insect by the exchangeable transplantation of larval gonads.

In the present study, the gametogenesis in the opposite sex was examined by the mating experiments. On the second day of the fifth instar larvae, one or two rudimentary gonads (ovaries or testes) were transplanted into the body-cavity of larvae in the opposite sex. Operated larvae were normally metamorphosed as did non-operated control insects. After eclosion, they were crossed with the non-operated another sex moths. They were also dissected to observe the gonads in the opposite sex. No abnormal fertility of spermatogenic cells in males which had been received an ovary or two ovaries was observed on the basis of either the number of eggs or the fertility of eggs laid by the mated females. The function of oocytes developed in the testis-transplanted females was also not different from the control regardless of the number of testes transplanted. These observations led to a conclusion that the gametes in recipients which had been transplanted opposite sex gonads can be developed normally. Immature oocytes in ovaries transplanted to the male larvae were apparently developed to almost normal mature oocytes and/or eggs in size. Growth of testes in the female was also very much of the control.

From these findings and others, it is likely that both non-transplanted and transplanated gonads can be developed free from different environmental condition, suggesting that sexual differentiation of gonads is not interfered under the sexualy different condition and/or by the sexual hormon-like substances.

Characterization of *fs*(1) MY-18 Mutant Rescued with the Transplantation of Normal Egg Cytoplasm in *D. melanogaster*

Masa-Aki YAMADA and Saburo NAWA

For the investigation of the interactions between nucleus and cytoplasm of egg during early development, X-linked maternal effect mutant, *fs*(1) MY-18, was isolated with EMS treatment. The characters of this mutant were as follows. 1) The locus of this gene was 11.3 on X chromosome. 2) The viability was normal in both sexes. This shows that this gene has no effect on the development from larvae to adults. 3) The eggs laid by the homozygous females were normal in shape and size but unable to deve-

lop into larvae even when fertilized with wild type sperms. The development of eggs was stopped at various stages of embryogenesis and in the most of dead embryos the morphorogical abnormalities were observed in the anterior region. 4) About 2% of embryos from eggs laid by homozygous females were rescured with the transplantation of normal egg cytoplasm (about 1% of egg volume), which was effective even after stored for 3 days at −20°C. 5) This mutant was neither auxotrophic nor temperature sensitive. 6) When the proteins of the ovary were analyzed by O'Farrell two-dimensional electrophoresis, the mutant ovary was deficient in one protein among about 230 protein spots detected. The isoelectric point of the protein was similar to vitellogenin. But its molecular weight was smaller than that of vitellogenin. It was conteined only in the insoluble fraction of ovary lysate in 0.8% NaCl solution, being dissolved by adding urea. But this protein was not detected in egg and other tissues of adult. It is considered that the protein is specific to ovary and acts during the oogenesis to produce essential components in early development. Further biochemical analysis is in progress.

V. CYTOGENETICS

F_1 Hybrids between Mauritius and Oceanian Type Black Rats and their Karyotypes

Tosihide H. YOSIDA

Mauritius type black rats were remarkable by the presence of 8 extra small acrocentric autosomes than the other black rats. A comparative karyotype analysis between the Mauritius and Oceanian type rats have revealed that those extra small acrocentrics of the Mauritius rat seemed to be originated from Robertsonian fission of two small metacentric autosome pairs, nos. 14 and 18, occurring in the Oceanian type rat. Thus, this mechanism induced the chromosome number of the Mauritius rat to be $2n=42$ (Yosida *et al.* 1979, Chromosoma **75**: 51–62). To obtain a evidence for the relation between these two types, cross experiments have been carried out between the Mauritius and Oceanian type rats. Six F_1 hybrids consisting 2 females and 4 males were thus obtained in two litters. Chromosome numbers in all F_1 individuals were found to be 40 as a result of a combination of each haploid set from both parents. The F_1 metaphase showed the constant occurrence of large metacentric M_1 and M_2 pairs, indicating that they are of the Oceanian origin. The presence of 7 small acrocentrics were remarkable in the hybrids. Among them three small acrocentrics, or pair no. 13 and the Y chromosome, were common to the both type rats, but the remaining 4 small acrocentrics were outstanding in the hybrid specimens, which were derived from the Mauritius rat.

The G-band analysis clearly showed that four small acrocentrics were homologous to the long and short arms of the small metacentric pair nos. 14 and 18. Each one partner of the small metacentrics of pair nos. 14 and 18 in the hybrids could be originated from the Oceanian type rat, while the small acrocentrics would be derivatives from the Mauritius type rat. All other chromosome pairs in the F_1 hybrids were homologous. The present hybridization experiment seems to provide somewhat confirmative evidence for the above presumption on that the Mauritius black rats were derived from the Oceanian type rat by chromosome fission.

Nucleolus Organizer Regions (NORs) in 7 *Rattus* Species and their Differentiation and Evolution

Tosihide H. YOSIDA

Nucleolus organizer regions (NORs) has been recognized as an important organization in the chromosome architecture, as well as are believed to be possible sites of rRNA genes. The NORs of the black rat (*R. rattus*) and the Norway rat (*R. norvegicus*) have been investigated by Yosida (1978, Proc. Jap. Acad., **54B**: 522–527). Taxonomically the above species are closely related with apparently similar karyotypes (Yosida *et al.* 1965, Chromosoma **16**: 70–78; Yosida and Amano 1965, Chromosoma **16**: 658–667): They had 42 chromosomes with 13 acrocentric or subtelocentric autosome pairs, 7 small metacentric autosome pairs and acrocentric X and Y chromosomes. According to him (Yosida 1973, Chromosoma **40**: 285–297), the karyotype of the Norway rat was recognized as to be derived from one of the polymorphic karyotypes in the Asian type black rat. In spite of the similarity of karyotypes in the black and Norway rats, the NORs were different between these two species with regard to their sites in the chromosomes. In the former species they were found in pair nos. 3, 8 and 13, while in the latter they were in pair nos. 3, 12 and 13 (corresponded to pair nos. 3, 11 and 12 in the standard karyotype nomenclature of the Norway rat). The present paper deals with the NORs in the other 5 *Rattus* species, *R. exlans*, *R. annandalei*, *R. losea*, *R. sabanus* and *R. neilli*, in comparison with those of the black and Norway rats. Among the 7 *Rattus* species 5 species (*R. rattus*, *R. norvegicus*, *R. exulans*, *R. annuandalei* and *R. losea*) had the same chromosome number ($2n=42$) with similar karyotypes. In *R. exulans* the NORs were found in the centromeric regions of pair nos. 3, 5 and 13. Interestingly the NORs occurring in pair nos. 3 and 13 were common in these three *Rattus* species, but the other one chromosome with the NORs was different from each other. It was found in pair no. 8 in *R. rattus*, pair no. 12 in *R. norvegenicus* and pair no. 5 in *R. exulans*. The NORs in *R. annandalei* and *R. losea* were characterized by being located in two chromosome pairs. In the former species they were in pair nos. 8 and 13, whereas the latter species showed in pair nos. 3 and 13. The karyotype of *R. sabanus* and *R. neilli* were considerably different from the above species. In the former species the NORs were found in centromeric resions of pair nos. 1, 3 and 5, while in the latter species they occurred in only two

chromosome pairs, nos. 1 and 3. Noticeable is the fact that the NORs were found in the distal end of pair no. 9 in these two species. Thus, these 7 *Rattus* species showed the NORs in pairs 1, 3, 5, 8, 9, 12 and 13. Among them, the NORs occurring in no. 3 were most frequently (6/7), those in no. 13 ranked second (5/7), and the NORs occurring in the other pairs were observed in one or two species. It is noticeable that the NORs are located in different chromosomes in each species. This feature is apparently species specific. Based on the above findings a conclusion would be possible that the differentiation of the nucleolus organizer regions on chromosomes is important to species differentiation.

Chromosome Constitution of *Rattus villosissimus* resulted from Robertsonian Fission

Tosihide H. YOSIDA

Chromosomes of *R. villosissimus* has previously been investigated by Barverstock *et al.* (1977) with a suggestion of the Robertsonian fission without banding analysis. In the present study the karyotype of this animal was reexamined by the conventional and G-banding karyotype analysis with a special attention toward a karyotypic comparison between this species and Mauritius type black rat with the Robertosonian fission. *Rattus villossimus* (*R. sordius villosissimus*), were obtained through the courtesy of Dr. C.H.S. Watts from Asutralia in 1972 and they had been bred in the author's laboratory.

The chromosome number of this species was determined as $2n=50$ which contained two large metacentrics which were derived from the Robertsonian fusion of acrocentric pair nos. 2 and 3 and also of acrocentric pair nos. 6 and 10. The karyotype peculiarity of this species was peculiar in the *Rattus* species, because 26 small acrocentrics and two small metacentrics were always included in the complement. Of the 26 small acrocentrics, two were of pair no. 13, but the remaining 24 chromosomes (12 pairs) were suggested to be derived from the centromeric fission of 6 small metacentric pairs (nos. 14 to 19). The other two small metacentrics were located at the pair no.20. Under this situation, the karyotype of this specis is to correspond to the basic karyotype of the *Rattus* species. Based on the arrangements of chromosomes due to the G-band characteristics it was revealed that twelve extra small acrocentric pairs just corresponded in their banding patterns to each

member of 6 small metacentric pairs (nos. 14 to 19) of the basic *Rattus* karyotype.

In the course of cytogenetical studies of the black rats, we (Yosida *et al.* 1979a, b, Yosida 1979) have chanced to find the chromosome-fission mechanism in the Mauritius black rats. Basically they had the Oceanian karyotype but were exceptional by having extra 8 small acrocentrics (4 pairs), which could have been resulted from the Robertsonian fission of two small metacentric pair nos. 14 and 18 originally presented in the Oceanian type black rat. The karyotype comparison between the Mauritius type black rat (*R. rattus*) and *R. villosissimus* revealed a similarity in their basic constitution. They had two large metacentric chromosomes, although the original elements forming two large metacentrics were dissimilar between two species, and the number of extra small acrocentrics occurred by Robertsonian fission of small metacentrics. In the black rat the dissociation occurred in two metacentric pairs (nos. 14 and 18), whereas in the *R. villosissimus* it was taken place in six metacentric pairs (nos. 14 to 19). Based on the comparative analysis of karyotypes among the *Rattus* species it is possible to state that the Robertsonian fusion mechanism plays an important role in karyotype evolution, but the Robertsonian fission takes also a part in evolutional changes of the karyotype and species.

A consideration on Relation between the Karyotype Evolution and the Mutagenic Environment

Tosihide H. YOSIDA and B. B. PARIDA[1)]

It is widely accepted that structural rearrangements in chromosomes have played a primary role in the development of variation of species. In the process of speciation and evolution, two events, one being at the genic level, and the other at the chromosomal level, are to be taken into consideration. The latter event deals primarily with changes of the phenotypical karyotype as a result of inversion translocation and others, and also those of the inner-structural karyotypes revealed by heterochromatin, the nucleolus organizer regions (NORs) and some others. Evidence favoring the species differentiation at chromosomal level has been availably providing recent karyological studies in three mammalian species; the black rat (*Rattus rattus*), the Indian spiny mouse (*Mus platythrix*) and the Indian muntjac

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(*Muntiacus munjak*). All the species under consideration have widely been distributing in Indian subcontinents. It seems very likely cytogenetically that their karyological changes in relation to evolution have occurres in southern India. It has been shown that in the black rats, the Ceylonese type rats having $2n=40$ and the Oceanian type rats having $2n=38$ had occurred in the southern India in association with the sequential Robertsonian fusion of certain chromosomes in the Asian type black rats with $2n=42$ (Yosida *et al.* 1974, Chromosoma **45**: 99–109). In the Indian spiny mouse ($2n=26$), the mechanism of tandem fusion between two elements of certain acrocentrics occurring in the house mouse (*Mus musculus*) having $2n=40$ seemed to take part in their development (Yosida 1979, Proc. Jap. Acad. **55B**: 270–274). The evolutional change of the Indian muntjac with $2n=6$ (♀) from the Reeve's muntjac (*Muntiacus reevesi*) with $2n=46$ has tentatively understood as a sequence of tandem fusion of certain acrocentrics (Wurster and Benirschke 1970, Science **168**: 1364–1366). To know the mechanism of such karyotype changes concentrately occurring in the Indian subcontinents is of utmost biological importance. Theoretically it seems probable that the changes have occurred in association with certain environmental mutagens. Such considerations are to be presented in the present paper on karyotype evolution, species differentiation and environmental mutagen with particular reference to the karyotypic rearrangements in the three species of Indian mammals concerned here.

Three examples mentaioned above clearly indicate that the karyotype evolution, after a long duration, ultimately leads to subspecies and species differentiation. Evidence is interesting showing that the evolutionary events concerned here are those occurring in the Indian subcontinent, especially in South India. Then a question arises as to factor or factors in India, particularly environmental, which influenced the germ cells to cause the karyotype evolution leading to species differentiation. There exist a few areas in the world which embrace a high natural radioactivity. One of these areas is said to occur in the Kerala State of South India (United Nations Report, 1966). The carrier of the radioactivity is known to be black monazite sand in the coast, which is derived from igneous rock-minerals containing thorium phosphate. Thorium is known as a radioactive element carrying a very long life (1.39×10^{10} years). This type of monazite sand is known to occur in various concentrations in other parts of South India including coastal areas of the Bay of Bengal like Gopalpur in Orissa State.

Based on the above discussion on the karyotype evolution in the black rats, Indian spiny mouse and Indian muntjac it makes possible that India, particularly South India, might had been a platform where cytogenetical events such as chromosomal breakages, fusion, inversion and translocation had taken place, probably under the natural mutagens. For the decided statements the further studies should be demanded on mammalian fauna in the above areas.

Finding of 1/12 Translocation in the Lewis-strain Rat

Tosihide H. YOSIDA

The present paper describes the translocation chromosomes observed in the Lewis-strain rat, along with data on its inheritance to their offspring. Among six Lewis strain rats (3♀ and 3♂ examined), 5 rats (2♀ and 3♂) had 42 chromosomes having the normal karyotype characteristic of the rat, while the remaining one female rat was remarkable by having the heteromorphic pair no. 1. The short arm of one member of the pair no. 1 was normal in length, whereas that of the othe member was constantly longer slightly than the normal partner. This rat had also a heteromorphic pair no. 12. The normal partner of the pair 12 was small subtelocentric, but the other partner was autstanding in being very small metacentric. The small metacentric pair 12 seemed to be derived from the deletion of the longer arm of the original subtelocentrics. The brocken end of the pair no. 12 thus formed seemed to be translocated to the short arm of one partner of the pair no. 1. The above relation between the pairs 1 and 12 was clearly defined by G-banding staining of these chromosome pairs.

In a cross of the translocation female rat with the normal male, 17 rats from 3 litters were obtained. Among them 9 rats showed the normal homologous pair nos. 1 and 12, while the remaining 8 rats had the 1/12 translocation. The rats with the 1/12 translocation and those with the normal karyotype appeared in almost the same ratio in number as expected. The partner of the chromosome with 1/12 translocation was normal as described above, but only a single rat was exceptional by showing mosaic cells; one of them had the normal partner to the 1/12 translocation as described above, whereas the other was characterized by the inversion partner. Details of the mosaic rat will be reported later.

Evolutionary Aspects of Mitochondrial DNAs in variant Types of Black and Norway Rats

Jun-Ichi HAYASHI[1], Hiromichi YONEKAWA[1], Osamu GOTOH[1], Yusaku TAGASHIRA[1], Kazuo MORIWAKI and Tosihide H. YOSIDA

Mitochondrial DNAs (mtDNAs) were prepared from varuous inbred and wild Norway rats, *Rattus norvegicus*, and from three types of black rats, *Rattus rattus*, (Asian type, Ceyloness type and Oceanian type). Intra- and interspecies divergence of their mtDNA sequences were calculated based on changes in restriction endonuclease cleavage sites. The extent of intraspecies divergence of black rats (about 8%) is much larger than that of Norway rats (1%) and the mtDNA of Asian-type black rats resembles the mtDNA of Norway rats more closely than it resembles the mtDNA of other types of black rats. These results strongly suggest that during the course of intraspecies differentiation of black rats, probably long after the separation of the three types of black rats, some Asian-type black rats were isolated sexually and formed a new species, Norway rats. On the basis of our observations we propose a hypothetical process to explain the evolution of animal mtDNA.

