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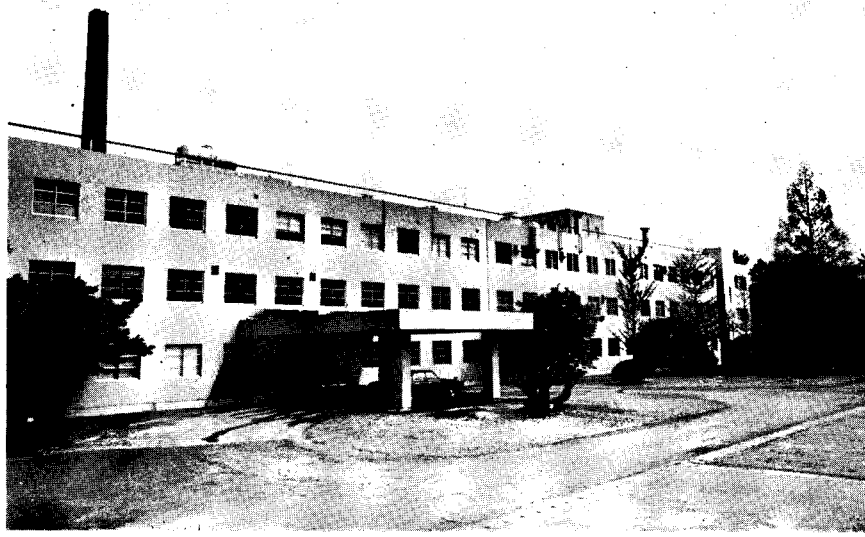

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1984

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GENERAL STATEMENT

In introducing the Annual Report of the institute that covers 1 year from January to December 1983, I should like to begin by paying tribute to my predecessor, Dr. Yataro Tazima, who directed the institute from March 1, 1975 to September 30, 1983. During that period Dr. Tazima had exerted himself for the development of the institute in many ways. In particular, in order to switch over the administrative category of the institute from the present system to that for joint use by universities, he made tenacious endeavor for the negotiation with the authorities concerned. As a consequence, the Ministry of Education, Science and Culture set up in April an *ad hoc* committee to investigate the reorganization plan of the institute (Chairman: Prof. Y. Yamamura, President of Osaka University). According to the report of the committee, this institute will have prospect to be reorganized into the one for joint use by universities from the next fiscal year. The institute conferred the title of Honorary Member on Dr. Tazima.

Having assumed, since October 1, heavy responsibility as director, I will do my best for further development of the institute so as to contribute more to the promotion of studies in genetics and related fields in this country. Since the foundation in 1949, the staffs of the institute have been cultivating the tradition to work assiduously in friendly rivalry with each other by breaking down academical cliquism or sectarianism. I was advised by Dr. Takashi Fujii, Chairman of the Board of Councillors of the institute, that the institute should carry on such tradition and independence. I will always keep his advice in mind.

Some of the events deserving special mention here are as follows. Dr. Tosihide H. Yosida, head of the Department of Cytogenetics, was awarded a Purple Ribbon Medal from the government for his long scientific contributions, including studies on the karyotypical evolution in Rodents. By the way, the same medal was awarded to Dr. Kan Oguma, who was the first director of the institute and teacher of Dr. Yosida. An Encouragement Award of the Association for the Promotion of Genetic Studies was given to Dr. Tomoko Ohta, head of the laboratory, Department of Population Genetics, for her theoretical studies on evolution and variation in

multigene families. Dr. Motoo Kimura, head of the Department of Population Genetics, published a book entitled "The Neutral Theory of Molecular Evolution" (Cambridge University Press, 1983). This theory has provoked a great deal of controversy among geneticists in the world since he first proposed it in 1968. Through 15 years ordeal, evident proofs supporting his theory are appearing one after another.

On April 23, the institute was opened to the general public. In addition to the exhibition of each department and moviefilms, a lecture was given by Dr. Yosida on "Chromosomes: From virus to humans." In the campus cherry blossoms of double-flowers species were in full bloom and the visitors numbered about 2500. On November 5, public lectures were given at the National Science Museum in Tokyo by Dr. Yukiaki Kuroda on "Cell genetics *in vitro*" and Dr. Yosida on "Chromosomes and evolution of organisms." More than 200 people listened to the lectures in spite of the weekend afternoon.

In regard to personnel change, Dr. Kin-ichiro Miura, head of the Department of Molecular Genetics, was appointed to professor of the Faculty of Technology, University of Tokyo in April; Dr. Kunitada Shimotohno, researcher of the same department, to laboratory head of the National Cancer Center Research Institute in March; and Dr. Kazuo Shinozaki, researcher of the department, to assistant of Faculty of Science, Nagoya University in April. Furthermore, Dr. Yoshibumi Komeda, researcher, Microbial Section, Genetic Stocks Center, was promoted to lecturer of Faculty of Science, University of Tokyo in November. For 15 years Dr. Miura had assumed leadership of the Department of Molecular Genetics and produced many achievements which added luster to the institute's reputation, for instance, he identified the cap structure of the 5′ end of messenger RNAs that is peculiar to eukaryote cells. Lastly, to our great sorrow, Dr. Takehiko Noguchi, researcher, Genetic Stocks Center, died of heart attack on November 24 at the age of 41. Dr. Noguchi had been studying on developmental genetics of mouse, especially on mutant strains with high frequency of testicular teratomas. Besides, he had been engaging in the work of practical use of keeping mouse stocks by freezing fertilized eggs. The sudden death of Dr. Noguchi, who had been expected much by the colleagues in and out of the institute, cannot be enough deplored.

We had many visitors from abroad as the last year, with whom informations and views on recent studies were exchanged actively. Among them,

Dr. Ick-dong Yoo (working in Dept. of Microbial Genetics) from Korean Ministry of Agriculture, Forestry and Fishery, Mr. T. J. L. van Hintum (Dept. of Applied Genetics) from Agricultural University in Wageningen, and Mr. Nai-Kai Zhu (Dept. of Induced Mutation) from Institute of Environmental Chemistry of Academia Sinica, were staying rather long for technical training and cooperative work.