Comparative Studies on Release of Enzymes from Platelets during Blood Clotting in the Black and Norway Rats

Toshihiro EMORI[2], Masakazu TAKAHASHI[2], Sumi NAGASE[2] and Tosihide H. YOSIDA

Increase of lactate dehydrogenase (EC 1. 1. 1. 28) and creatine kinase (EC 2. 7. 3. 2) in serum and plasma level during blood clotting was compared in the black rat (*R. rattus flavipectus*) and the Norway rat (*R. norvegicus*, JCL-SD strain). Both the enzyme level increased by the release from platelets in process of clotting in rat as previously described (Emori T. *et al.* 1978, Exp. Animals **27**, 168), but the change in black rat was very slight.

Owing to search for the reason to prove the difference in this phenomenon, physiological and chemical properties of blood from both the animals were compared, and it was demonstrated that the content of plasma fibrinogen in the black rat is higher than that in the Norway rat, while the platelet

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number contained in each ml of blood and the enzyme activities in each platelet are not different between them. The analysis of the membrane proteins from platelets of both the animals by SDS-polyacrylamide gel electrophoresis showed that the fractional value of glycoproteins was found to be different between them.

Establishment of Cell Lines from *Millardia meltada* and Examination of Endogenous Virus

Haruo SUGIYAMA[1], Masuo YUTSUDO[1], Kumao TOYOSHIMA[1], Tosihide H. YOSIDA and Yoshiaki MURATA[2]

Millardia meltada, a member of Muridae, is a wild rodent found in fields in India, and this rat was established as a useful laboratory animal by Yosida, one of the present author (Yosida 1978, Lab. Anim. **12**: 73–77). It is intermediate in size between a mouse and a rat, and its diploid cells have 50 chromosomes. It is still uncertain whether *M. meltada* is more closely related to mice or to rats. There are, of course, no tumor virlogical observations on *M. meltada* at all. If *M. meltada* is to be used as a laboratory animal for cancer research, it is especially interesting to know whether it has endogenous type-C RNA virus, because various classes of oncovirus have been isolated not only from laboratory rodents but also from wild ones. The object of this work was (1) to establish cell lines from *Millardia* embryos, (2) to induce endogenous virus from *Millardia* cells, and (3) to examine whether *Millardia* cell DNA has any nucleotide sequence homologous with murine leukemia virus (MuLV) and rat endogenous virus.

For the above studies four cell lines were established from this animals; an untransformed cell line (MM-D), a non-producer (NP) cell line transformed with murine sarcoma virus (MM-CL4), and SV40-transformed cell lines (MM-8, MM-663). The MM-D line is epithelioid and contact-inhibited, whereas MM-CL4, MM-8, and MM-663 lines are fibroblastic and grow piling up. No endogenous virus was induced in these cell lines by a long-term culture with various inducers. *Millardia* cell DNA has no nucleotide sequence homology with cDNA of either murine leukemia virus or rat endogenous virus.

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Acquired Resistance and Immune Responses of Eight Rat Strains to Infection with *Angiostrongylus cantonensis*

Kentaro YOSHIMURA[1], Hiroko AIBA[1], Nakami HIRAYAMA[2] and Tosihide H. YOSIDA

Strain-dependent differences in susceptiability to a wide variety of parasitic infections have been found in various experimental animal models. These differences are thought to be possibly determined by hostgenetic factors among which immune responsiveness would be one of the most important components. However, only limited information is available on a possible association between the host susceptibility and immune responsiveness. Up to the present, however, no specific data are available on not only differences in susceptibility to *Angiostrongylus cantonensis* of different inbred rat strains but also the functional relationship between the host immune responses and susceptibiluty. As a prelude to such an exploration, the present study was attempted to compare the susceptibility and immune responses of eight inbred strains of rats, ACI (F102), BUF (F66), LEW9 (F17), NIG(F33), Tokyo (F50), WKA(F202), WKS(F14) and W/Ms (F71), to infection with *A. cantonensis*. Consequently, it was found that both BUF and ACI strains acquired less protective immunity than other strains and, conversely, Tokyo and NIF strains yielded higher resistance. The remaining strains showed various intermediate degrees of acquired resistance. Striking strain differences in pattern of reagin production were found; Tokyo was a high responder while ACI was a low responder. In contrast, the kinetics of indirect hemagglutination (IHA) antibody response was essentially the same in all the strains examined. The IHA antibody titers were generally high in both Tokyo and NIG. Histopathologically, ACI was distinct from NIF in that fibrotic reactions around the nodules of developing eggs in the lung parenchyma were almost undiscernible. These data suggest that genetic factors would be involved in acquired resistance and reaginic antibody responses to *A. cantonensis* infection in rats.

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Karyotypes of Four Carangid Fishes

Makoto MUROFUSHI[1] and Tosihide H. YOSIDA

The present paper deals with the cytogenetical study of four carangid fish species, *Trachurus japonicus*, *Caranx equals*, *C. sexfasciatus*, and *Alectis cilialis*, obtained in the west coast of Izu Peninsula, Shizuoka Prefecture. Diploid chromosome numbers from kidney cells in the four carangid fish species were all 48. The karyotype of *Alectis cilialis* was the simplest form consisting of all acrocentric pairs ranging from large to small in size. On the other hand, the karyotypes of two *Caranx* species (*C. equula* and *C. sexfasciatus*) were similar to the above species, except the largest pair (no. 1) which was characterized by subtelocentrics. The other 23 pairs of these species were acrocentrics. The remaining one species (*Trachurus japonicus*) had the same chromosome number as the above three, but the karyotype was markedly different from the other species. Fifteen pairs (nos. 1 to 15) were biarmed chromosomes. Among them two pairs (nos. 2 and 11) were metacentrics, and seven pairs (nos. 1, 6, 10, 12, 13, 14 and 15) were submetacentrics, and six pairs (nos. 3, 4, 5, 7, 8, and 9) were subtelocentrics. The remaining nine pairs (nos. 16 to 24) were acrocentrics, although very short arms were sometimes found in some chromosome pairs (nos. 16, 20 and 22). Sex chromosomes could not be identified in any of the four species examined. Based on the karyotype characteristics it is clearly shown that two species (*Caranx equula* and *C. sexfasciatas*) with one subtelocentric (pair no. 1) seems to be derived from *Trachurus japonicus* with the acrocentric pair by pericentic inversion. The third type of carangid species, *Trachurus japonicus*, which had fifteen biarmed pairs, might have been resulted from the inversion of the other acrocentric pairs included in the former species. From the view point of the karyotype analysis, three species of *Alectis* and *Carax* seemed to be closely related, but *Trachurus* is rather far from the above three.

High Incidence of Urethan-induced Pulmonary Tumor in the Intersubspecies Hybrids between Laboratory Mouse and Japanese Wild Mouse

Kazuo MORIWAKI, Toshihiko SHIROISHI and Nobumoto MIYASHITA

Genetic regulatory systems governing developmental processes in mamma-

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lian forms seem to be considerably stable. In these systems, regulatory genes and structural genes are possibly couple quite precisely which could not allow the occurrence of viable mutations. In the intersubspecies hybrids (A×B), however, it is possibly assumed that a regulatory gene derived from A subspecies can not completely control a structural gene from B subspecies and *vise versa*, if the divergence time between the two subspecies is fairly large. This is sometimes favorable for the development of tumors. We have already estimated the divergence time between Japanese wild mouse, *Mus musculus molossinus*, and the laboratory inbred mouse, most probably

Table 1.

Mouse strains	Number of mouse observed	Pulmonary nodles per mouse
ICR	18	2.7±0.88
(ICR×MOL. TEN)F_1	25	5.8±2.08
Mol. Ten	7	0.2±0.34

Urethan treatment: Subcutaneous injection, 1.0 mg/g body weight, 17–19th day of gestation.

Scoring of nodules: 5 months age.

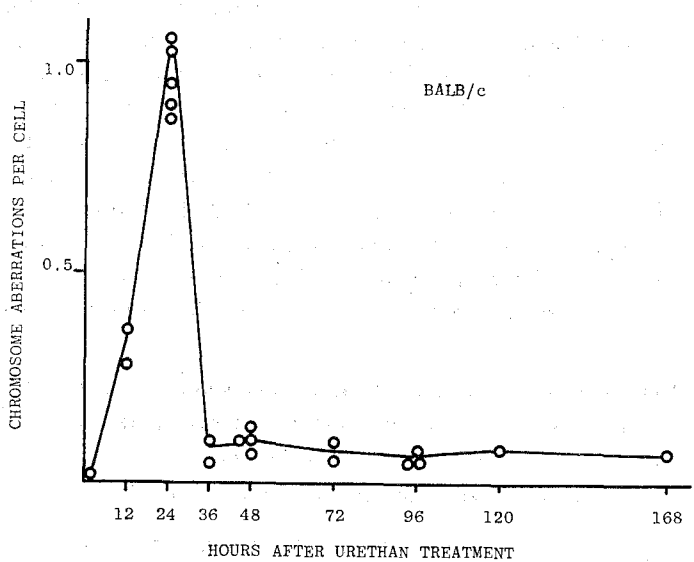

Fig. 1.

Table 2.

Mouse strain	Chromosome breaks per cell after urethan treatment				
	0	12	24	36	48 (hr)
BALB/c	0.0	0.10	*0.55*	0.02	0.06
C. Ten F_1	0.0	0.20	*0.69*	0.09	0.04
Mol. Ten	0.0	0.34	*1.59*	0.72	—
B. Ten F_1	—	0.20	*1.06*	0.11	—
C57BL/10	0.0	0.28	*9.72*	0.11	—

Urethan dose: 1.5 mg/gB.W. Age: 3 weeks.

originated from *M. m. domesticus*, roughly $1\text{–}2\times10^6$ years.

In the present study, *molossinus* mouse strain Mol. Ten, the laboratory strain ICR and their hybrids were used for the transplacental administration of 10% urethan solution (1 mg/g B: W.) at around 17–19 days of gestation. Five months after birth, the number of pulmonary tumor foci was scored. Table 1 summarizes the results demonstrating a significantly higher susceptibility of F_1 hybrid to urethan-induced pulmonary tumor.

In order to examine whether such a high susceptibility to urethan in the hybrids as shown above is related to the changes of genetic materials in the earlier stage of urethan action *in vivo* or not, we observed chromosome aberrations in the bone marrow cells at the several points shortly after the urethan administration. In this experiment, we employed 3 weeks youngs and urethan dose of 1.5 mg/g B.W. Chromosome breaks per cell were scored at 12, 24, 36 and 48 hours after subcutaneous urethan injection. As shown in Fig. 1, a peak of chromosome bleaks sharply appears at 24 hours. Comparing the effect of urethan on the chromosome aberrations at this stage, we could not recognize any augmentation of them in the F_1 hybrids between either BALB/c $\times$ Mol. Ten and C57BL/10$\times$Mol. Ten (Table 2). This may suggest that the higher incidence of pulmonary tumors in the F_1 hybrids is probably caused by the later stage of tumor development.

Urethan-Induced Chromosome Aberrations in Various Mouse Strains Suggesting an Inverse Relationship between Chromosome Changes and Tumorigenesis

Kazuo MORIWAKI, Toshihiko SHIROISHI, Nobumoto MIYASHITA and Tomoko SAGAI

It has been already known that A and BALB/c strains are highly susceptible to urethan-induced pulmonary tumor and C57BL is lowly susceptible. Japanese wild mice seem to be quite resistance to the urethan action which are demonstrated in this Ann. Rep. (Moriwaki *et al.*). Comparing the frequency of chromosome breaks in their bone marrow cells 24 hours after the urethan administration, we obtained the results suggesting an inverse relationship between chromosome aberration and tumorigenesis (Table 1). As this result seems to be contradictiory to the present understanding on the relation between chromosome changes and tumor development, we examined the error-prone DNA repair which was said to probably concern with tumorigenesis (Kondo, 1977). Treatment with caffeine, an inhibitor

Table 1.

Mouse strain	Chromosome breaks per cell				Urethan induced tumorigenesis
	0	12	24	36 (hr)	
A/J	A/J	—	*0.55*	—	+++
BALB/c	0.0	0.10	*0.55*	0.02	++
C57BL/10	0.0	0.28	*0.72*	0.11	+
Mol. Msm	—	—	*0.89*	—	±
Mol. Ten	0.0	0.34	*1.56*	0.72	±

Urethan dose: 1.5 mg/gB.W.. Age: 3 weeks.

Table 2.

Treatment	Aberrant cell percentage 24 hours after urethan and caffeine treatment
Control	0.00
Caffeine	0.01
Urethan	19.7±7.9
Urethan+caffeine	23.9±8.3

Urethan dose: 1.5 mg/gB.W.
Caffeine dose: 0.1 mg/gB.W. at 0.8 and 16 hours after urethan injection, s.c.

of error-prone DNA repair, could somewhat increase the urethan-induced chromosome aberrations (Table 2).

This finding might imply that on the carcinogen treatment, error-prone DNA repair introduce elevated tumorigenesis on one hand, but on the other hand seemingly decreased chromosome aberrations by the DNA repair even if it is error-prone.

On the Precocious XY Separation at the First Meiotic Metaphase of Mouse Spermatocytes

Yoichi MATSUDA, Hirotami T. IMAI, Kazuo MORIWAKI, and Kyoji KONDO[1)]

Recently we found an unique phenomenon that the X and Y chromosomes separate highly frequently (ca. 70%) at meiotic metaphase I of the F_1 hybrid males between laboratory mice (BALB/c) and Japanese wild mice (*Mus musculus molossinus*). The same phenomenon was also observed in the F_1 hybrids between BALB/c and various subspceis *M. m. castaneus*, *M. m. bactrianus*, and *M. m. urbanus* (Table 1). On the other hand, the frequencies of the XY separation were only 21.1% in Japanese wild mice and 12.0% in BALB/c. These observations suggest that the highly frequent separation of the XY is characteristic to the F_1 hybrids between subspecies in the

Table 1. Frequencies of the precociously separated XY at the first meiotic metaphase of spermatocytes in the wild and laboratory strain mice and their F_1 hybrids

Materials used	Number of individuals examined	Number of cells observed	% of the separated XY
Japanese wild mice (*M. m. molossinus*)	30	3804	21.1±21.6
Laboratory mouse strain (BALB/c)	18	3175	12.0± 4.5
F_1 hybrids			
BALB/c×*M. m. molossinus*	36	5324	73.2± 9.6
BALB/c×*M. m. castaneus*	8	800	81.9± 1.1
BALB/c × *M. m. bactrianus*	5	628	78.2± 6.4
BALB/c×*M. m. urbanus*	6	722	67.3± 3.9
BALB/c × *M. m. domesticus*	12	1491	6.5± 2.3

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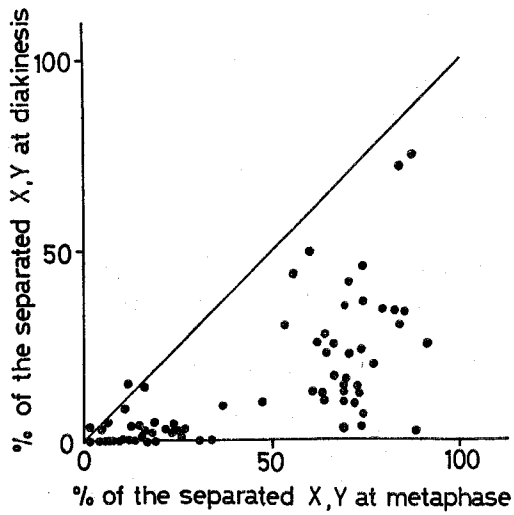

Fig. 1. Correlation of the XY separation in spermatocytes compared between diakinesis and metaphase I.

genus *Mus*. In connection with this, one exceptional case was observed in the F_1 hybrids between BALB/c and *M. m. domesticus*, where the value was extremely low (6.5%). Such exceptional data may be consistently interpretable by the generally accepted assumption that the laboratory inbred mouse strains probably originated from the European wild mice (*M. m. domesticus*).

To know the initial stage of the XY separation, we compared the frequency of the separated X and Y chromosomes between diakinesis and metaphase I. As demonstrated in figure 1, there is a clear tendency that those individuals showing high separation values at metaphase I also show high values at diakinesis, though the latter is lower (about half) than the former in most cases. These data suggest that the XY separation strats at least at early diakinesis and accelerates during metaphase I. Besides it is well known fact that the X and Y chromosomes usually separate completely during the first meiotic anaphase stage. Therefore, we conclude that the XY separation we found falls under the category of "precocious separation", but does not mean non-pairing of the sex chromosomes at zygotene stage.

VI. MUTATION AND MUTAGENESIS IN ANIMALS

In Vitro Complementation of Repair Enzyme Activities in Cellular Extracts from Ataxia Telangiectasia Fibroblasts

Tadashi INOUE, Akiko YOKOIYAMA and Tsuneo KADA

Ataxia telangiectasia is an autosomal recessive disease of man characterized by telangiectasia, immunodeficiency, cancer-proneness, and hyper-sensitivity to ionizing radiation. We previously suggested that the hypersensitivity of ataxia cells to ionizing radiation may be ascribed to the deficiency in some kind of repair enzymes, which are assayed in a newly developed system using gamma-irradiated colicin E1 DNA and purified DNA polymerase I.

In the present study, we applied this system to examine whether the extracts from various ataxia strains complement each other *in vitro*. The strain used were AT3BI, AT5BI and AT4. AT4 was kindly donated by Prof. Sasaki of Kyoto University. The experiments yielded the results that AT3BI and AT4 complemented each other as well as AT3BI and AT5BI. However, AT5BI and AT4 did not complement. These results suggest that there are at least tow complementation groups in ataxia telangiectasia; one contains AT3BI and AT4, and another contains AT5BI.

Human Enzymes Functioning in Repair of Gamma-Ray-Induced DNA Damage

Tadashi INOUE and Tsuneo KADA

Many kinds of enzymes are though to be functioning in repair of DNA damage especially of those which induced by ionizing radiation, since the radiation produces various types of lesion in base and sugar moieties as well as scissions in the phosphodiester linkage. In order to purifiy and characterize the repair enzymes, we developed an assay system for repair enzymes in which the capacity of cellular extracts to enhance to priming activity of gamma-irradiated DNA for purified DNA polymerase was measured. Application of this system to the cellular extracts of fibroblasts from patients with genetic disorder ataxia telangiectasia revealed that the ataxia cells

are deficient in some kind of repair enzyme activity (Biochim. Biophys, Acta **479**: 497). Ataxia telangiectasia is an autosomal recessive disease characterized by hypersensitivity to ionizing radiation, telangiectasia, immunodeficiency and an increased frequency of malignancy. It is therefore of interest to examine the relationship between these symptoms and enzymatic defect. Tissue culture cells, however, provide too little materials for extensive enzymatic characterization, so we have thus turn to human placenta to study further the physical and catalytic properties of human repair enzymes.

The placental repair enzyme activity was separated into several distinct fractions by means of column chromatographies. One of them was purified to approximately 400-fold by successive application of ammonium sulfate fractionation, DEAE-cellulose column chromatography, gel-filtration and affinity chromatography on gamma-irradiated DNA-cellulose. Though the preparation is not yet homogeneous, it significantly enhanced the priming activity of gamma-irradiated DNA without any effect on unirradiated DNA. Neither phosphatase nor exonuclease activity was detected in the preparation. The preparation exhibited endonucleolytic activity to acid/heat-treated DNA. This activity was detected only when Mg^{2+} was present in the reaction mixture.

The Mutagenic Activity of Pyrrolizidine Alkaloids in the Silkworm Germ-Cells

Akio MURAKAMI, Tsutomu FURUYA[1] and Toshiko OZAWA

Pyrrolizidine alkaloids are widely distributed in plants. Many pyrrolizidine alkaloids are carcinogenic or carcinostatic in experimental animals and mutagenic in various organisms including Drosophila. In silkworm *Bombyx mori*, no information on the mutagenicity of the alkaloid has been reported. The purpose of the present experiment was to investigate the mutagenic activity of pyrrolizidine alkaloids in *Bombyx*: namely to test whether or not the alkaloids induce recessive visible mutations in the pupal germ-cells by the egg-color specific locus method.

The pyrrolizidine alkaloids tested in this experiment were clivorine, ligularidine and seneciphylline. The first two alkaloids were extracted from *Li-*

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gularia dentate and the last one was from *Sececio cannabifolius*. Each compound was suspended in 0.5% carboxyl methyl cellulose (CMC) with 0.85% NaCl solution and were administered by injection into the abdomen of wild-type (*C108*×*Aojuku*)F_1 silkworms at the mid-stage pupa. They were then mater individually to the marker stock homozygous for *pe* and *re* loci to measure the frequency of egg-color mutations. Seven concentrations of clivorine were used: 0.001, 0.01, 0.1. 1.0, 10.0, 20.0 and 40.0 μg per capita. Ligularidine was tested at two concentrations of 20 and 40 μg/capita and seneciphylline was tested only at a concentration of 20 μg/capita. A 0.025 ml of 0.5% CMC solution per capita was applied to control.

The treatment of pupal oocytes and spermatozoa with clivorine at lower concentrations ranged from 0.001 to 1.0 μg per pupa did not induce the egg-color specific locus mutations. On the contrary, when applied high doses of 10, 20 and 40 μg of clivorine per capita, a significant increase in the frequency of the egg-color mutations was observed. The non-induction of mutations by the alkaloid at lower doses may be due to the fact that this compound is not sufficient to cause enough mutational events to result in the recessive visible mutations, and suggesting that a threshold dose might be existed in the dose-response relation. The frequency of egg-color mutations after ligularidine administration was slightly higher in the treated than in the control group. It is well known facts that both clivorine and ligularidine are promutagens and the mutagenicities of these alkaloids are dependent on the metabolic activation. Accordingly, silkworm pupae were postulated to contain enzymes or an enzyme-like system capable of converting the parent alkaloid into a mutagenic substance. None of the tested seneciphylline concentration at 20 μg/capita was increased the mutation frequency.

The result of the present experiment suggests that the pyrrolizidine alkaloids with otonecine as the necine base were mutagenic to germ-cells of silkworms, while the alkaloid with retronecine as base was not mutagenic. This mutagenic response of the pyrrolizidine alkaloid to the germ-cells of the silkworm seems to have something in common with that of Salmonella test system coupled with the mammalian metabolic activation system (Yamanaka, *et al.*, 1979). In this context, the silkworm has proved to be a useful insect species as well as Drosophila for biological monitoring of the pyrrolizidine alkaloid which requires metabolic activation for demonstration of their mutagenic activity.

Mutagenicity of Two Phosphoramides, TEPA and HMPA, in the Silkworm Germ-Cells

Akio MURAKAMI and Toshiko OZAWA

Triethylene phosphoramide, TEPA, is widely used in the chemical industry. TEPA and HMPA, hexamethyl phosphoramide, a nonalkylating analog of TEPA, have been given attention on the biological properties as either insect chemosterilants or cancer chemotherapeutic drugs. The mutagenicity of both phosphoramides has been demonstrated in various organisms from bacteriophages to mammals. In Drosophila, these compounds induced a high frequency of recessive lethal mutations, dominant lethals and other chromosomal events. The genotoxicity of TEPA was about 100 times more efficient than HMPA in the sperm of *Bracon hebetor* (R. M. Watrous, Brit. Ind. Med. **4**: 111 (1947)). Domesticated silk moth (*Bombyx mori*), as one of the mutagenicity testing insects, may differ from Drosophila and *Bracon* in the metabolism and eating habits; thus, mutagenic response to chemical compounds is not necessarilly expected to be identical in these insect species. Therefore, we tested the mutagenic activity of these known promutagens, TEPA and HMPA, on germ-cells of the silkworm using the egg-color specific locus method.

Both phosphoramides were dissolved in 0.85% NaCl solution and were administered by injection method to the pupae of wild-type (*C108*×*Aojuku*) F_1 silkworms at 0.5, 1.0, 2.5, 5.0, 10.0 and 25.0 μg/capita, they were mated with marker stocks having a double recessive egg-color genes, *pe* and *re*, for evaluation of mutagenic activity of the compounds. The germ-cell stages in pupae are prophase I oocytes and spermatozoa for female and male, respectively.

After TEPA treatment a significant increase of mutations at both *pe* and *re* loci was observed in pupal oocytes and spermatozoa. However, the increase was non-linear in relation to the doses studied. The genotoxicity of TEPA for pupal germ-cells was more pronounced than that of Diethylsulfate (DES), often equalling or surpassing that of DES. On the contrary, with the positive result in other insect test systems, the frequency of egg-color mutations after HMPA administration was not significantly increased in the treated than in the control group, regardless of type of germ-cells. This finding means that the silkworm pupa is lacking in enzymes or an enzyme system capable of converting or demethylating HMPA into muta-

genic intermediates. From the present experience and others, it can be said that test organisms for the mutagenicity assay of environmental chemicals should include, at least one species having an ability to activate the given parent chemical for biologically active intermediate(s).

The Mutagenicity Test of Some Flavonoids in Pupal Germ-Cells of the Silkworm, *Bombyx Mori*

Akio MURAKAMI and Toshiko OZAWA

The flavonoids are universally distributed among vascular plants including mulberry tree, and the mutagenicities of several of them have been reported in various mutation test systems. Most of the flavonoids are a so-called promutagen, i.e., not mutagenic in itself, but it requires metabolic activation for conversion into a mutagenic substance. The silkworm feeds on leaves of the mulberry trees. The mulberry leaves contain a various kind of the flavonoids. It is of interest to test whether ot not the flavonoids are mutagenic to the silkworm. This communication concerns the mutagenic activity of four different flavonoids, quercetin, quercitrin a glycoside of quercetin, rutin a rutinoside of quercetin and morin, in pupal germ-cells of the silkworm by the egg-color specific locus method. All compounds tested were purchased from Wako Pure Chemicals, Osaka.

Each chemical was dissolved in 0.025 ml of 0.5% CMC solution, and applied by injection into the abdomen of mid-stage pupae of wild-type [*C108*× *Aojuku*] F_1 silkworms in four different concentrations, 100, 200, 400 and 600 μg per capita for evaluation of the mutagenic effect. Control pupae were given the same volume of 0.5% CMC solution. After emergence, they were allowed to mate with the marker stock homozygous for egg-color genes, *pe* and *re*, and recessive visible mutations were scored. The number of whole- and fractional-body mutants detected was recorded separetely for each locus and the over-all frequency of mutations was calculated.

The frequency of egg-color recessive visible mutations at both loci in the pupal oocytes and spermatozoa after the flavonols, quercetin and mori, administration was not significantly higher in the treated than in the control group. These flavonols were mutagenic to Salmonella tests in the presence of mammalian metabolic activation systems (Brown, Muta. Res. **75**: 243 (1980)). The mutagenicity of the two flavonol glycosides, quercitrin (quercetin-3-0-rham) and rutin (quercetin-3-0-glu-rham), were also not muta-

genic in both sex germ-cells of the silkworm pupae.

Negative results with this test system could mean either that the flavonoids are not mutagenic in the silkworm germ-cells or that the flavonoids are mutagenic but not detected as such by this procedure. The silkworm feeds innoxiously on mulebrry leaves which contain a various kind of flavonoids. In addition, isoquercitrin, a flavonoid, in mulberry leaves was identified as one of biting factors in the silkworm (Hamamura, 1970). Morin showed also the biting activity in the insect but not found in mulberry leaves (Hamamura, 1970). Such being the context, it would seem highly probable that, in the long evolutional process, silkworms have been acquired an ability to take mulberry leaves without any detrimental effect to maintain their life. Accordingly, it can be said that the silkworm seems to be disqualified for the mutagenicity test of flavonoids and their related compounds regardless of the presence or absence of enzymes or an enzymelike system capable of converting the parent compounds into a metabolic active intermediate.

Strain Differential Activity for Metabolic Activation of 2-Acetylaminofluorene in the Silkworm

Akio MURAKAMI and Michiko GOTO

Many environmental chemicals defined as promutagens exert their mutagenic effect on the experimental organisms only after their conversion into active intermediates. The development of sensitive assay systems and/or biological materials for such promutagens is urgently necessary. The present study aims to select the sensitive silkworm strains or stocks having a highly metabolic activity for making promutagens to form mutagenic active intermediates.

Microsomal S9 fractions from the 5th instar whole larva in seven stocks of of the silkworm (*Bombyx mori* L.) were compared for their ability to metabolize parent chemicals to mutagenic substances. Drug metabolic activity of the S9 fraction was measured by the His^+ *Salmonella* TA 98/silkworm microsome system according to Sakamoto *et al.* (1977) with small modifications of the method developed by Ames *et al.* (1975). In this system, the metabolic activity was assayed as the mutagenicity of the chemical to *Salmonella* or the increase of His^+ independent revertant colonies on minimum glucose media as compared with controls without the microsomal

S9 fractions. 2-Acetylaminofluorene (AAF), one of typical promutagens, was used as the test chemical. In *Salmonella* TA 98, AAF is not mutagenic in the absence of the activation systems, but mutagenic in the presence of the systems. The preparation of silkworm microsomal S9 fractions was carried out as described by Ames *et al.* (1975). The S9 fractions were obtained from five original stocks (C108, *old* C108, Aojuku, Nihonnishiki, and Kojiki), an F_1 hybrid of Aojuku × C108 and a mutant stock, *rb* (red blood).

The results obtained in this study indicate that the AAF metabolic activity among 7 stocks investigated was C108 as the highest activity and *old* C108, F_1 hybrid of Aojuku × C108, Aojuku, Nihonnishiki, *rb*, and Kojiki in this order regardless of sexes. S9 fractions taken from female larvae seemed to have somewhat higher the activity for AAF metabolic activations than those of males except for the cases of Nihonnishiki and *rb*. The activity of S9 fractions taken from larvae of C108 and *old* C108 was three times higher than that of liver S9 from rats which had been treated with an enzyme inducer polychlorinated biphenyl and that of those from F_1 hybrid of *Aojuku* × *C108*, Aojuku, Nihinnishiki, and *rb* was almost the same as that of rat livers. The S9 fraction from Kojiki alone was less in activity than that from rat livers. In any case, it can be said from the present study that the AAF metabolic activation system in the silkworm larval microsome S9 fractions is considerably active.

Mutagenic Effects of X-Rays at a Dose Down to 25 R in Adult Oocytes of the Silkworm

Akio MURAKAMI and Toshiko OZAWA

The greater use of X-radiation, radioisotopes in medicine, and of nuclear power for electricity generation bring foward an important social problem on the genetic effects of radiation at low dosage levels. However, little information on this subject has been reported in higher organisms. Among them, available experimental results of Drosophila males provided evidence that a linear relationship between doses and mutation frequencies can be applied to low doses down to around 5 to 8R. Unfortunately, no sufficient study on the dose-response relations at low-level radiations in female germ-cells has been reported. In the silkworm, the dose-response curve for egg-color mutations induced by X- or gamma-rays for prophase I oocytes was non-linear over the high dose ranges studied from 500 to 15000 R

(Murakami, 1971) as reported for Drosophila oocytes (Parker, 1960). This report communicates about the genetic effects of low-level X-radiation on silkworm female germcells by the specific locus method. To make low dose experiements, it requires a large number of individuals to be observed in order to find a reasonable number of mutants. For this silkworm prophase I oocytes in adults just before entering into meiotic division process are suitable because collection of a large number of homogeneous materials is easy and that this stage oocytes are fairly sensitive to radiation. In addition, the egg-color specific locus method as the mutation detection system allows to easily detect the visible complete or mosaic mutants among a large number of F_1 individuals or diapaused eggs.