E. Matsunaga

STAFF

Director

MATSUNAGA, Ei, D. Med., D. Sc.

Members

1. ***Department of Morphological Genetics***

KURODA, Yukiaki, D. Sc., Head of the Department

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INOUE, Tadashi, D. Ag.

The 2nd Laboratory

KADA, Tsuneo, D. Sc., Head of the Laboratory

The 3rd Laboratory

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The 2nd Laboratory

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9. ***Department of Population Genetics***

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The 2nd Laboratory

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10. ***Department of Molecular Genetics***

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The 2nd Laboratory

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KUSUDA, Jun, D. Ag.

Plant Section

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Microbial Section

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NISHIMURA, Akiko

12. ***Experimental Farm***

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MIYAZAWA, Akira

13. ***Department of Administration***

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OOIDE, Yukio, Chief of the Finance Section

Honorary Members

KIHARA, Hitoshi, D. Sc., Director of the Kihara Institute for Biological Research, Member of Japan Academy, Emeritus Professor of Kyoto University
SAKAI, Kan-ichi, D. Ag.
MORIWAKI, Daigoro, D. Sc., Emeritus Professor of Tokyo Metropolitan University
OSHIMA, Chozo, D. Sc.
OKA, HIKO-Ichi, D. Ag.
TAZIMA, Yataro, D. Ag.

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YOSIDA, Tosihide H., Managing Director, Head of the Cytogenetics Department
SINOTO, Yosito, Manager
WADA, Bungo, Manager, Emeritus Professor of University of Tokyo
TAZIMA, Yataro, Manager
OSHIMA, Chozo, Manager

PROJECTS OF RESEARCH FOR 1983

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)
Cytogenetics in the silk worm (MURAKAMI)
Pheno-genetics of the silkworm (MURAKAMI)
Radiation- and chemical-induced mutagenesis in the silkworm (MURAKAMI)

Department of Cytogenetics

Studies on chromosome evolution and species differentiation 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)
Genetic study on the subspecies differentiation of mouse (MORIWAKI)
Immunogenetical study on the MHC functions in mice (MORIWAKI)
CYTOGENETICAL study of ants (IMAI)
Cytogenetical studies on *Drosophila* (YAMAMOTO)
Cytogenetical study of fishes (YOSIDA)

Department of Physiological Genetics

Behavior genetics of *Drosophila* (WATANABE and OSHIMA)
Evolutionary and ecological genetics of *Drosophila* (WATANABE)
Population genetics of *Drosophila* (WATANABE)
Theory of population genetics and speciation (MARUYAMA)

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

Behavioral genetic studies in animals (FUJISHIMA)
Theoretical studies on breeding techniques (IYAMA)
Genetic studies of trees in natural forest (IYAMA)
Evolutionary studies on wild and cultivated rice species (MORISHIMA and SATO)
Ecological genetic studies in weed species (MORISHIMA)
Genetic effects of environmental pollution on plant population (IYAMA and MORISHIMA)

Department of Induced Mutation

Molecular mechanisms of radiation- and chemical-induced mutations (KADA, SADAIE, INOUE and TEZUKA)
Enviromnental mutagens, desmutagens and antimutagens (KADA)
Radiation genetics in mice (TUTIKAWA)
Biochemical factors involved in cellular repair of genetic damage and induced mutagenesis (INOUE and KADA)
Genetics of *Bacillus subtilis* (SADAIE and KADA)
Molecular mechanisms of unicellular differentiation in *Bacillus subtilis* (SADAIE)

Department of Human Genetics

Genetic and cytogenetic studies on retinoblastoma and Wilms' tumor (MATSUNAGA, NAKAGOME and HORAI)
Clinical cytogenetic studies of congenital abnormalities in man (NAKAGOME)

Molecular cytogenetic studies of human chromosomes (NAKAGOME)
Studies on DNA polymorphisms in human populations (HORAI and MATSUNAGA)

Department of Microbial Genetics

Genetic regulaotry mechanisms of DNA replication in *E. coli* (YASUDA)
Genetic regulatory mechanism of cellular division in *E. coli* (HIROTA, NISHIMURA and YAMADA)
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, OHTA, TAKAHATA and AOKI)
Studies on molecular evolution from the standpoint of population genetics (KIMURA, TAKAHATA and OHTA)
Theoretical studies on the evolution of multigene family (OHTA)
Theoretical studies on the evolution of altruism (AOKI)

Department of Molecular Genetics

Studies on primary structure of DNA (SOEDA)
Transformation of human lung and rat fibroblast cells with viral promoter DNA (SOEDA)
Biogenesis of papovaviruses (SOEDA)

Genetic Stocks Center

Studies and conservation of germplasm resources in rice and wheat species (FUJII and SANO)
Specificity of mutagen to tolerance in higher plants (FUJII)
Exploitation of genetic ability of nitrogen fixation in Graminae (FUJII, SANO and IYAMA)
Studies on genetic differentiation in rice (SANO)

Cytogenetic studies of Norway rats and establishment of the chromosomal mutant stocks (YOSIDA)

Establishment of new experimental animals from wild rodents (YOSIDA and MORIWAKI)

Studies on chromosomal polymorphism in Drosophila (INOUE)

Analysis of fibroin genes of silkworm and its relatives (KUSUDA)

Coordination of flagellar formation and cell division in *E. coli* (NISHIMURA, and HIROTA)

Synthetic ColE1 plasmids carrying genes for cell division in *Escherichia coli* (NISHIMURA)

RESEARCHES CARRIED OUT IN 1983

I. MOLECULAR GENETICS

An Improved Method for Shotgun DNA Sequencing

Shuhei YASUDA, Naohiro NAKAYAMA, Hiroyuki JIKUYA
and EIICHI SOEDA

Shotgun DNA sequencing is one of the most rapid and easiest methods of determining the sequence of a large DNA molecule (Deininger, P. L., Anal. Biochem., **129**, 216–223, 1983). It involves choosing clones randomly from a shotgun library of DNA fragments cloned into a M13 phage vector, sequencing them by the "dideoxy" method and, finally building up to the complete sequence by overlapping the subfragment sequences. The optimum library should contain recombinants with inserts which span the whole region of the DNA of interest, share short pieces of overlapping sequences with each other and are present in roughly equal proportions in the library. Difficulty occurs in cloning DNA larger than 1 kb (kilo base) in length into M13 vectors, and sequences smaller than 0.4 kb should be avoided since this is the maximum length that can be read from a polyacrylamide sequencing gel. Thus, in order to construct such a library, it is important to dissect DNA randomly so as to fall within this size range and process them for blunt-end ligation to M13 vectors. Probably, the combination of physical shearing by sonication and repair by T4 DNA polymerase is one of the best procedures to achieve it. However, the cloning efficiency by this method was rather low as compared with that obtained with use of restriction fragments.

In order to promote shotgun DNA sequencing further, the method for construction of M13 phage clone libraries was modified, including the shearing of DNA into appropriate sizes by sonication and the processing of the sheared DNA for blunt-end ligation to the phage vectors.

By changing the volume of DNA solution applied as well as time and amplitude of sonication, the DNA was dissected randomly to generate fragments predominantly 0.4–1 kilo bases in length, this size range being suitable

Table 1. Effect of the processings of sonicated DNA on cloning efficiency

	Sonicated λDNA (20 ng)			λDNA/*Alu*I (20 ng)
	Nuclease P1	T4 pol.	Nuclease P1 & T4 pol.	
White	9	30	113	251
White Plaque	9	30	113	251
Blue Plaque	111	87	130	51

The sonicated DNA was processed to generate blunt-ends by T4 DNA polymerase, nuclease P1 and both, respectively, and cloned. The cloning efficiency is represented by the number of white plaques. Phage λDNA/*Alu*I fragments bearing flush-ends are used as a positive control.

for shotgun DNA sequencing. Furthermore, by introducing nuclease P1 treatment prior to the repair of the sheared DNA by T4 DNA polymerase, the cloning efficiency increased three fold compared to when T4 DNA polymerase was used alone (Table 1).

An Altered DNA Sequence Encompassing the *ras* Gene of Harvey Murine Sarcoma Virus

Shuhei YASUDA, Masato FURUICHI and Eiichi SOEDA

Harvey murine sarcoma virus (Ha-MuSV) was originally isolated from tumours induced by inoculation of Moloney murine leukemia virus (Mo-MuLV) into rats. This virus was able to transform fibroblast cells in culture and induce sarcomas in susceptible mice. In the virus-infected cells, a transformation specific protein with a molecular weight of 21 kilo daltons, p21, was produced. The closed circular DNA intermediates of the viral genome have been isolated from the infected cells and cloned molecularly and physically characterized. Studies on transforming ability of the subgenomic DNA permitted its localization within the *Sma*I-*Pst*I fragment in the proximal half of the viral genome.

The DNA fragment encompassing the *ras* gene of Harvey murine sarcoma virus was sequenced and the coding region of a transforming protein, p21, was assigned to the sequence (Figure). Examination of uncleotide sequence, taken together with the result of analysis of the *ras* mRNAs, has revealed that p21 is encoded from a continuous coding region starting with the 5′ proximal initiation codon but is not a processed protein. How-

```
      AGACCCCGCTCTAGTGGCAGTGTGTTGGTTGATAGCCAAAGTTAATTTTTAAAA   54

CATAGTGTTTTGGGGGTTGGGGATTTAGCTCAGTGATAGAGCTCTTGCCTAGCAAGCGCA  114
                  CC
AGGCCCTGGGTTCGGTCCCCAGCTCTGAAAAAAAGGAAAGAGAAACAAAACAAAAACATA  174

TAGTGTTTTATCTGTGCTTATGCCCGCAGCCCGAGCCGCACCCGCCGCGGACGGAGCCCA  234
  A   A
TGCGCGGGCCCAGTCGGCGCCCGTCCGCGCCCCCGCCCTGCCCCGGCCCCGGCCCCGGGG  294

GCAGTCGCGCCAGCAAGCGGTGGGGCAAGAGCTCCTGGTTTGGCAGCCCCTGTAGAAGCG  354

ATG ACA GAA TAC AAG CTT GTG GTG GTG GGC GCT AGA GGC GTG GGA   399
Met Thr Glu Tyr Lys Leu Val Val Val Gly Ala Arg Gly Val Gly    15

AAG AGT GCC CTG ACC ATC CAG CTG ATC CAG AAC CAT TTT GTG GAC   444
Lys Ser Ala Leu Thr Ile Gln Leu Ile Gln Asn His Phe Val Asp    30

GAG TAT GAT CCC ACT ATA GAG GAC TCC TAC CGG AAA CAG GTA GTC   489
Glu Tyr Asp Pro Thr Ile Glu Asp Ser Tyr Arg Lys Gln Val Val    45

ATT GAT GGG GAG ACG TGT TTA CTG GAC ATC TTA GAC ACA ACA GGT   534
Ile Asp Gly Glu Thr Cys Leu Leu Asp Ile Leu Asp Thr Thr Gly    60

CAA GAA GAG TAT AGT GCC ATG CGG GAC CAG TAC ATG CGC ACA GGG   579
Gln Glu Glu Tyr Ser Ala Met Arg Asp Gln Tyr Met Arg Thr Gly    75

GAG GGC TTC CTC TGT GTA TTT GCC ATC AAC AAC ACC AAG TCC TTT   624
Glu Gly Phe Leu Cys Val Phe Ala Ile Asn Asn Thr Lys Ser Phe    90

GAA GAC ATC CAT CAG TAC AGG GAG CAG ATC AAG CGG GTG AAA GAT   669
Glu Asp Ile His Gln Tyr Arg Glu Gln Ile Lys Arg Val Lys Asp   105

TCA GAT GAT GTG CCA ATG GTG CTG GTG GGC AAC AAG TGT GAC CTG   714
Ser Asp Asp Val Pro Met Val Leu Val Gly Asn Lys Cys Asp Leu   120
  T  G
GCC GCT CGC ACT GTT GAG TCT CGG CAG GCC CAG GAC CTT GCT CGC   759
Ala Ala Arg Thr Val Glu Ser Arg Gln Ala Gln Asp Leu Ala Arg   135
    Gly
AGC TAT GGC ATC CCC TAC ATT GAA ACA TCA GCC AAG ACC CGG CAG   804
Ser Tyr Gly Ile Pro Tyr Ile Glu Thr Ser Ala Lys Thr Arg Gln   150

GGT GTA GAG GAT GCC TTC TAC ACA CTA GTA CGT GAG ATT CGG CAG   849
Gly Val Glu Asp Ala Phe Tyr Thr Leu Val Arg Glu Ile Arg Gln   165

CAT AAA CTG CGG AAA CTG AAC CCG CCT GAT GAG AGT GGC CCT GGC   894
His Lys Leu Arg Lys Leu Asn Pro Pro Asp Glu Ser Gly Pro Gly   180

TGC ATG AGC TGC AAG TGT GTG CTG TCC TGA CACCAGGTGAGGCAGGGACC   944
Cys Met Ser Cys Lys Cys Val Leu Ser Ter                       189

AGCAAGACATCTGGGGCAGTGGCCTCAGCTAGCCAGATGAACTTCATATCCACTTTGATG 1004
  G  CG
TCCCTGCTCCCCCAATTCTGCCAATCCCCCTGCCTGCA                       1042
```