The materials used in this experiment were newly emerged the wild-type (*C108* × *Aojuku*) F_1 virgin female moths and were irradiated with various doses of X-rays (180 kVp, 25 mA, 1.0 mm Al filter and a dose-rate at 300 R/min). Applied doses were 25, 50, 100, 200, 300, 400 and 500 R. Non-irradiated female moths were used as control. Each locus mutation frequency was calculated as the total frequency of complete and mosaic mutants.

It was clearly observed a locus differential sensitivity between the *pe* and *re* loci for both control and experiment groups. Spontaneous frequency was almost 10 times higher at the *pe* locus than at the *re* locus as in other experiments reported elsewhere. At the *re* locus, a linear relationship between egg-color mutation frequencies and X-ray doses was observed with the dose over range studied and indicating that no threshold dose at this locus in mutagenic effects of low doses of radiation down to 25 R. While, at the *pe* locus, the frequencies of mutations at 25 and 50 R were no more different from an extraordinarily high spontaneous mutation frequency which estimated to be around a 10×10^{-5}. This may be not due to mutational incidence, but to abnormal fertilization such as androgenesis. Accordingly, at the *pe* locus, the linear dose relationship was applied to a dose down to no more than 100 R.

Large scale experiments at the *re* locus on the mutagenic effects with further low-level radiations in the oocytes system of silkworms are in progress.

X-Ray-Induced Recombinational Events between Z and W Chromosomes in Oogenic Cells of the Silkworm (*Bombyx Mori* L.)

Akio MURAKAMI and Akio OHNUMA

The physical induction of recominational events between Z and W chromosomes has been investigated in the female silkworm, in which it occurs scarcely spontaneous crossing-overs. Females (*sch*$^{+}$/W^{pe+}: *pe*/*pe*) having a W-chromosome marked with *pe*$^{+}$ gene and the 5th chromosome homozygous for *pe* (yellow-white egg: 5–0.0) received a single dose of X-rays (1,000 R) at various developmental stages from first instar larvae to late stage pupae. They were crossed to tester males (*sch*/*sch*: *pe*/*pe*) homozygous for both *sch* (sex-linked chocolate: 1–21.5) and *pe* genes.

The results indicated that X-rays clearly increase the occurrence of recombinational events in oocytes after irradiation of silkworms from the late stage larvae to mid-stage pupae, but slightly increase in oogonia from first to early third instar larvae. The most sensitive germ-cell stage to X-ray-induced recombinational events was observed in oocytes of early stage pupae (153.4×10^{-5}) and that was delayed for a few days as compared with the exchange type recombinations in the 5th chromosome. The frequency of recombinants recovered in oocytes of late stage pupae was reduced to the level of the first instar larvae. The incidence of recombinational events between sex-chromosomes was several times more frequent than that of the 5th chromosome (Murakami, 1976).

Genetic analysis of the recombinants recovered is not yet completed, it can be said, however, that the femaleness deciding factor(s) is localized in the inside segment rather than the distal Portion of the W chromosome as inferred by Tazima (1946). In addition, it can be deduced from the analysis that the factor suppressing the maleness or development of male sex organs and gametes may be located in the W-chromosome.

Chromosomal Aberrations in *Drosophila simulans*

Yutaka INOUE

Natural and radiation induced chromosomal aberrations were studied in *D. simulans* and *D. melanogaster*. These sibling species are similar in morphology, but cytologically different by a single inversion in the right arm of

the third chromosome. In natural populations of *D. simulans*, no polymorphic inversions have been detected. Only four unique inversions were reported by Dobzhansky (Biol. Rev. **14**: 339, 1939) and Itoh (DIS **55**: 64, 1980). On the other hand, a total of eleven kinds of the polymorphic inversions were observed in *D. melanogaster* on the both arms of the second and third chromosomes. In addition, the unique inversions were found in frequency of 0.26% per autosome arm (Inoue and Watanabe, Jap. J. Genet. **54**: 83 1979). A total of 734 iso female lines of *D. simulans* from Japan, Africa and Australia were examined and the four inversions were observed (In(3L)68D: 70C, In (3L)64B; 70C, In(3R)86A; 93D. In(3LR) 67F; 83E). Their frequency was 0.07% per autosome arm and it was much lower than that found in *D. melanogaster* population. Males from 16 geographical strains of Japan were irradiated by X ray (4000 rads) and mated to non-irradiated females of the respective species. Chromosomal aberrations induced in the germ cells of the males were examined in F_1 larvae. The frequencies of aberrations were 8%(24/300) for *D. melanogaster* and 4% (11/300) for *D. simulans*. The results were rather consistent with Lemke *et al.* (DIS **53**: 159 1978), but were different from those of Woodruff and Ashburner (Am. Nat. **112**: 456, 1978) in which they showed the frequency was almost equal for the both species.

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN MICROORGANISMS AND PLANTS

Natural and Environmental Antimutagens

Tsuneo KADA, Hajime MOCHIZUKI, Masako HARA, Akiko YOKOIYAMA, Tadashi INOUE, Kenji KANEKO, Hiroyuki MINAKATA and Yoshito SADAIE

A number of environmental antimutagens were detected by assaying, in four bacterial systems, capacities for reducing; (a) UV- or gamma-ray-induced reverse mutations in *E. coli* WP2 B/r *try*, (b) high spontaneous histidine reversions in *B. subtilis* strain NIG1125 possessing an altered DNA polymerase III, (c) spontaneous reversions in *Salmonella typhimurium* TA100 possessing a plasmid pKM101, and (d) streptomycin-dependent→independent mutations in strains derived from *Salmonella* TA98 and TA100.

The followings are examples that were concluded as antimutagens; some bio-essential metal compounds such as cobaltous chloride, extracts from placenta tissues (human, monkey, dog, rabbit, rat, mouse); extracts from Japanese green tea plants; extracts from leaves of some lily species; water or methanol extracts of some crude drugs; etc. 5-Fluorouracil was used as a positive control.

Antimutagenic Effect of Cobaltous Chloride on Various Mutator Strains

Tadashi INOUE, Kayoko SHIMOI and Tsuneo KADA

We previously reported that the presence of cobaltous chloride in post-treatment media markedly diminished the MNNG- as well as gamma-ray-induced reverse mutations in *Escherichia coli* WP2 B/r *trp*. In order to elucidate the detailed mechanisms of the antimutagenesis by cobaltous chloride, we examined the effect of the metal compound on the following four mutator strains whose molecular bases for mutator activity are well characterized. (1) *Bacillus subtilis* NIG 1125 (*mut-1*). The *mut-1* cells produce an error-prone DNA polymerase III which is a chromosome replicating enzyme of the bacterium. (2) *E. coli* SG 902 (*dna E*). This strain is an

analogue of *B. subtilis* NIG 1125, *i.e.*, the mutator activity is ascribed to temperature sensitive error-prone DNA polymerase III. (3) *E. coli* DM 1187 (*lexA spr tif sfi*). In this strain, the error-prone DNA repair pathways (SOS-functions) appear to be expressed constitutively without any inducing signals. (4) T4 L56 (ts). This phage also produces an error-prone DNA polymerase. The error-proneness is attributed to decrease activity of proofreading exonuclease (3′ to 5′ exonuclease) of the enzyme.

Among these mutator strains, *B. subtilis* NIG 1125 and *E. coli* DM 1187 were found to be very susceptible to the metal compound with respect to their spontaneous mutability. The mutation frequency of histidine reverse mutation was reduced by the metal compound to one-twentyth and one-seventh in *B. subtilis* NIG 1125 and *E. coli* DM 1187, respectively. However, in *E. coli* SG 902 and T4 L56, which possess mechanisms for mutator activity very similar to those of *B. subtilis* NIG 1125, no antimutagenic activity of the metal compound was detected.

From these data, we suggest that the metal antimutagen may correct error-proneness of DNA replicating enzymes by improving their fidelity in the DNA synthesis, but the susceptibility of the enzyme to the metal compound is species specific.

Mutagenic Effect of L-ethionine in Soybean and Maize

Taro FUJII

Ethionine, a methionone analogue, is a well known carcinogen inducing predominantly liver-cancer. It is known that about 80% of the carcinogens have mutagenic activities whereas a number of carcinogenic substances remain non-mutagens. A lack of mutagenic activity of ethionine has been documented in mammalian and microbial test systems, except in *Bacillus subtilis* assay (Kada *et al.*, 1980, Chemical Mutagens **6**: 149). Strain T-219 of soybean with a marker Y_{11} and maize heterozygouns for the Yg_2-gene were used, and mutagenicity of L-ethionine was examined. In the experiment with soybean, seeds were soaked in 0.5–2.5 mg/ml concentrations and increase in mutation frequency was measured by the increase in numbers of spots per leaf. Increase of spot frequency was observed with higher concentrations, and repeated experiments with concentrations ranging 0.25–2.0 mg/ml, also showed consistent results. Spot frequencies from these two experiments showed clear dose-related frequency relationships. To

obtain the threshold dose of the substance for mutation induction, much lower concentrations, viz., 0.05–0.20 mg/ml, were applied. No increase in spot frequency was observed with these low concentration treatments.

Maize seeds were treated with concentrations ranging 0.025–5.0 mg/ml. The frequency of somatic mutation of the Yg_2-gene, characterized by yellow-green stripes, did not differ between the water steeped sample and those treated at rather lower concentrations. At 1.0 mg/ml treatment, however, an appreciable increase of striping frequency per leaf was observed, and the frequency increased linearly at higher concentrations. These results indicate that L-ethionine induces somatic mutations in maize at concentrations higher than 1.0 mg/ml. In spite of many negative results, the present results with soybean and maize demonstrate that L-ethionine has mutagenic activity in higher plants. Frequencies of three spot types due to forward and back mutations, and somatic crossing over in soybean, and forward mutation frequency in maize, induced commonly by physical or chemical mutagens, were increase with L-ethionine treatments.

Identification of Newly Induced Waxy Mutant Gene in Maize

Etsuo AMANO

Among many marker genes in maize, waxy (*wx*) is a very clear and dependable endosperm marker. It is one of the few loci which have been studied their intralocus fine structure. The enzyme produced from this locus has been reported. Mutations can be induced in this locus by seed treatment with a chemical mutagen, EMS, or less frequently with ionizing radiations. The newly induced *wx* mutant gene could be detected in endosperm after pollination with tester *wx* pollen. But this pollination not only upset purity of the stock used but also complicated isolation of the new *wx* gene. The mutant kernels detected contain both tester *wx* and newly induced *wx* genes. They can be separated by back cross to original *Wx* stock followed by self pollination in the next generation. By this way, new *wx* or tester *wx* used can be brought into homozygotes respectively. The problem is how to distinguish new *wx* mutant line from the tester *wx* line in the siblings. There are some flanking markers available on the chromosome 9 where *wx* locates. However, since they are some distances apart from *wx* locus, in either side of *wx*, they can only help as a primary proof for the new *wx* gene. The best guarantee will be the characteristics of the

new *wx* gene itself. Mutant site or position of the alteration in *wx* cistron may be one of the factors for identification, but it requires detailed examination of fine structure analysis. Polypeptide analysis or DNA base sequence analysis will be the ultimate proof of the new *wx* mutant gene, but they are much more laborious tasks. For practical purposes, leakyness of *wx* phenotype seemed to be a good help. Phenotype of *wx* mutant is expressed as decreased level of amylose fraction in starch. This changes the color of staining by iodine from dark blue to reddish-purple. Some of the EMS induced *wx* mutants showed intermediate color suggesting that they might be missense mutation. These intermediate or leaky phenotype could serve as a proof for the new *wx* mutant gene. When such intermediate phenotype was so clear that it could be noticed by visual examination, it is easy to separate the new *wx* mutant from tester *wx*. For nearly complete *wx* mutant, dual wave length photometry was applied. In some of the tested mutant, aleuron layer was colored to purple or brown by other marker genes. However, the photo-metrical measurements of iodine stained endosperm starch solution were not disturbed very much, and some possible "slightly intermediate" *wx* mutant could be separated.

Phenotypic Expression of *Wx* Gene in Cereals

Etsuo AMANO

Waxy (*wx*) starch mutants which can synthesize no or only small amount of amylose component of starch, are found in several species of cereal crops. They can be induced artificially with radiations or more efficiently with chemical mutagens. During examinations of these waxy mutant, gene dosage effect in endosperm was noticed in rice. In the mutant segregating populations, M_3, M_4, or F_2, endosperms could be classified visually into 1 full waxy, 1 intermediate waxy and 2 nonwaxy groups. The last group still had some deviation in turbidity in endosperm. Dual wavelength analysis which was a modified blue value method and was developed to measure the waxy phenotype quantitatively, supported the results of visual classification. Such gene dosage effect was shown neither in maize nor barley, in which clear and theoretical 3: 1 segregations were observed both in visual and instrumental examinations.

In F_2 population of *Oryza perennis* (*Wx*) × *Oryza sativa* (*wx*) segregation ratio of *Wx* and *wx* was distorted probably due to low seed fertility or small-

ness in numbers of endosperms examined. However, as for the gene dosage effect, there was no intermediate phenotype. In a F_2 population of *Wx/wx* heterozygote of *Oryza glaberrima*, another cultivated rice species, segregation pattern was the same as other maize or barley. Only *Wx* genes in Japonica type *Oryza sativa*, Norin No. 8 and Taichung 65, showed the gene dosage effect producing *wx* heterozygotes with intermediate phenotypes. Heterozygosities of these intermediate kernels, selected visually, were confirmed in the following generation where all of them segregated in 2: 1: 1 ratios.

These results may suggest that at least *Wx* genes of some of the Japonica rice have different degree of dominance over *wx*, from other starchy cereal or even from other *Oryza* relatives. In general, fully matured storage starch in plants contains about 25% amylose. It would be interesting to suppose that a single normal *Wx* gene could synthesize amylose up to 25%, but by sone kind of mutation occurred during the evolution, *Wx* gene in Japonica rice lost some of its function so that full three *Wx* genes were required in endosperm to synthesize the normal amount of amylose. To test this hypothesis, a wide survey of the gene dosage effect of *Wx* gene in other varieties of rice is in progress.

VIII. POPULATION GENETICS (THEORETICAL)

Efficiency of Truncation Selection

James F. CROW and MOTOO KIMURA

Truncation selection is known to be the most efficient form of directional selection. When this is modified so that the fitness increases linearly over a range of one or two standard deviations of the value of the selected character, the efficiency is reduced, but not greatly. When truncation selection is compared to a system in which fitness is strictly proportional to the character value, the relative efficiency of truncation selection is given by $f(c)/\sigma$, in which $f(c)$ is the ordinate of the frequency distribution at the truncation point and σ is the standard deviation of the character. It is shown, for mutations affecting viability in *Drosophila*, that truncation selection or reasonable departures therefrom can reduce the mutation load greatly. This may be one way to reconcile the very high mutation rate of such genes with a small mutation load. The truncation model with directional selection is appropriate for this situation because of the approximate additivity of these mutations. On the other hand, it is doubtful that this simple model can be applied to all genes affecting fitness, for which there are intermediate optima and antagonistic selection among components with negative correlations. Whether nature ranks and truncates, or approximates this behavior, is an empirical question, yet to be answered. For details, see *Proc. Natl. Acad. Sci. USA* 76, 396–399.