Fig. 1. The nucleotide sequence of the fragment encompassing H-*ras* gene of Ha-MuSV. The nucleotide sequence of the *Acc*I-*Pst*I fragment was determined by the methods of Maxam and Gilbert and of Sanger *et al.*, and compared with the sequence reported earlier. The differences in nucleotide sequences from the published one are indicated above the sequence line. Arrows (▼) indicate the positions where nucleotides were added to the original sequence. The deletion of dinucleotide CC occurred between position 134 and 135 shown in the box. The predicted amino acid sequence of p21 is also shown below the sequence line with one replacement of glycine by alanine at position 122.

ever, there were found several differences between the sequence published by Dhar *et al.* (Science, **217**, 934–937, 1982) and ours, including 9 deletions, 7 substitutions and 2 insertions of nucleotides in the published sequence of 997 nucleotides in length. Among these, one of the substitutions occurring in the coding region resulted in amino acid replacement of glycine by alanine at position 122 of p21. The latter suggests that p21 is encoded continuously from the 5′ proximal ATG triplet in the single open reading frame.

This work was published in Nucleic Acids Research, **12**, 5583–5588 (1984).

The Human c-Ha-*ras*2 Is a Processed Pseudogene Inactivated by Numerous Base Substitutions

Eiichi SOEDA, Masaki KAGIMOTO,[1] Jun MIYOSHI[2] and Yoshiyuki SAKAKI[2]

Normal human DNA contains sequences related to retroviral oncogenes, and activation of these proto-oncogenes is considered to be involved in expression of the transformed phenotype in naturally occurring tumors. The transfection assay of NIH 3T3 cells with tumor DNA disclosed activated versions of *ras*-related proto-oncogenes, comprised of at least three members (c-Ha-*ras*, c-Ki-*ras* and N-*ras*), in human solid tumors, cell lines of epithelial origin and certain types of hematopoietic malignancies. Based on hybridization analysis, the human genome was found to contain additional cellular homologues of transforming genes of the Harvey and Kirsten murine sarcoma viruses. As characterization of normal *ras*-related sequences and determination of their exact numbers might be of clinical significance in the diagnosis and prevention of cancer, we attempted to identify homologous sequences by screening a human genomic DNA library using the v-Ha-*ras* probe at various hybridization stringency. Among these clones, the c-Ha-*ras*2 gene, is reportedly located on the X-chromosome and has lost introns. There has heretofore been no information on its precise gene structure and oncogenic potential. We have determined the nucleotide sequence of the c-Ha-*ras*2 and demonstrate that it is a processed

[1] First Department of Internal Medicine, Kyushu University, Faculty uf Medicine, Fukuoka 812.

[2] Research Laboratory for Genetic Information, Kyushu University, Faculty of Medicine, Fukuoka 812.

pseudogene surrounded by several direct repeats and contains numerous base substitutions as well as a notable mutation (AGT at codon 12 of the p21 protein) responsible for oncogenic conversion of the known *ras* genes. (For detail, see Nucleic Acids Research, **12**, 1821–1828, 1984)

DNA Rearrangement Affecting Expression of the BK Virus Transforming Gene

Eiichi SOEDA, Sumie WATANABE,[1] Seijiro UCHIDA[2] and Kunito YOSHIIKE[1]

The BK virus (BKV) mutant, designated pm-522, has a number of characteristics distinct from those of the wild-type (WT) BKV. On human embryonic kidney (HEK) cell cultures, pm-522 forms turbid plaques smaller than clear plagues of WT BKV. This mutant is approximately five times as tumorigenic in hamsters as WT BKV and can induce hamster insulinomas, which so far have not been induced by the plague isolates of WT BKV. Despite its somewhat inefficient growth in HEK cells, BKV pm-522 produces many more foci of permanently transformed cells in hamster or rat cell cultures than WT BKV does. Apparently, pm-522 is a host range mutant that can express its transforming gene in nonpermissive cells more efficiently than WT BKV.

The high transforming capacity of pm-522, compared with that of WT BKV, is probably ascrible to the capacity of unintegrated pm-522 genomes to express continuously their early functions in the nonpermissive cells and to transform them transiently before stable transformation is established. Rat cells infected with either pm-522 or WT BKV contain unintegrated BKV DNA in their nuclei during the first 2-week period, before foci of transformed cells become detectable. The free viral genomes continue to be transcribed to direct synthesis of T antigen, which renders the cells transiently transformed, in pm-522 infection. In WT infection, however, transcription of T antigen gene occurring, initially seems to be repressed soon after infection and T antigen production cease. Apparently, WT BKV is susceptible to some repressive mechanism, which pm-522 can escape, in rat cells.

BKV pm-522 has a deletion at approximately map unit 0.72 in the non-

[1] Department of Enteroviruses, National Institute of Health, Tokyo 141.

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coding region (topographically, the putative control region for early transcription) near the origin of DNA replication, within the *Hind*III C segments. Construcion and characterization of recombinant viruses between pm-522 (*Hind*III C fragment) and wt-501 (*Hind*III ABD fragment) has shown that *Hind*III-C (450 base pairs around the origin of replication) of pm-522 contains the mutation responsible for both the altered plaque morphology and the high transforming capacity. Therefore, sequencing the *Hind*III C segment is expected to correlate the change in nucleotide sequence to biological functions of BKV.

In the present study, we compared the nucleotide sequence of wt-501 *Hind*III C segment with that of pm-522 *Hind*III-C, which contains the mutation responsible for the altered plaque type and trasforming capacity. The difference between the two BK viruses was the local DNA rearrangement (deletions and duplications) that had occurred in the putative control region for early transcription in pm-522 DNA. Whereas wt-501 had three sets of 68-base pair repeats (the central set had a deletion of 18 base pairs) in this region, pm-522 had one set of 68-base pair unit and two sets of shorter 37 base pair repeats. Three BK virus mutants, forming clear large plagues like those of wt-501 but capable of tranforming rat cells, were derived from the recombinant virus carring the *Hind*III C segment of pm-522. These mutants had further duplications of shorter segments originating from the pm-522 sequence in the putative early control region. (For detail, see J. Virol., **51**, 1–6, 1984)

II. MICROBIAL GENETICS

The *Escherichia coli* Origin of Replication: Essential Structure for Bidirectional Replication

Mituru TAKANAMI, Satoshi TABATA, Atsuhiro OKA, Kazunori SUGIMOTO, Hitoshi SASAKI, Seiichi YASUDA and Yukinori HIROTA

The 245 base-pair *oriC* sequence of the *E. coli* K-12 chromosome was subjected to localized mutagenesis *in vitro* which uses sodium bisulfite, and a large number of mutants carrying a single to multiple GC to AT changes were isolated. The replicating ability of these mutants was assayed by introducing into cells, and the correlation between the position of mutation and replicating function was analysed. Combining the results obtained from this and previous studies together, we concluded that *oriC* provides multiple interaction sites precisely separated by spacer sequences, and initiation factors cooperatively interact on these sites to form an initiation complex.

The functional role of the 245 bp *oriC* sequence was investigated by using an *in vitro* replication system and various Ori^+ and Ori^- plasmids. The result of analysis clearly indicated that DNA replication started at a region near, but outside, the 245 bp *oriC* sequence, and proceeded bidirectionally. The replicating function was reduced by mutations introduced only in *oriC*, but not by sequence replacements in the flanking regions. The result indicates that the 245 bp *oriC* sequence contains information enough for directing bidirectional replication in the vicinity of *oriC*. It was also noted that initiation of DNA synthesis at the specific region required the *dnaA*-complementing fraction from cells harboring a *dnaA*-carrying plasmid. Based on the above observations, a possible model for bidirectional replication directed by *oriC* is discussed. (For detail, see "Mechanisms of DNA Replication and Recombination", pp. 257–273, Alan R. Liss Inc., New York, 1983)

Escherichia coli uvrD Mutants with Thermosensitive DNA-dependent Adenosine Triphosphatase I (Helicase II)

Evelyne RICHET, Yukinobu NISHIMURA, Yukinori HIROTA and Masamichi KOHIYAMA

Three mutants producing thermosensitive DNA-dependent Adenosine triphosphatase (ATPase) I were screened from a collection of temperature sensitive mutants of *Escherichia coli* K12. ATPase I purified to near homogeneity from one of the mutants (JE10100) possesses both thermosensitive DNA-dependent ATPase and DNA helicase activities. We have shown that ATPase I is encoded by the *uvrD* gene as first suggested by Oeda *et al.* (1982): (i) the thermosensitive ATPase I mutation present in JE11040 lies in or very close to the *urrD* gene, (ii) ATPase I activity is absent in *uvrD*210, *uvrD*156, and *uvrD*252 mutants. Thus the thermosensitive mutations correspond to new *uvrD* mutations. However, the mutation present in JE11040 confers neither UV sensitivity nor mutator phenotype at high temperature. Evidence is presented that the mutant ATPase I is stabilized *in vivo* at 42°C. (For detail, see Mol. Gen. Genet. **192**, 378–385, 1983)

Overlapping of the Coding Regions for α and γ Components of Penicillin-Binding Protein 1 b in *Escherichia coli*

Jun-ichi KATO, Hideho SUZUKI and Yukinori HIROTA

The mode of biosynthesis of penicillin-binding protein (PBP)-1b in *Escherichia coli* was investigated by use of the plasmid carrying the *ponB* (PBP-1b) gene region. Analyses of the products synthesized in minicells and *in vitro* showed that PBP-1b was synthesized as two molecular species corresponding to the α and γ components of PBP-1b. The coding regions for the α and γ components were located within the ca. 3.7 kb *MluI–Hin*cII fragment and transcribed in the direction from the *Hin*cII to the *Mlu*I site. The capacity for producing the α component was abolished by a deletion extending to the *Mlu*I site ca. 0.7 kb inward from the *Hin*cII end of the ca. 3.7 kb fragment; the remaining 3.0 kb region with the *Mlu*I site at both ends directed the production of the γ component alone. The production of the γ component was enough to correct all the known defects caused by a *ponB* mutation. In addition to these results, the analyses for cross-reacting materials produced