Fixation of a Deleterious Allele at One of Two "Duplicate" loci by Mutation Pressure and Random Drift

Motoo KIMURA and Jack L. KING

We consider a diploid population and assume two gene loci with two alleles each, A and a at one locus and B and b at the second locus. Mutation from wild-type alleles A and B to deleterious alleles a and b occurs with mutation rates v_a and v_b, respectively. We assume that alleles are completely recessive and that only the double recessive genotype *aabb* shows

a deleterious effect with relative fitness $1-\varepsilon$. Then, it can be shown that if $v_a > v_b$ mutant *a* becomes fixed in the population by mutation pressure and a mutation-selection balance is ultimately attained with respect to the *B/b* locus alone. The main aim of this paper is to investigate the situation in which $v_a = v_b$ exactly. In this case a neutral equilibrium is attained and either locus can drift to fixation for the mutant allele. Diffusion models are developed to treat the stochastic process involved whereby the deleterious mutant eventually becomes fixed in one of the two duplicated loci by random sampling drift in finite populations. In particular, the equation for the average time until fixation of mutant *a* or *b* is derived, and this is solved numerically for some combinations of parameters $4N_e v$ and $4N_e\varepsilon$, where v is the mutation rate ($v_a = v_b \equiv v$) and N_e is the effective size of the population. Monte Carlo experiments have been performed (using a device termed "pseudo sampling variable") to supplement the numerical analysis. For details, see *Proc. Natl. Acad. Sci. USA* 76, 2858–2861.

Model of Effectively Neutral Mutations in which Selective Constraint is Incorporated

Motoo KIMURA

Based on the idea that selective neutrality is the limit when the selective disadvantage becomes indefinitely small, a model of neutral (and nearly neutral) mutations is proposed that assumes that the selection coefficient (s') against the mutant at various sites within a cistron (gene) follows a Γ distribution; $f(s') = \alpha^\beta e^{-\alpha s'} s'^{\beta-1}/\Gamma(\beta)$, in which $\alpha = \beta/\bar{s}'$ and $\bar{s}'$ is the mean selection coefficient against the mutants ($\bar{s}' > 0$; $1 \geqq \beta > 0$). The mutation rate for alleles whose selection coefficients s' lie in the range between 0 and $1/(2N_e)$, in which N_e is the effective population size, is termed the effectively neutral mutation rate (denoted by v_e). Using the model of "infinite sites" in population genetics, formulas are derived giving the average heterozygosity ($\bar{h}_e$) and evolutionary rate per generation (k_g) in terms of mutant substitutions. It is shown that, with parameter values such as $\beta = 0.5$ and $\bar{s}' = 0.001$, the average heterozygosity increases much more slowly as N_e increases, compared with the case in which a constant fraction of mutations are neutral. Furthermore, the rate of evolution per year (k_1) becomes constant among various organisms, if the generation span (g) in years is inversely proportional to $\sqrt{N_e}$ among them and if the mutation rate per

generation is constant. Also, it is shown that we have roughly $k_g=v_e$. The situation becomes quite different if slightly advantageous mutations occur at a constant rate independent of environmental conditions. In this case, the evolutionary rate can become enormously higher in a species with a very large population size than in a species with a small population size, contrary to the observed pattern of evolution at the molecular level. For details, see *Proc. Natl. Acad. Sci. USA* 76, 3440–3444.

Population Genetics of Multigene Family with Special Reference to Decrease of Genetic Correlation with Distance between Gene Members on a Chromosome

Motoo KIMURA and Tomoko OHTA

A mathematical method is developed which enables us to treat exactly the process of coincidental evolution under mutation, unequal intrachromosomal crossing-over as well as ordinary crossing-over between homologous chromosomes in a finite population of the effective size N. It makes use of finite difference equations involving two quantities denoted by f_i and ϕ_i, in which f_i is the identity coefficient of two gene members that are i steps apart on the same chromosome and ϕ_i is that of two members i steps apart on two homologous chromosomes. When the number of genes (n) per family is large, the finite difference equations can be approximated by ordinary second-order differential equations which can then be solved analytically. Results obtained by the present method are compared with the corresponding results previously obtained by one of us (Ohta, Genet. Res. **31**: 13–28) using conventional diffusion models of gene frequency changes in population genetics. For details, see *Proc. Natl. Acad. Sci. USA* 76, 4001–4005.

An Extension of a Model for the Evolution of Multigene Families by Unequal Crossing-over

Tomoko OHTA

Evolution of a multigene family is studied by using approximate diffusion model. It is assumed that the multigene family is undergoing continuous *interchromosomal* unequal crossing-over, mutation and random frequency drift. The equilibrium properties of the probability of gene identity (identity

coefficient) are investigated, using two measures: identity probability within and between chromosomes. The measures represent average identity coefficient of genes within a family in one chromosome and that of gene families between two homologous chromosomes. The means, the variances and the covariance of these two measures of identity coefficient are obtained. It is shown that the means and the variances are generally smaller than those prepredicted in the previous model assuming intrachromosomal (sister chromatid) unequal crossing-over (Ohta, Genet. Res. **31**: 13–28). For details, see *Genetics* 91: 591–607.

Genetic Variability Maintained in a Finite Population under Mutation and Autocorrelated Random Fluctuation of Selection Intensity

Naoyuki TAKAHATA and Motoo KIMURA

By using the diffusion equation method, the level of genetic variability maintained under mutation pressure in a finite population is investigated, assuming autocorrelated random fluctuation of selection intensity. An appropriate mathematical model was formulated to treat the change of gene frequencies, incorporating mutation pressure and fluctuating selection. Extensive Monte Carlo simulation experiments were also performed to supplement the theoretical treatments. Excellent agreement between the two results suggests the validity of the present diffusion model for the autocorrelated selection. One of the most important findings from the simulation studies is that mutations and random sampling drift largely determine the level of genetic variability, and that the presence of autocorrelated selection can significantly lower genetic variability only when its strength, as measured by the cumulative variance of selection intensity, is larger than about 10^3 times the mutation rate. It is pointed out that the effects of both mutations and random sampling drift have to be incorporated in order to assess the role of various factors for the maintenance of genetic variability in natural populations. The present finding is favorable to the neutral theory of molecular evolution. For details, see *Proc. Natl. Acad. Sci. USA* 76, 5813–5817, 1979.

IX. POPULATION GENETICS (EXPERIMENTAL)

New Inversions of *Drosophila melanogaster* spreading over Japan

Yutaka INOUE, Kendo TSUNO and Takao K. WATANABE

A new inversion on the left arm of the second chromosome, *In*(2*L*)*W*28C; 32C, was firstly found in Katsunuma in 1972. Thereafter, it has been detected there in frequency of about 3 or 4 percent which is the next most frequent inversion to *In*(2*L*)*t* on the arm. The *W* inversion was also found in other eastern Japan than the Katsunuma; Sapporo (1977), Tochigi (1978, 1979), Niigata (1978), Shiojiri (1977, 1978, 1979), Mishima (1978), Ito (1975), Shimoda (1975). Association between some electromorphs and the *W* ininversion was examined among 48 *W* inversions ever found in the Katsu-Katsunuma; 42 was α-*Gpd*^{F}-*Adh*^{F}, 5 was α-*Gpd*^{F}-*Adh*^{S} and 1 was α-*Gpd*^{S}-*Adh*^{F}.

Another new inversion on the left arm of the third chromosome, *In*(3*L*) *Y*68F; 75C, was firstly found in the Katsunuma in 1975. Thereafter, it has been detected there in frequency of about 2 or 3 percent which is surpassing the old inversions, 3*LP* and 3*LM*, on the same arm. The distribution of the *Y* inversion is relatively wider than the *W* inversion; from Sapporo (1977) to Tottori (1979) and Osaka (1979). Association between *Est*-6^{S} and the *Y* inversions seemed to be exclusive in the Katsunuma (1979) population.

These two inversions, *W* and *Y*, might have occurred in some middle part of Japan in comparatively lately and is spreading over Japan. Papulations carrying these inversions have also carried the other polymorphic old inversions simultaneously. This situation suggests that a new inversion can survive to become a polymorphic inversion where the old inversions already exist.

Inversion Polymorphism in some African, New Guinean and Philippine Populations of *D. melanogaster*

Yutaka INOUE and Takao K. WATANABE

A total of 70 *Drosophila melanogaster* females were collected at six localities of Africa, New Guinea and Phillippines in 1979. They were indivi-

Table 1. Frequencies of inversions detected in some African, New Guinean and Philippine populations of *D. melanogaster*

Locality	No. of tested lines	No. of inversion carrying lines	% of polymorphic inversion/chromosome arm							
			2Lt	2RNS	3LP	3LM	3RP	3RC	3RK	3RMo
Mombasa, Kenya	4	1*	0	0	0	0	0	0	0	0
Nairobi, Kenya	27	14	18.5	0	1.9	0	7.4	3.7	5.6	0
Tananarive, Madagascar	13	7	26.9	0	0	0	0	3.9	0	0
Wau, Papua New Guinea	6	6	33.3	41.7	25.1	8.3	58.3	0	0	0
Port Moresby, Papua New Guinea	18	18	66.7	47.2	44.4	2.8	83.3	0	0	0
Manila, Philippines	2	1	0	0	25.0	0	25.0	0	0	0

* Unique endemic inversion: In (3L)62D-68A.

dually sent to Japan as isofemale strains. One larva was sampled from each strain and examined the salivary gland chromosomes. Table 1 shows the result. Every population carried inversions although the number of tested lines was small. Most populations except Mombasa were characterized by the maintenance of polymorphic inversions. Among them, two New Guinean populations showed the highest level of the inversion contents when compared with the Ishigaki, Japan (Inoue and Watanabe, Jap. J. Genet. 54,69. 1979) and the Florida, U.S.A. (Mettler *et al.*, Genetics, **87**: 169, 1979) populations. *In*(**3***R*)*K* is restricted its distribution in Africa and U.S.A., and it was confirmed here. Other polymorphic inversions are spread over the world, but the lacks of *In*(**3***R*)*Mo* in every locality and *In*(**3***R*)*C* in New Guinea may be ascribed to the small sample of the present study (Supported by MEJ 404149 & 504344).

X. EVOLUTIONARY GENETICS

Courtship Song and Mating Preference in *Drosophila melanogaster*, *D. simulans* and their Hybrids

Masaoki KAWANISHI and Takao K. WATANABE

Intra-specific variations of the courtship song (inter-pulse interval: *ipi*) of *Drosophila melanogaster* and *D. simulans* males were investigated. The variation among strains was small in *D. melanogaster* (31.3–34.8 msec, $n=14$), whereas it was much larger in *D. simulans* (42.8–56.8 msec, $n=55$). The average *ipi* was 32.8 msec for *D. melanogaster* and 49.1 msec for *D. simulans*, and the distributions between the two sibling species were non-overlapping.

Courtship song (*ipi*) and mating preference of hybrids between *D. melanogaster* and *D. simulans* were investigated. The *ipi* of hybrid males carrying the X chromosome either of *D. simulans* or of *D. melanogaster* is intermediate between that of the two parents, suggesting that the autosome determination with incomplete dominance. On the other hand, the mating preferences of hybrid males measured by the number of copulated pairs within 30 minutes differed by the X chromosomes carried by the males; the hybrid males carrying the X chromosome of *D. simulans* copulated with *D. simulans* females exclusively while the hybrid males carrying the X chromosome of *D. melanogaster* preferred to copulate with *D. melanogaster* females. These mating successes were primarily due to male mating discriminative abilities, since the hybrid males with the *D. simulans* X did not court *D. melanogaster* females while the hybrid males with the *D. melanogaster* X rather couted *D. melanogaster* females though the discrimination was weak.

Chromosome Analysis of Hybrid Male Sterility between *D. simulans* and *D. mauritiana*

Tsuguhiko TAKAMURA and Takao K. WATANABE

Interspecific cross between two sibling species, *D. simulans* and *D. mauritiana*, produces fertile females and sterile males (David *et al.*, J. Genet. **62**: 93, 1976). We set up a line of *D. simulans* marked with *y*, *bw*, *st* on the first, second, and third chromosome respectively. This *simulans* was crossed to

wild *mauritiana* and F_1 females were backcrossed to *y*: *bw*: *st simulans*. Segregated 8 types of males were examined whether seminal vesicles contained motile sperm or not as a criterion of fertility

In the cross between F_1 females of *simulans*♀ × *mauritiana*♂ and marked *simulans*, fertility of male progenies were: (*y*: *bw*: *st*) 16.5%, (*y*: *bw*)6.67%, (*y*: *st*)23.76%, (*y*) 7.55%, (*bw*: *st*) 3.88%, (*bw*)1.92%, (*st*) 3.91%, (+) 0.97%. In the cross, reciprocal F_1 females × marked *simulans*, the fertilities were 10.00%, 4.00%, 11.00%, 3.00%, 2.00%, 0.00%, 1.00%, 2.00%, for each phenotype respectively. If we hypothesize additivity in the contribution of each chromosome homozygous for marked chromosome, the contribution of the first chromosome, for example, to the fertility is calculated by 1/4[(*y*: *bw*: *st*)−(*bw*: *st*)+(*y*: *bw*)−(*bw*)+(*y*: *st*) − (*st*)+(*y*) − (+)]. The chromosomal contributions calculated in such a way are 11.00, −1.78, 7.72 for 1st, 2nd, and 3rd chromosome respectively in the first cross and 5.75, −0.25, 3.75 in the reciprocal one. These figures means that the first and third chromosome contribute largely to male fertility but the second chromosome does not or even worse when it becomes homozygous with *simulans* chromosome.

Even (*y*: *bw*: *st*) males from the first cross recovered its fertility only 16.5% though the three chromosomes and cytoplasm are all *simulans*. This was probably because the breakage of coadapted systems by the crossing over in the F_1 females. To conferm this, recombination ratio in the F_1 hybrid was obtained between *y* and *w*, and *y* and *v* on the first chromosome. Crossing over value between *y* and *w* was 5.34% in the hybrid while 9.74% in the control *simulans*. Between *y* and *v* was 29.36% in F_1 and 40.53 in *simulans*. Thus the crossing over takes place in high frequency in F_1 females though the ratio was smaller than that in *simulans*. The reason why there is a difference between reciprocals are not answered yet.

Host-dependent Evolution of Three Papova Viruses

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A marked similarity has been observed in the organization of genomic information between two of the papova viruses, SV40 and polyoma virus (Py), suggesting that these viruses may have diverged from a common ancestor during evolution. However, distinct differences between them and also

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between them and the human papova virus, BKV, are observed, for example, in their interactions with several animal cells and the immunological cross-reaction between viral proteins. Results from DNA-DNA hybridization experiments suggest that homologies between the viral DNAs are small, although the search for homology has depended largely on the stringency of the experimental conditions. It has also recently been pointed out that in stringent hybridization conditions, greater than 85% homology is required for the observation of double-strandedness, and that the use of less stringent conditions reveals more extensive homology between Py and SV40 than had earlier been reported. Nonetheless, the above data did not seem to allow detailed discussion of the relationships between papova viruses. We therefore turned to a comparison of the nucleotide sequences of the viral genomes and found striking homologies around the DNA replication origins

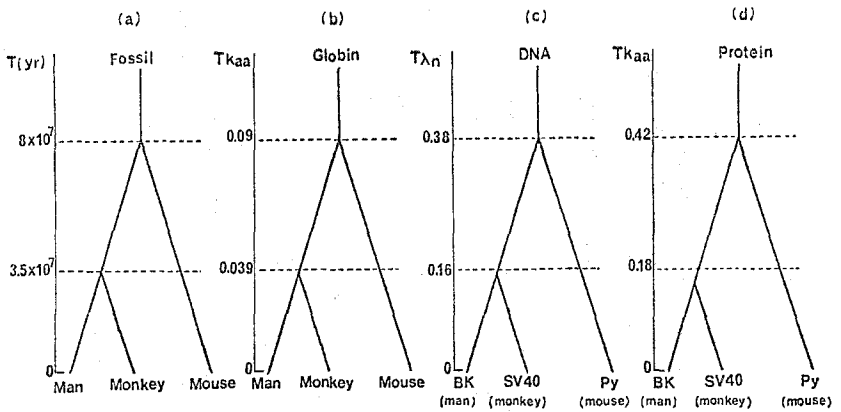

Fig. 1. Phylogenetic trees for hosts and for viruses. a. For the hosts, based on palaeontological evidence; each branch of the tree represents evolutionary divergence time. b. For the homologous globin amino acids; each branch represents the evolutionary distance measured by κT. c. For the viruses based on nucleotide sequence; each branch represents the evolutionary distance measured by λT. d. For the viruses based on the amino acid sequence comparisons, each branch represents the evolutionary distance measured by κT. The dotted horizontal lines indicate the relative points where the branch should occur if the molecular evolutionary distances among the viruses agreed completely with the branchings of the hosts. As the dotted lines show only relative locations, one of the two lines can be placed at an arbitrary location. We have therefore adjusted the branchings between Py and SV40 (BKV) to the same height of the primates-rodent branching in a.