in correspondence to the various deletions indicated that the coding regions for the α and γ components overlapped and that the N-terminal portion was responsible for the difference between the two components. The distal region about 0.7 kb long inward from the *Mlu*I end of the *Mlu*I-*Hin*cII fragment was dispensable for producing the functional PBP-1b, although the PBP-1b produced was curtailed. By a larger distal deletion reaching almost to the middle of the *Mlu*I-*Hin*cII fragment, the polypeptide produced for PBP-1b lost the ability to bind penicillin and still retained a low but significant activity for glycan synthesis. We suggest, therefore, that the polypeptide portion required for transglycosylase activity resides on the N-terminal half of PBP-1b, followed by the middle portion necessary for penicillin-binding and the C-terminal part dispensable for the function of PBP-1b. (For detail, see Mol. Gen. Genet. **196**, 449–457, 1984)

Glycan Polymerase with No Penicillin-binding Activity in *Escherichia coli*

Hiroshi HARA, Taro UEDA and Hideho SUZUKI

The enzymes involved in the last stage of peptidoglycan synthesis in *Escherichia coli* have been found among penicillin-binding proteins (PBPs): first PBP-1b and subsequently PBP-1a and PBP-3 were identified as peptidoglycan synthetases that carry out both transglycosylation and transpeptidation. PBP-1b appeared to be a major peptidoglycan synthetase, bacause the membrane fractions of mutants defective in PBP-1b were almost unable to support *in vitro* peptidoglycan synthesis. We found, however, that the activity for peptidoglycan synthesis was detectable even in the membrane fractions defective in PBP-1b on addition of non-ionic polar substances like glycerol to the reaction mixture. The investigation of the glycerol-stimulated activity suggested that two or more glycan polymerases other than PBP-1b were responsible for the activity and that a glycan polymerase with no penicillin-binding ability may exist in *E. coli*. This glycan polymerase with no penicillin-binding ability has no transpeptidase activity and consequently should require cooperation of a cross-linking enzyme to serve sacculus synthesis. (For detail, see "The Target of Penicillin, Proceedings of International FEMS Symposium on the Murein Sacculus of Bacterial Cell Walls" Eds. R. Hakenbeck *et al.*, pp. 583–588, Walter de Gruyter, Berlin, 1983)

A Novel Glycan Polymerase that Synthesizes Uncross-Linked Peptidoglycan in *Escherichia coli*

Hiroshi HARA and Hideho SUZUKI

A simple and efficient procedure to assay peptidoglycan synthesis *in vitro* was established. By this procedure, a novel activity for glycan polymerization in *Escherichia coli* was found in the fraction containing no detectable penicillin-binding protein (PBP). This polymerase activity was relativery insensitive to moenomycin, showed requirement for Ca^{2+} or Mn^{2+} but not for Mg^{2+}, and led to production of uncress-linked glycan chains. These properties distinguished the glycan polymerase from the activities shwon by the fractions containing PBPs. The glycan polymerase catalyzing polymerization of glycan units from lipid intermediates was purified and identified as a protein of 34 kdal. (For detail, see FEBS Letters **168**, 155–160, 1984)

A *Bacillus subtilis* Gene Involved in Cell Division, Sporulation and Exoenzyme Secretion

Yoshito SADAIE and Tsuneo KADA

To elucidate the divisional control of sporulation in *Bacillus subtilis*, we examined the effect of temperature sensitive septum initiation mutations on sporulation and competent cell formation and found that the *div*-341 mutation (Miyakawa *et al.* Mol. Gen. Genet. **181**: 207) resulted in the early *spoO* phenotype at an intermediate permissive temperature (37°C) where vegetative growth was largely intact. Sporulation, exoenzyme production and competent cell formation were reduced at 37°C in the mutant. The *div*-341 mutation was found closely linked to the *sacU*^{h}32 mutation (Kunst *et al.* Biochimie **56**: 1481) by transformation which results in pleiotropic phenotype: hyperproduction of exoenzymes and derepressed sporulation in the presence of excess nutrients. A double mutant (*div*-341 *sacU*^{h}32) retained the *sacU*^{h}32 property of hyperproducer of exoenzymes even at 37°C and showed the *sacU*^{h}32 property of sporulation at 30°C and an intermediate property at 37°C. The filamentous growth at higher temperature (40°C) of the *div*-341 strain could not be recovered by the introduction of the *sacU*^{h}32 mutation into the strain.

From the opposing properties of both mutations and their close linkage

we conclude that the *sacU* gene might be a regulator gene for the *div*-341 structural gene and that derepression of the *div*-341 gene, which is caused by the *sacU*^{h}32 mutation, might be responsible for the high production of exoenzymes and high incidence of sporulation in the presence of excess nutrients. The *div*-341 gene product might be involved in a step required for the excretion of some exoenzymes and cell surface proteins. Upon starvation for nutrients, the *div*-341 gene might be derepressed to carry out early sporulation events. (Mol Gen. Genet. **190**: 176).

Flagella Are Formed at the Late Stage of Cell Cycle Just before the Septum Formation in *Escherichia coli*.

Akiko NISHIMURA and Yukinori HIROTA

We discovered a phenomenon in *E. coli* that the formation of flagella is coupled with cell division (Nishimura and Hirota, Ann. Rept. Natl. Inst. Genet. **32** (1981), 31–35). In this paper, we report that flagella in *E. coli* are formed at the late stage of the cell cycle, just before septum formation.

Formation of flagella in wild type cells, PA3092, was studied as follows. PA3092 was exponentially grown in broth at 30°. The cells were negatively stained with phosphotungstic acid (pH 7.0) and electron micrographs were taken. On the EM photographs, the number of flagella per cell (N) and the cell length (l) was measured. The total number of cells measured was 372. These cells in random population were classified into 12 groups by cell length (l_t). The average number of flagella per cell length, D_t ($=N_t/l_t$), was calculated for each cell length group (l_t). The cell length (l_t) was used as one of the factors which possibly indicate the stage in the cell cycle, because rod cells like *E. coli* grow lengthwise without changing thickness. If flagella are formed continuously abreast of increase in cell length, D_t would be constant irrespective of cell length. On the contrary, if flagella are only formed at a special stage of the cell cycle, D_t would decrease in inverse proportion to the increase in cell length.

The results are as follows. The cells in the smallest class ($l_0=1.56$ μm), i.e. the class of newborn cells, were found to have the highest value of D_t ($D_0=5.23/\mu$m). The larger the cell size (l_t) became, the lower the value of D_t became in inverse proportion to cell length. D_t increased again after the cell size reached 1.7 times of the cell size of newborn cells. A septum was observed in a portion of the cells in this class. The cells which have a

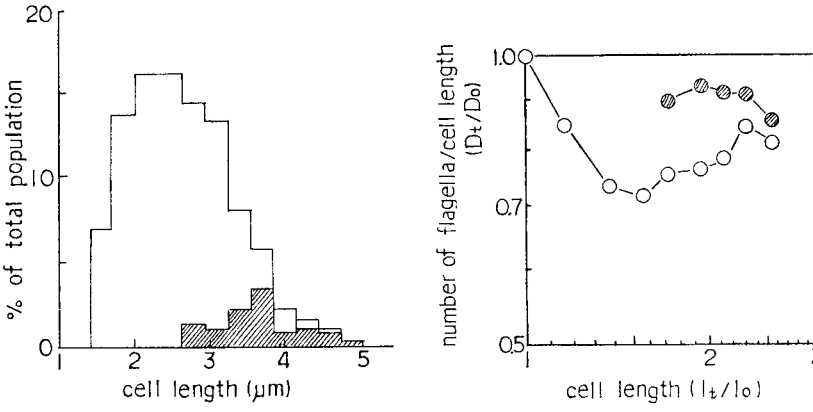

Fig. 1. Length (l_t) distribution and the number of flagella per cell length (D_t) of *E. coli* PA3092. (A) length measurements of exponentially growing wild type cells at 30°. The total number of cells measured was 372. The average length of the cells was 2.59 μm. The hatched area represents the distribution of dividing (constricting) cells (5.15% of total); the average length was 3.53 μm. The average length of the newborn cell (7.00% of total) was 1.56 μm. The length distribution sloping on right side indicates the exponential increase of cell length. (B) logarithmic plot of the relative number of flagella per cell length (D_t) versus relative cell length (l_t) in each length class. The average number of flagella per cell length of newborn cells (D_0) was 5.23 μm.

septum between the two daughters had almost the same value of D_t as newborn cells.

From these results, we suggest that, during the cell cycle, the flagella are formed at the stage of septum formation. After the completion of cell division, formation of flagella is suppressed.

III. BIOCHEMICAL GENETICS AND IMMUNOGENETICS

Conserved and Variable Antigenic Specificities on the H-2 Derived from a Japanese Wild Mouse, MOL.SGR

Kazuo MORIWAKI, Tomoko SAGAI, Nobumoto MIYASHITA, Hitoshi SUZUKI and Toshihiko SHIROISHI

Eight monoclonal antibodies of mMS series have recently been prepared using B10.MOL-SGR strain (This Report, No. 33 1983). We surveyed the presence of reactivities with these mMS antibodies in various mouse subspecies in the world and also in another species, *Mus speciligus*. As demonstrated in Table 1, an H-2 antigen detected by mMS 38 seems to be most conservative in the present survey. It was able to react with all the *Mus musculus* subspecies examined at the higher phenotypic frequency and moreover reacted with the other species, *Mus speciligus*. K33 antigenic specificity detected by monospecific conventional antibody was also widely observed not only in *Mus musculus* species but in *M. speciligus* (This Report, p. 00). Southern blot analysis using a H-2K DNA probe demonstrated that regardless of their subspecies, all mice having K33 determinant commonly shared 2.5 Kb DNA fragment digested with BglII restriction enzyme.

The antigens detected by mMS 1 and 37 were also relatively conservative. They were distributed within *Mus musculus* species at the higher frequency,

Table 1. Frequency distribution of MOL-SGR H-2 specificities detected with mMS series monoclonal antibodies in various wild mice

Name of species or subspecies	No. of Sample	Phenotypic frequencies								
		Monoclonal antibodies (mMS)								cAb
		1	37	38	24	25	42	39	40	502
M. m. molossinus	36	.89	.36	.69	.06	.06	.28	.17	.64	.36
M. m. subsp. CHN	15	.23	.37	.67	.00	.00	.00	13	.20	.13
M. m. castaneus	19	.79	.74	1.00	.00	.00	.42	.47	.42	.34
M. m. musculus	8	.88	.63	.63	.00	.00	.25	.63	.25	.13
M. m. domesticus	17	.23	.35	.59	.06	.12	.00	.00	.00	.18
M. speciligus	13	.00	.00	.31	.00	0.0	.00	.00	.00	.00

but not in *M. speciligus*. The most variable determinant examined was that detected by either mMS24 or mMS25. It reacted only with the donor strain and DOM.PGN strain which derived from Canadian wild population in the Ontario State. These two mouse strains are evolutionarily remote each other by around one million years (Yonekawa *et al.* 1980). The antigenic determinant on H-2 molecule detected by mMS 24–25 is considered to be highly variable. But, presumably it could remain unchanged by chance in the both lineages of wild mice, even though they have been separated long time ago.

Founder Effect in the Frequency Distribution of Mouse H-2 Observed in the Japanese Wild Populations

Kazuo MORIWAKI, Tomoko SAGAI and Nobumoto MIYASHITA

Extensive polymorphism of H-2 class I antigens has already been demonstrated both in the laboratory mouse strains and the European wild populations. We have surveyed the wild populations of Asian mice, mostly Japanese ones, by using monospecific antisera directed to both of the well-known H-2 private specificities and the newly identified ones in Japanese mice.