Table 1. Comparison of nucleotide and amino acid sequences among the three virus species BKV, SV40 and polyoma virus (Py)

Virus lineage	Gene	Probability of identity		Linearized evolutionary distance*		No. of nucleotides examined
		P^n DNA	P^a Protein	$2\lambda T$ DNA	$2kT$ Protein	
Py/SV40	Small t	0.453	0.306	0.98	1.25	513
	Large T	0.501	0.423	0.82	0.89	1,881
	VP2/3	0.501	0.434	0.82	0.86	885
Py/BKV	Small t	0.470	0.322	0.92	1.19	513†
	Large T	0.425	0.407	1.09	0.93	1,380†
	VP2/3	0.469	0.341	0.92	1.12	510†
BKV/SV40	Small t	0.709	0.709	0.37	0.35	516
	Large T	0.721	0.753	0.35	0.29	1,533
	VP2/3	0.764	0.758	0.28	0.28	534

* This is divergence time (T) multiplied by rate (λ or k).

† The line-up of homologous peptides of BKV/Py is very similar to that oberved in Py/SV40. Approximation to maximum homology of BKV/Py was mainly obtained by substitution of homologous peptides of BKV/SV40 into those found for Py/SV40.

of these viral species. Recently, the complete sequences of SV40, Py and BKV DNAs have been elucidated, providing insight into the evolutionary relationships of these viruses.

BKV, SV40 and Py are easily isolated from healthy human, African green monkey and mouse tissues, respectively. They grow *in vitro* to high titres on cells from these species; therefore, we consider these to be their natural hosts. From the DNA sequence homology, we have calculated relative evolutionary distances by the method of Zuckerkandel and Pauling (Table 1), and constructed a phylogenetic tree for the viral species. For the hosts, the evolutionary time and the relationships between species have been well documented from palaeontological studies, based on the fossil record (Fig. 1).

From coincidence of the phylogeny, we conclude that each of these viral species has evolved from a common ancestor and diverged with its host organism.

This work has been reported in Nature, **285**, 165–167 (1980) and was presented at the Imperial Cancer Research Fund Tumor Virus Meeting on SV40, polyoma and adenoviruses, Cambridge, England 1979.

XI. HUMAN GENETICS

Hereditary Retinoblastoma: Host Resistance and Age at Onset

Ei MATSUNAGA

Age-specific incidence data from 244 bilateral and 31 familial unilateral cases of retinoblastoma were analyzed. In the bilateral cases, a high intraclass correlation between ages of the patients at diagnosis in the right and left eyes was noted, even after removal of the bias due to the fact that the diagnosis for each eye is never independent. This finding indicated strongly that the age at onset was largely determined by host factors common to both eyes. The mean ages of the patients at diagnosis of the inherited cases among the bilateral or unilateral group varied consistently with parental phenotype. This finding implied that inherited host resistance played an important role in the latency period for the gene carriers. The fraction of cases not yet diagnosed fitted well to a negative exponential distribution, and in the less susceptible group with hereditary unilateral cases, tumor formation may have been suppressed about twice that in the most susceptible group with bilateral cases. It was argued that the presumed second hit initiating tumor formation in the gene carriers was not a mutational process, but probably an error in the process of differentiation that could be suppressed completely in the most resistant group who could remain unaffected, but that could not be suppressed completely in the less resistant group. For details, see *J. Natl. Cancer Inst.* **63**: 933–939, 1979.

Threatened Abortion, Hormone Therapy and Malformed Embryos

Ei MATSUNAGA and Kohei SHIOTA

Causal relations between maternal genital bleeding, supportive hormone therapy and external malformations of the embryos were investigated with special reference to the critical period of oraganogenesis. This was done using morphological and obstetrical data obtained by Nishimura and his associates from 667 undamaged embryos derived from induced abortions

whose mothers had genital bleeding in early pregnancy. In addition, data from 90 embryos with polydactyly and 38 with limb reductions in the Nishimura collection were used for case history studies. Evidence was presented to demonstrate that, for major malformations such as CNS anomalies, cleft lip, polydactyly and limb reductions, maternal genital bleeding was not a cause but a consequence of the conception of an abnormal embryo. No indication was revealed that exogenous female hormones currently used in Japan for preventing miscarriages could produce major malformations recognizable at the embryonic stage, including limb reductions, nor salvage the severely malformed embryos. This does not however mean to exclude the possible relationship of progestogens/estrogens intake during early pregnancy with an increased incidence at birth of certain internal and/or external malformations. It was suggested that most, if not all, of the minor anomalies observed at certain embryonic stages are kinds of normal variants without any functional impairment of embryonic development. For details, see *Teratology* **20**: 469–486, 1979.

Suppressed Centromere and the Loss of "Centric Dot" in Dicentric Chromosomes

Yasuo NAKAGOME

In 1979, Eiberg (Nature **248**: 55, 1974) described a technique called Cd band which stained two small dark spots on both sides of a centromere (kinetochore). The Cd denoted a centric dot and presumably represented either kinetochore itself (Evans, H. J. & Ross, A.: Nature **249**: 861, 1974) or condensed chromatin related to a kinetochore (Roos, U. P.: Nature **254**: 463, 1975).

In 1976, Nakagome *et al.* described a child with multiple congenital anomalies caused by an unusual translocation resulting a dicentric chromosome: tdic (7: 15)(p21; p11) (Nakagome, Y. *et al.*: Clin. Genet. **9**: 621, 1976). Both centromeres of a no. 7 and a no. 15 were included in the tdic chromosome but it assumed monocentric appearance. Only the centromere derived of the 7 showed characteristics of a centromere. The other was presumably not functioning [pseudodicentric chromosome; psu dic (7)t (7; 15) (p21; p11)]. In a C-banded (CBG) metaphase, both active and inactivated (suppressed) centromeres were positively stained. When stained with the Cd-band technique, however, only the active (derived of a no. 7) centromere was

positive. No Cd positive material was detected at the site of inactivated centromere. Later, Mattei *et al.* (Hum. Genet. **50**: 33, 1979) and Maraschio *et al.* (Hum. Genet. **54**: 265, 1980) reported similar results.

In the present study, three cases of pseudo-dicentric chromosome, 3 cases of i(Xq) and a case of r(6) (p25q27) were examined using a variety of banding techniques including GTG, QFQ, AT-specific(DAPI), distamycin-DAPI, CBG, Ag-I and Cd. In a case of Xq+, karyotype was found to be 46, X, psu dic(X) (pter→cen→q22:: q22→pter), i.e., one of the two centromeres were inactivated. Both active and inactive centromeres were stained positively by the C-band but the Cd staining revealed dark spots only on the site of an active centromere. A case of psu dic(21) was found to have a karyotype 46, XX, psu dic(21)(pter→cen→q22:: q22→pter). Both ends of the psu dic were C-positive. The Ag-I staining also revealed nucleolus-organizing regions on both ends, however, the Cd staining revealed dark spots at only the site of an active centromere. In a 3rd case, the karyotype was identified as 47, XY, +psu dic(9) (q2101) based on several different banding techniques (Abe *et al.*, Ann. Genet. **20**: 111, 1977). Both centromeres were C-positive and brightly fluoresced in a distamycin-DAPI stained metaphase. In only one of them, were Cd spots detected.

In 2 of 3 cases of i(Xq), a C- and Cd-positive site was detected on the i(Xq). In one case, two C-positive but only one Cd-positive site was detected on an i(Xq). The two C-positive sites were very close each other and sometimes they appeared fused. According to Hsu, L.Y.F. *et al.* (J. Med. Genet. **15**: 222, 1978), of 30 cases with i(Xq), 21 showed two C-positive spots. In all of them, only one constriction (centromere) was visible. No Cd-band studies were carried out. In a r(6), some of the metaphases contained a dobule-sized ring. They always had two C or Cd spots in accordance with the presence of two visible centromeres. No single sized rings had two C or Cd spots.

We do not still know the exact nature of Cd-positive material, however, it appears that the detection of Cd-positive material corresponds to the presence of an active centromere and the loss of the former to the supression of the latter. In a case of dicentric i(Xq), whether the presence of two active centromeres is compatible with the survival of the dicentric due to the close proximity of both centromeres, is to be determined.

A New Approach in the Evaluation of Chromosome Variants in Man (II and III)

Yasuo NAKAGOME, Jun-ichi AZUMI, Shigehiro OKA and Ei MATSUNAGA

A new approach is proposed in the evaluation of chromosome variants in man. A scanning microdensitometer was used in the determination of the size (area) of a variant and results were assigned into five classes based on the difierence from an average in terms of standard deviation. In the first part of the present series, results abtained in C variants of nos. 1, 9 and 16 were described (Azumi *et al.*, Jap. J. Hum. Genet. **24**: 99, 1979).

In the present study, the procedure was applied to LBA-treated metaphases. LBA stood for late-replicating band detected by BUdR-acridine and intended to detect variable segments on the basis of their DNA-replicating pattern. It was shown to be very effective for chromosome pairs lacking established Q or C variable segments, e.g., nos. 2, 5, 6, 7, 8, 10, 11, 12, 17, 18, 19 and 20 (Nakagome *et al.*, Hum. Genet. **38**: 307, 1977). Metaphases were obtained from twelve unrelated individuals. Chromosomes were identified based on their Q-band pattern. The size (area) of an LBA-bright centromeric heterochromatin (variants) was measured using a microdensitometer, expressed relative to that of euchromatic segment of the given chromosome, and were assigned into five classes as described in the first part (for example those within $\pm$1SD of an average was assigned into class 3).

A total of 87 LBA-variants were detected in twelve individuals, i.e., each of twelve pairs with no established variable segment revealed 4 to 10 variants by the present method. Acrocentrics with Q-variable sites, i.e., pairs 13, 14, 15, 21 and 22, were also studied. A total of 47 LBA-variants were detected (for details, see Azumi *et al.* (part II) Hum. Genet. **55**: 75, 1980 and Oka *et al.* (part III) Hum. Genet. **55**: 327, 1980).

XII. BEHAVIORAL GENETICS

Selection for the Learning Ability of Mice*

Tohru FUJISHIMA

A selection has been made bidirectionally for the discriminated avoidance performance (DAR, %) of mice (60–70 days of age) in the second of two daily training sessions, each comprising fifty learning trials. The fundamental stock was constructed from crossing of four inbred mouse strains (C3H/HeMs, SWM/Ms, C57L/JMs and D103/Ms), each having different characteristics of DAR measured with an automated Y-type maze apparatus (cf. Ann. Rep. **25**: 85).

The data for the fifth selected generation were obtained in this year. They indicated that the cumulative genetic gain for the average of males and females was 10.8, and the difference in avoidance between the DAR High line and the DAR Low line (High line minus Low line) was 20.9, while the difference in discrimination was −3.5. They also showed that the superiority of the Low line over the High line in discrimination was more considerable in males than in females. These results suggested that the selection for higher DAR resulted in the higher avoidance ability of mice rather than the higher discriminatory ability.

Genetic Difference in Oviposition Cycle in *D. melanogaster*

Tsuguhiko TAKAMURA

By artificial selection, two lines which are different in oviposition site preference was set up (Takamura and Fuyama, Behav. Genet. **10**, 105, 1980). One prefers paper placed on the medium (P line) and the other prefers medium (M line). The oviposition behavior of these two lines were observed and compared in a small chamber which contained a cube medium half of which was covered with paper. Two clear difference was detected between these two lines.

Before oviposition, *D. melanogaster* females always probe the surface of

* This work was supported by a grant from the Ministry of Education (No. 339026).

substrate with its ovipositor and if the site is judged suitable, they deposit eggs. The first difference was seen in the frequency of the first probing which took place on the paper. Ninety-five percent of the probing of the P line took place on the paper half but only 60% for the M line.

There are three sequels after first probing took place on the paper. 1) Oviposition on the paper, 2) Repetition of probing until the fly comes to the medium half, then oviposition, 3) Giving up oviposition after several probings. P line went into these three sequels almost in the same frequency. But M line never deposit on the paper even if the first probing was released on the paper, that is, the frequency of 1) is zero. 2) was 64.2% and 3) 35.8%. If the first probing was started on the medium (5% for P line and 40% for M line), both line deposit eggs 100% on the medium. The eggs which are not laid in the first cycle after probing on the paper, about 30% 95×0.53) for P and about 20% (60 ×0.36) for M, are recycled into the oviposition cycle from the beginning again. If we denote this recycling ratio r, and the proportion of eggs deposited on the paper after the first cycle a_p and that on the medium a_m, the over all egg production on the paper is expected with $a_p/1\text{-}r$ and on the medium $a_m/1\text{-}r$.

It was known that tarsus amputation deprived discriminating ability of of P line and ovipositor cautelization reduced that of M line. If the first probing is released by the sensory imput from the tarsi, and tarsus amputation makes the frequency of the first probing on the paper and on the medium equal, this strongly influence P line. If ovipositor cauterization renders the frequency of three sequels equal after probing on the paper, it influences M line largly. And these hypothesis meet well to the experimental results cited above (Takamura and Fuyama, Behav. Genet. **10** 1980, Takamura, this report, **29**).

Consequently, there are at least two components in the oviposition behavior of *D. melanogaster* which was genetically controlled and changeable by artificial selection. One is where the first probing takes place, which must be mediated by tarsus sensory organs and the other is actual oviposition released by the sensory imput from ovipositor. These two factors might play important roles in the choice of oviposition site of *D. melanogaster*.

Differentiation of Oviposition Thrusting Power in *D. melanogaster* Species Sub-group

Tsuguhiko TAKAMURA

In *D. melanogaster* it was found that there were large amount of genetic variation in the tendency of inserting eggs during oviposition (Takamura, Jap. J. Genet. **55**: 91, 1980). The character was tested easily by letting flies oviposit on the medium half of which was covered with paper. If the fly has strong tendency to bury eggs, they does not deposit on the paper because it is impossible to insert eggs into it. They only lay eggs on the medium half. However if the fly does not gave such a strong tendency, it deposit eggs also on the paper in a certain proportion. Using this method, two siblings of *D. melanogaster*, *D. simulans* and *D. mauritiana*, were examined whether there are intraspecfic genetic variations or not. Also 6 members of *melanogaster* species sub-group were compared in the tendency of burying eggs.

Little genetic variation was found among 40 and 17 isofemale lines of *D. simulans* and *D. mauritiana* respectively. Almost all the lines laid eggs on the medium half. This is a striking contrast to *D. melanogaster*. Then, more than 60 flies of each 6 species of *melanogaster* species sub-group were individually examined for their oviposition site preference either paper or medium. Except *melanogaster* all the species laid eggs on the medium. This means *D. melanogaster* is a exceptional species in the group that only shows genetic variation of the character.

The strength of egg-inserting tendency is measured more purely by the following method. After oviposition on the medium the surface is treated by NaOH for 20 minutes. After that the surface is washed by tapping water. This treatment washed away the unburied eggs. So the proportion eggs left after the NaOH treatment shows the degree of strength of the burying tendency. In order to compare the strength of the thrusting power of the 6 species, NaOH treatment experiments were done using mediums of different agar concentrations ranging from 0.8% to 4%. The larger the concentration, the harder the medium became. The sequence in the thrusting power of the 6 species was, *D. teissieri*$\leqq$*D. malanogaster* (line prefering paper)$<$*D. melanogaster* (line prefering medium)$\leqq$*D. yakuba*$<$*D. simulans*$<$*D. mauritiana*$<$*D. erecta*. Except two cosmopolitan species, *melanogaster* and *simulans*, 4 others are African endemic species. Some of them

are known to have specific host plants. It was postulated that in the process of speciation and habitat differentiation of the 6 species, the increase and decrease of oxiposition thrusting power played an important role.

Effect of UV Treatment on the Hatchability of Buried and Unburied Eggs of *D. melanogaster*

Tsuguhiko TAKAMURA

Takamura (Japan J. Genet **55**: 91) reported that there was a large amount of genetic variation in natural population of *D. melanogaster* in the tendency of egg-insertion when they oviposit. Some isofemale lines from Katsunuma showed strong tendency to bury eggs but some did not. However nothing was known as to the adaptive meaning of the behavioral difference and any difference in any fitness components of these two egg condition has not been examined either. One possible environmental facter causing difference in fitness is ultra violet lay. Here reported is a effect of UV treatment on hatchability of eggs laid by flies of different tendency of inserting eggs. Used lines, M8, M9 (having strond tendency to bury eggs), P8 and P9 (having not so strong tendency to bury eggs) were derived from the artificially selected lines for their oviposition site preference (Takamura and Fuyama Behav. Genet. **10**, 1980)

UV, 25 erg/mm^2/sec., was irradiated for 110 second on the surface of medium where eggs were already laid by above 4 lines. Number of eggs which did not hatch after 36 hours of storation of the medium in a humidified container at 25°C was counted. The hatchabilities were 5.34 ± 1.68, 7.25 ± 2.31, 47.94 ± 2.37, and 58.19 ± 2.35 for P8, P9, M8, M9 lines respectively. While control viabilities with no UV treatment were 87.35 ± 4.10, 94.15 ± 3.54, 96.36 ± 0.78, 82.67 ± 1.96 for each line in the same order. There is a possibility that M line eggs are physiologically resistant to UV. Thus, the UV treatment were done on eggs deposited on the paper, where it is impossible for flies to bury eggs. In this case the hatchability was around 10% in both P and M lines. Therefore, the large difference in hatchability after UV treatment was due to the effect of insertion of eggs into the medium. The medium protected the eggs from the UV. Of course, there is no possibility for eggs to be exposed to such a strong UV used in the experiment in natural condition but if the dosage of UV and its effect has strong correlation, week natural UV may cause difference in fitness such as hatchability,

preadult viability, and fertility between buried and unburied eggs or larvae and adults from these eggs. However, it dose not explain the polymorphism of the character in natural population.

XIII. ECOLOGICAL GENETICS

Observations on Rice Species and Accompanying Weeds in the Hilly Area of Tropical Asia

Hiko-Ichi OKA, Hiroko MORISHIMA and Yoshio SANO

In the eary dry season (October–November) of 1979, we travelled in the hilly areas of Nepal, India and Thailand for ecological genetics studies of rice species and accompanying weeds. Our trip was realized under a grant for overseas field research of the Ministry of Education. Our objectives were: a) observations on the diversity of rices and weeds influenced by environmental conditions, b) reexamination of the place and conditions of the initiation of rice domestication, and c) collection of seed samples of wild and cultivated rice species for future studies. Observations were recorded at 36 sites for wild rice and at 52 sites for cultivated rice. At each site, recordes were taken regarding physiography, degree of disturbance and water condition of the habitat, and plant height and percent cover of rice species and major weed species. In rice fields, conditions of cultivation were also recorded. As to weeds, a total of 147 species were identified by using herbarium specimens taken at each site. Extracts from our field notes are briefly summarized below.

1) *Oryza perennis* (Wild): a) This species was frequently found in marshy lowlands but never in the hilly area except in the Jeypore Tract, Orissa,. In the foothill of the Himalayas (Terai), wild rice was not found. b) Niche differentiation between the perennial and annual types of *O. perennis* was observed. The perennial type was found in deep swamps which appeared to be relatively stable, while the annual type was found in temporary shallow swamps which were parched in the dry season and frequently disturbed by man. The perennial type tended to be associated with perennial companion species, while the annual type with annual species. In a small marsh near Bhbaneswar, Orissa, we observed an annual type growing on the fringe and a perennial type in the center. c) Intermediate perennial-annual types were observed in disturbed habitats, and their habitats showed a high diversity of coexisting plant species. d) In the rice fields directly seeded, plants of *O. perennis* were often found occurring as weeds. They appeared

to have absorbed genes from *O. sativa* and have become weedy types adapted to the conditions of rice fields. e) We observed in Thailand that a roadside population of perennial type became extinct as replaced by sedge species possibly because of environmental pollution and disturbance, and that an annual population declined along with an increase of perennial weed species, probably because of relaxation of habitat disturbance.

2) *Oryza sativa* (Cultivated): a) Different types of rice culture were observed; lowland fields with varying water depths, terraced fields (upto 2000 m in Nepal), and upland fields. In accordance with diverse environments, different varieties were grown. b) Several seed characteristics were examined to estimate the Indica-Japonica differentiation. Varieties collected from the hilly area in Nepal were mostly of Japonica type, and some from nothern Thailand appeared to be intermediate Indica-Japonica types. Among native varieties of Nepal and Manipur, negative phenol reaction, long apiculus hair and round spikelet shape were frequently found, but these characters were not inter-correlated so as to make the distinction of Indica and Japonica types possible. c) Populations of *O. sativa* grown in the hilly area were polymorphic in seed characters, but no trend to Indica-Japonica differentiation could be recognized within populations. In the highland of Nepal, farmers sometimes grew mixture of two varieties. Populations collected in nothern Thailand were glutinous or mixtures of glutinous and non-glutinous plants. d) Within-populational variability as shown by the generalized variance of length and width of spikelets tended to be positively correlated with species diversity of coexisting weed species.

We came to the view that the Terai region might be not the place of origin of cultivated rice, since there was found no wild rice which could be the progenitor of cultivated rice, although some authors (e.g. Chang 1976) considered the foothill of the Himalayas to be the homeland of cultivated rice. During this trip, 37 populations of *O. perennis*, and 51 populations and 190 strains of *O. sativa* were collected. They are preserved in our Genetic Stocks Center.

An Experiment on Population Growth of Wild Rice in Greenhouse

Hiroko MORISHIMA

In order to examine how a perennial and an annual strain of *Oryza perennis* interact in their vegetative and reproductive phases, and how they respond

to habitat disturbance, two strains, W120 (perennial) and W106 (annual), were planted in pure stand and in mixture in concrete beds prepared in a greenhouse. The planting was made in 1978 summer with two replications. At maturity, the seeds were left to natural shedding, and biomass was recorded for the wild rice and also for naturally emerged weeds. Then, the field was divided into disturbed (the plants were cut and the soil was dug with a hoe) and non-disturbed plots. The fields were not watered during the winter-spring season to simulate the water condition in their natural habitats. In the second season, the following characters were recorded; number of surviving plants, number of recruited plants from soil-buried seeds, number of buried seeds per unit area, and dry weights of vegetative and reproductive parts of the plants. The results obtained are summarized as follows:

1) The perennial strain was a strong competitor against the annual strain in their vegetative growth. 2) With weeds, however, the annual strain showed a strong competitive ability than the perennial strain. 3) About 40% of annual plants survived after seed shedding because of a low seed fertility caused by low temperatures in winter. In the non-disturbed annual plot, the population consisted of both original and recruited plants, while in the disturbed plot the plants were exclusively recruited ones. 4) The survival of perennial plants was about 75%, but they produced no seedling. Their dry matter production was generally greater in non-disturbed plot than in disturbed plot, but their reproductive effort (proportion of panicle weight to total plant dry weight) was markedly higher in the disturbed plot. 5) In the non-disturbed plot where the two strains were mixed, the population in the second year consisted of perennial plants only, while in the disturbed plot both the perennial and annual strains coexisted.

The data thus indicated that perennial habit (vegetative propagation) was advantageous in stable environment, and annual habit (seed reproduction) was advantageous in disturbed environment.

Copper Tolerance and Reproductive Competition in Barnyard Grass

Hiroko MORISHIMA and Hiko-Ichi OKA

The strains of barnyard grass (*Echinochloa crus-galli*), C and D, were grown in 1978 in copper-polluted (*ca* 300 ppm) and control plots, and their seeds were left to natural shedding. Strain C was tolerant of copper as compared

with D, but was a weak competitor with D in vegetative growth. This year, the plants occurring from naturally sown seeds were observed for plant number and biomass at maturity. Records were also taken for other weed species naturally emerged. In general, strain D was found to dominate over C. In the polluted plot, however, C was not inferior to D so much. The dry matter weights of C and D coexisting on the same sampling site were negatively correlated among sites in the control plot, but they were positively correlated in the polluted plot. This indicated that the mode of competitive interaction between the two strains was affected by copper treatment. The total biomass of other weed species showed no significant difference between the polluted and control plots. Comparing the two plots, however, we found marked difference in the relative dominance of component species suggesting a change in community structure caused by copper treatment.

Copper Tolerance and Reproductive Competition in Four Annual Weeds

Hiroko MORISHIMA and Hiko-Ichi OKA

Last year (1978), the seeds of *Cyperus difformis* (Cy), *Fimbristylis miliacea* (Fm), *Centipeda minima* (Ce) and *Vandellia angustifolia* (Va) were sown in mixture (1 : 1) in all possible binary combinations on normal and toxic (200 ppm copper) soils prepared in trays. Their seeds were left to natural seeding from which the plants occurred in the second (this) year. In both the first and second years, observations were recorded for their number and total dry weight at seed maturity. Their relative dominance was evaluated by the ratio of total dry weight of coexisting two species. On the normal soil, their dominance was higher in the order Fm$\fallingdotseq$Cy>Ce>Va in the first year, and Cy$\geq$Fm$\geq$Ce>Va in the second year. Phenotypic plasticity, which was greater in the order Cy>Fm>Ce>Va, could have played some role in their adaptation under the competitive condition. On the toxic soil, dominance relation was Cy>Ce>Fm>Va in both the first and second years. This was in agreement with the order of their copper tolerance, Cy>Ce>Fm$\fallingdotseq$Va, which was evaluated on the basis of "toxic-normal ratio" in plant weight in pure stand.

Variation in Competitive Ability among *Oryza perennis* Strains

Yoshio SANO and Hiroko MORISHIMA

To examine how competitive ability as shown by neighbour effect is associated with the variation in reproductive strategy, a mix-planting experiment was carried out with 26 strains of *Oryza perennis* (wild) ranging between perennial and annual types and two strains of *O. sativa*. They were tested in pure stand and mixture with a test-strain in a concrete bed with automatic shortday control. As the test-strain, a strain of Asian perennial type of *O. perennis*, W120, was used; many plants having the same genotype were obtained from regeneration of stem cuttings taken from one plant of the strain, and they were planted in each row alternately with the seedlings of the strain to be tested with an interval of 10 cm. The dry weight at seed maturity was recorded in each strain. Competitive ability was estimated in terms of $(X_{ij}-X_{ii})-(X_{ji}-X_{jj})$, where X_{ij} is the measurement of strain i in mixture with j (test-strain) and X_{ii} represents that in pure stand.

The strains tested showed a wide range of variation in competitive ability. In general, perennial wild strains were stronger competitor with the test-strain than annual wild strains. A negative correlation ($r=-0.41$, significant at 5% level) was found between competitive ability and reproductive effort (proportion of seed weight to total plant dry weight in pure stand). As reported previously (Ann. Rep. **27**: 92 & 28, 109), the same set of strains had been tested for competitive ability with two different test-strains, a *sativa* strain (Taichung 65) and an annual wild strain (W107). The results obtained with different test-strains were not consistent; when tested with a perennial atrains, perennial strains were stronger in competition than annual strains, while annual strains were stronger than perennial strains when tested with an annual strain. The *sativa* test-strain can be considered as a perennial type. This complex of variations in competitive ability seems to have resulted from differentiation of *O. perennis* strains in adaptive strategy, and its analysis is left for studies in the future.

Neighbour Effects between Plants of *Oryza sativa* and *O. glaberrima* Grown in Mixture in Nigeria

Yoshio SANO and Hiroko MORISHIMA

Two rice species, *Oryza glaberrima* and *O. sativa*, are often planted in mix-

ture in West Africa. Seeds of coexisting plants of the two species, sampled from a upland and a lowland field in Nigeria and multiplied in Misima, were used for this experiment; the material consisted of four strains, G_1 (*glaberrima*, upland), G_2 (*glaberrima*, lowland), S_1 (*sativa*, upland), and S_2 (*sativa*, lowland). The four strains were tested in a mixture-diallel experiment on a controlled soil moisture gradient to examine neighbour effect, which was evaluated in terms of ($\log X_{ij} - \log X_{ii}$) where X_{ij} stood for the dry weight of plant i neighboured by j. The data showed that, although lowland strains were generally strong competitos with upland strains, the neighbour effects markedly differed according to strain combinations and water conditions. In the mixture of two species collected from the same field, *O. sativa* tended to be a strong competitor against *O. glaberrima* under wet conditions, but was not so under dry conditions. This trend was particularly obvious when upland strains of the two species were in mixture. On the other hand, lowland strains of the two species appeared to be cooperative in mixture, showing a R.Y.T. higher than unity (R.Y.T.$=1/2(X_{ij}/X_{ii}+X_{ji}/X_{jj})$; relative yield total). These changes in neighbour effect suggest that the responses to neighbouring plants differ according to water conditions.

XIV. APPLIED GENETICS

Effect of Assortative Mating on the Multigenic Character in a Finite Population

Shinya IYAMA

Computer simulation was carried out to inquire into the effect of assortative mating on the genetic structure in a finite population when assortment was based on the character controlled by many genes. Assortative mating was assumed to take place on the quantitative character governed by 31 equally effective independent genes. No other selection was not assumed in the simulation. In a randomly mating population of finite size, it has been known that the genetic variability decreases with generations and finally the population reaches a fixation into a single genotype. The results of simulation showed that the assortative mating accelerated the speed of loss of variability in the population (Table 1). Though the temporary increase in genetic variability was observed in the early generations of assortative mating, but differentiation was not attained and the population became fixed into any single genotype between both extremes. When the more similarity

Table 1. Percentage of fixed loci in successive generations of assortative mating based on the character governed by 31 equally effective independent genes. Initial gene frequency=0.5. Average of 10 runs.

Generation	Population size=40			Generation	Population size=200		
	Assortative mating*		Random mating**		Assortative mating*		Random mating**
	I	II			I	II	
10	4.0	0.7	0 (0)	50	0	0	0 (0)
20	21.8	9.7	1.4 (1.1)	100	31.6	3.7	0.7 (1.1)
30	43.5	15.5	6.5 (5.9)	150	78.6	29.0	7.1 (5.9)
40	59.2	31.0	14.3 (13.3)	200	90.3	47.5	12.3 (13.3)
50	77.4	37.4	18.4 (21.8)	300	96.3	73.3	32.3 (30.1)
100	99.2	72.3	57.1 (57.1)	400	97.2	86.6	43.9 (45.0)
150	100.0	91.0	77.0 (77.0)	500	100.0	94.5	58.1 (57.1)

* I: Perfect assortative mating; II: Assortment included three adjacent classes of phenotype to both sides. ** Theoretical values are shown in parentheses.

required for assortment, the faster the fixation. It was unlikely that the assortative mating resulted differentiation of genotypes even for multigenic character when the population was of finite size.

Changes in Isozymes and Some Quantitative Characters Observed in Hybrid Populations of Rice

Hiroko MORISHIMA and Toru ENDO

The wild rice, *Oryza perennis*, has many alleles at various loci controlling isozymes, but only a part of the variation is found in cultivated rice, *O. sativa*, derived from the former species. To look for the reason why domestication results in reduction of allelic variations, hybrid populations from wild and cultivated strains were propagated in bulk from F_2 to F_6 generation, and the frequencies of alleles at two loci (Px_1 and Acp_1) controlling peroxidase and acid phosphatase isozymes in the F_6 population, respectively, were compared with those observed in F_3.

The data showed that two alleles, Px_1^{2A} and Acp_1^1, tended to increase, both being possessed by the *sativa* parents. In F_3, the seeds for the next generation were harvested by two methods, one from the seeds remaining on panicles at maturity and the other from the seeds shed on the soil, and the same procedure was taken in each group for successive generations. In general, the genotype of populations shifted towards the cultivated type in response to the pressure of cultivation. The populations raised from the seeds remaining on panicles more rapidly shifted to cultivated type than those from shed seeds, in certain quantitative characters such as degree of shedding and those concerning yielding potential. Further, the former populations differed in the frequencies of isozyme alleles from the latter. It appeared that a gene for earliness was linked with Acp_1.

Nitrogen Fixation in the Rhizosphere of Cultivated and Wild Rice

Yoshio SANO, Taro FUJII, Shinya IYAMA, Yukinori HIROTA, and Kazuo KOMAGATA

To look into variations among genotypes of N_2 fixing ability in the rhizosphere of paddy rice, a modified assay system using the acetylene reduction technique was devised in order to measure large numbers of plants easily. Acetylene reducing activity was detected immediately after incu-

bation and increased almost linearly.

The activity measured by the present method was compared with that obtained by the whole plant assay system previously reported (Ann. Rep. **28**: 114). Correlation between the readings obtained by the two methods from 21 cultivars was highly significant (r=0.74, d.f.=19), indicating that the detachment of the shoot did not seem to affect the acetylene reducing ability in the rhizosphere when the activity was measured within 2 hr of incubation. The present assay system was successfully used to examine the N_2 fixing ability of a number of strains. Among 47 *O. sativa* strains, acetylene reducing ability per plant was significantly correlated with the activity per g dry root but not with dry root weight. This suggested that the activity per plant is independent of the quantity of root. Then acetylene reducing activity per g dry root was compared among strains. The activity showed a wide range of variation from 81 to 2148 nmol per hr per dry root. In addition, the activities of *O. sativa* and *O. glaberrima* strains (cultivated) were significantly higher than those of *O. perennis* and *O. punctata* strains.

To assess seasonal and diurnal changes in acetylene reducing ability, a rice strain C5444 which showed a relatively high activity, was used to measure the activity at different stages. At each stage, four plants were examined six times every 4 hr after noon. The activity was low in the early stages of growth and gradually increased as development advanced. Maximum activity was observed during flowering though the diurnal fluctuation also increased after flowering. The activity tended to be higher at night than in the daytime.

Nitrogen fixing bacteria were isolated from the rhizosphere of C5444 on a nitrogen-free agar medium. Isolated bacteria was subcultured several times with the same media, and N_2 fixers were purified. They were spiral, filamentous, or rod shapes. The results indicated that various species of N_2 fixers existed in the rhizosphere of rice.

Analysis of Genes Controlling the F_1 Pollen Sterility between *Oryza sativa* and *O. glaberrima*

Yoshio SANO

The F_1 plants between the two cultivated rice species, *Oryza sativa* and *O. glaberrima*, are highly pollen-sterile although the chromosomes normally pair in meiosis. They can be backcrossed as some embryosacs remain

functional. As reported previously (Ann. Rep. **25**: 85 & **27**: 80), isogenic F_1-sterile lines having the genetic background of *sativa* (Peiku, Acc. 108, an Indica type from Taiwan) and of *glaberrima* (W025 from Guinea) parent were isolated from B_8F_2 plants, respectively. They were self-fertile and showed semi-sterility in the F_1 plants when crossed with the respective parental strains. But the F_2 plants were fully fertile. This F_1 sterility was favorably explained by a "one locus sporo-gametophytic interaction" model of sterility genes, which assumes that Acc. 108 and W025 have S_1^a S_1^a S_2 S_2 and S_1 S_1 S_2^a S_2^a, respectively, and that if a S_1 or S_2 gene is prepresent in the maternal tissue, gametes with S_1^a or S_2^a deteriorate.

A following-up experiment was initiated in 1975 by using a Japonica strain (Taichung 65 from Taiwan) as the *sativa* parent and W025 as the *glaberrima* parent, following the same procedure as in the preceding experiment. The backcrossing experiment showed a different pattern of segregation from that observed before. Out of 51 B_9F_1 plants, 27 were fertile and 24 were semi-sterile, giving a 1 : 1 ratio. When the semi-sterile B_8F_1 plants were pollinated as the pollen parent, all the B_9F_1 plants were semi-sterile. It was also observed that the partially pollensterile plants had a seed fertility exceeding 90 percent and their selfing produced fertile and semi-sterile plants in a 1 : 1 ratio. This indicates that megaspores were not affected by the sterility genes.

This F_1 sterility can be explained by assuming that Taichung 65 and W025 have S_3^a S_3^a and S_3 S_3, respectively, and that if a S_3 gene is present in the maternal tissue, pollen with S_3^a deteriorates (one locus sporo-gametophytic inter-interaction). In addition, the heading date of plants having S_3 S_3^a or S_3^a S_3^a was about 10 days earlier than those having S_3 S_3. This difference was not observed in shortday conditions, suggesting that a recessive gene (*se*) controlling photosensitivity (carried by W025) was closely linked with the S_3 gene.

Adaptation to High Ambient Temperature of Japanese quail

Takatada KAWAHARA

F_1 hybrid birds of domestic Japanese quail were used for preliminarily examining their adaptability to the high ambient temperature. The number of female quails examined was 36 birds for temperature treatment and 51 birds for non-treatment control, respectively. The experiment was carried

out for one hundred and forty days from 231 to 370 days after hatching with three consecutively changed ambient temperatures. For the pre-temperature treatment, the temperature was kept at 25°C±2°C (relative humidity: 60±10%) for 17 days. Then it was gradually changed to 35°C within 6 days, for the first high temperature treatment, where it was kept at 35°C±2°C (50±10%) for 23 days, then again changed to 39°C within 3 days for the second high temperature treatment, where it was kept at 39°C±2°C (45±10%) for 20 days and returned to 25°C within 2 days. After then, the temperature was kept at 25°C±2°C (60±10%) for 69 days. The control group was reared under fixed temperature conditions: 25°C±2°C and relative humidity 60±10%. Photoperiod applied was 18-hour light and 6-hour darkness throughout the experiment. Food (20% crude protein) and water were supplied *ad libitum*.

Almost all birds showed a panting under either of the two high temperature treatments. Body weight also decreased under high ambient temperature treatment, to 89.2% at 35°C and to 86.7% at 39°C of those of the control. Egg production rate of both groups in the pre-treated period (25°C) was ranging from 65.3% to 84.7%. During the first high temperature treatment (35°C) it decreased to minimum 36.1% (control: 75.5% to 89.2%), and during the second high temperature treatment (39°C), it decreased further to minimum 20.8% (control: 79.4% to 88.2%). But the egg production rate was recovered in the later period of high temperature treatment, to 60% at 35°C and to 52% at 39°C, respectively. When temperature conditions returned from 39°C to the moderate temperature 25°C, the egg production rate recovered to the level of control in 20 days. Egg weight was reduced under high temperature conditions, the average being 8.50 g for 35°C treatment (9.24 g in a control) and 8.17 g for 39°C treatment (9.29 g in a control). Differences between the treated and the control groups were statistically significant at the 1% level. When temperature conditions returned from 39°C to the moderate temperature 25°C, the egg weight rapidly recovered to the level of the control.

Under the ambient temperature conditions as high as the body temperature, egg production rate and egg size of domestic Japanese quails decreased but egg production rate recovered gradually in the later period of the treatment.

Domestication in Japanese quail, *Coturnix coturnix japonica*

Takatada KAWAHARA

According to tradition, Japanese quail has been domesticated for the last several hundred years in Japan. The birds were loved for its countrified crow by the Samurai caste in the feudal days before the close of 19th century, but after the opening of the present century, they have become economically important for producing eggs and meat. Recently, they achieved rapid progress as an experimental animal. However, the domestic Japanese quails are very similar in appearance to the wild ones, and it is often impossible to distinguish clearly one from the other. Both groups are very different in some physiological as well as growth properties.

This experiment was carried out to find the genetic changes in wild Japanese quails reared for ten generations under domestic conditions. Examination was made on several characters of the birds which had been reared without conscious selection since they were captured in the field. Eggs laid during the ages of 20 to 35 weeks were used to obtain the birds for the next generation.

The results indicated that the most striking changes of the quails toward the domestic type occurred several characters: to early sexual maturity and increased fertility, hatchability, viability, egg production rate, fitness index (fertility $\times$ hatchability $\times$ viability $\times$ egg production rate) and body size. Decrease in the variability of various characters was also observed in progressive generations. The degree of change in performance with progress of generation was measured by the linear regression of a given trait on generation. Number of generations required to reach the level of domestic birds was estimated for various traits based on the regression. It ranged from 12 to 64 generations. In fitness index, for instance, it was estimated as 24 generations.

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ABSTRACTS OF DIARY FOR 1979

February	9	254th Meeting of Misima Gene ticists'Club
	13	255th Meeting of Misima Geneticists' Club
	23	256th Meeting of Misima Geneticists' Club
	28	144th Biological Symposium
March	1	145th Biological Symposium
	8	257th Meeting of Misima Geneticists' Club
	12	258th Meeting of Misima Geneticists' Club
	15	259th Meeting of Misima Geneticists' Club
	23	146th Biological Symposium
	30	260th Meeting of Misima Geneticists' Club
April	19	147th Biological Symposium
May	2	148th Biolobical Symposium
	21	149th Biological Symposium
	25	150th Biological Symposium
July	13	261st Meeting of Misima Geneticists' Club
	28	262nd Meeting of Misima Geneticists' Club
August	3	151st Biological Symposium
	14	152nd Biological Symposium
September	6	153rd Biological Symposium
	27	154th Biological Symposium
October	25	155th Biological Symposium
	25	156th Biological Symposium
December	3	263rd Meeting of Misima Geneticists' Club

FOREIGN VISITORS IN 1979

January	10	LAMSEEJAN, S., Kasetsart University, Thailand
		CAMPIRANAN, A., Kasetsart University, Thailand
February	10	SUEOKA, N., University of Colorado, U.S.A.
	28–March 1	RAMEL, C., University of Stockholm, Sweden
March	1	PITTENDRIGH, C. S., Stanford University, U.S.A.
April	21	PRICE, C. A., University of Rutgers, U.S.A.
May	2	SOKAL, R. R., University of New York, U.S.A.
	17	SORIANO, J. D., University of the Philippines, Philippines
	19	SEARLE, T., MRC Radiobiology Unit, England
	19–21	SCHAECHTER, M., University of Tufts, U.S.A.
	24–June 5	CROW, J. F., University of Wisconsin, U.S.A.
June	1	HAYNES, R. H., University of Yoke, Canada
August	14	NAKADA, D., University of Pittsburgh, U.S.A.
	14–15	MILLER, J. R., University of Columbia, Canada
September	3–March 30, '80	PARIDA, B. B., University of Utkal, India
	5–6	BARLOW, B. A., University of Flinders, Australia
		ROJANAPO, W., Nat'l Cancer Institute, Thailand
October	1–3	HARLAN, J., University of Illinois, U.S.A.
	15	RUFFIE, J., Centre d'Hemotypologie du CNRS, France
		SCHMID, D. O., München University, Germany
	22	PAVAN, C., University of Sao Paulo, Brazil
		PATERNIANI, University of Sao Paulo, Brazil
		IKUTA, H., University of Sao Paulo, Brazil
	24–27	POULSON, D. F., University of Yale, U.S.A.

	25–26	de GROUCHY, J., Lab. de Cytogenetique, Hopital des Enfants-Malades, France
November	8	WATSON, J. D., Cold Spring Harbor Laboratory, U.S.A.

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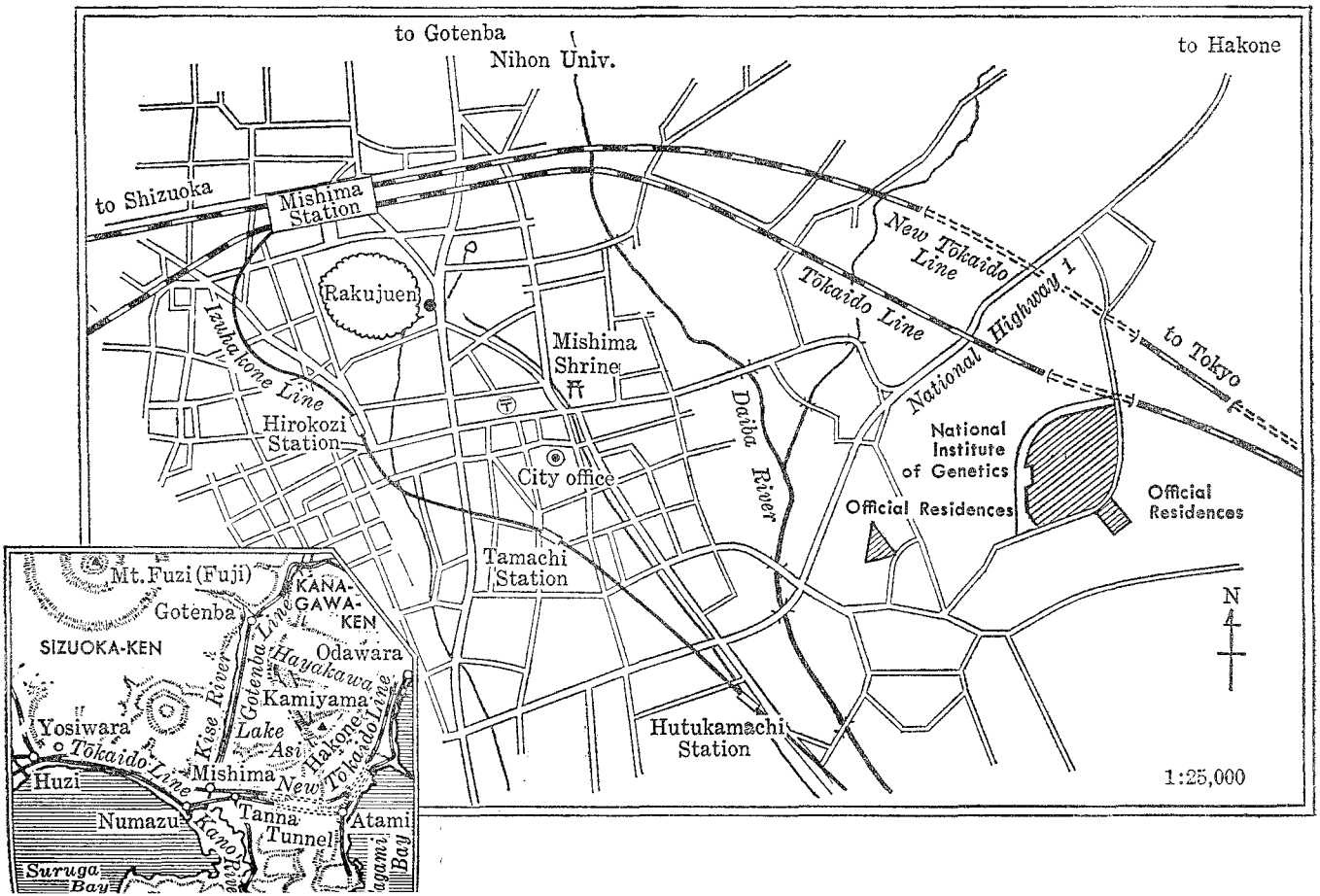
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