As shown in Table 1, the Japanese wild populations exhibited relatively higher frequencies of several H-2 specificities such as K26, D32, K20, KD501, KD507, K509 and K510. Phenotypic frequencies of all these specificities were more than 10% which was apparently higher than those in Chinese wild population. On the other hand, the number of H-2 specificities detected was less in China mainland than in Japan. Probably the Japanese populations were originated from a small fraction of the Chinese populations. This has been also suggested from the cytogenetical survey of chromosome C band patterns both in Japanese wild mice and Chinese ones. No such differences in frequency distribution of H-2 were observed between the European population and their Canadian derivatives which we surveyed in this study. Presumably the founder of the Canadian population had a size large enough to eliminate founder effect, when they were transfered from Europe.

Table 1. Frequency distribution of H-2 antigenic specificities in various wild mice

Name of species & subspecies	No. of sample	Phenotypic frequencies H-2 haplotype and antigenic specificities																			
		b		d		f		k		q		u									
		K	D	K	D	K	D	K	D	K	D	K	KD	KD	KD	KD	K	K			
		33	2	31	4	26	9	23	32	17	30	20	501	502	503	507	509	510			
M. musculus																					
molossinus	115	.00	.00	.00	.00	.26	.10	.00	.13	.00	.01	.16	.43	.08	.02	.11	.16	.40			
subsp. CHN	41	.12	.05	.05	.00	.24	.07	.00	.05	.00	.05	.15	.27	.12	.00	.00	.05	.15			
castaneus	28	.18	.04	.00	.04	.29	.36	.14	.39	.00	.04	.21	.42	.00	.07	.04	.11	.21			
*domesticus**	49	.12	.06	.20	.04	.10	.12	.00	.02	.00	.04	.36	.16	.02	.00	.00	?	?			
*domesticus***	320	.08	.03	.15	.16	.07	.02	.06	.05	.02	.06	.01									
M. speciligus	14	.88	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00			

* Collected from Ontario, Canada.

** Götze *et al.* 1980

MS-Series Monoclonal Antibodies Directed to the H-2 and Ia Antigenic Determinants of B10.MOL-SGR Mouse

Tomoko SAGAI, Toshihiko SHIROISHI and KAZUO MORIWAKI

B10.MOL-SGR (H-2^{wm7}) carries the H-2 complex of Japanese wild mouse and its intra-H-2 recombination frequency is extremely high (About 3%. See Nature 300: 370–372, 1983). As previously reported, we have obtained several hybridoma cell lines secreting monoclonal antibodies to H-2 gene products of B10.MOL-SGR (This Report No. 33, 1983). Recently we have established total 19 hybridoma clones and analysed strain distribution of their specificities. The intra-H-2 subregions corresponding to each of those specificities were decided using B10.MOL-SGR recombinants. As shown in Table I, 8 clones are secreting antibodies directed to class I antigen and the others those to class II antigens. Among the

Table 1. Characterization of monoclonal antibidoes against B10.MOL-SGR

	Hybridoma designation	Recipient	Tentative antigenic determinant	Specificity	Cross reactive haplotype
	MS1	B10	ms1	$K^{wm7}D^{wm7}$	d, k, f, r, v, p
	MS37	B10	ms2	$K^{wm7}D^{wm7}$	k, q, r, v
	MS38	B10	ms3	$K^{wm7}D^{wm7}$	k, q, s, r, v, p, u, (d)
Class I	MS24	B10×B10.A	ms4	K^{wm7}	none
	MS25	B10×B10.A	ms4	K^{wm7}	none
	MS42	B10	ms5	K^{wm7}	f, v, p
	MS39	R212	ms6	D^{wm7}	(f, v, p)
	MS40	R212	ms7	D^{wm7}	k, f, r, j, p, (b, d)
	MS34	B10.D2	ms1		none
	MS2	B10	ms2		v
	MS15	B10.A	ms2		v
	MS43	B10	ms2		v
	MS17	B10.A	ms3	$I\text{-}A^{wm7}$	q, v, p
Class II	MS28	B10×B10.A	ms3	or	q, v, p
	MS33	B10.D2	ms3	$I\text{-}E^{wm7}$	q, v, p
	MS10	B10.A	ms4		b, d, q, v, p
	MS12	B10.A	ms5		f, q, v, j
	MS35	B10.D2	ms6		b, k, q, s, r, v, j, p, u
	MS36	B10.D2	ms7		b, f, q, s, r, v, j, p

former antibodies, MS-24 and 25 (H-2K region specific) did not react with any inbred and B10.MOL congenic strains tested. They seem to be highly specific to the H-2 molecule of B10.MOL-SGR, while MS-1 directed to both H-2 K and D region was crossreactive with all the B10.MOL congenic strains tested. The latter antibodies for class II were classified to 7 groups by their strain distribution pattern. MS-34 monoclonal antibody was specific to B10.MOL-SGR and the other reacted with B10.SM, B10.MOL-TEN2 and B10.MOL-NSB, each of which has different class I antigens. Those antibodies should be useful for studying the mechanism of high recombination in B10.MOL-SGR and surveying H-2 complex in the wild mice as well.

Effect of H-2 Complex on the Incidence of Urethan-Induced Mortality and Malformation in the Mouse Embryos

Nobumoto MIYASHITA, Shinji NITO* and Kazuo MORIWAKI

Teratogenecity of carcinogens and the similarity in the mechanism of carcinogenesis and teratogenesis have been studied by many investigators. Genes, however, which regulate the susceptibility to carcinogen-induced teratogenesis have not been well determined. We studied the effect of H-2 gene complex on the urethan-induced malformation in mouse embryos by using H-2 congenic strains. Urethan (ethyl carbamate) was used as a teratogenic agent because it can penetrate the placental barrier and reach the embryos at any stage of pregnancy.

Six to eight week-old virgin females of B10.A and B10 strain were used. These mice were weighed and mated from 4 p.m. to 12 p.m. with male mice and their vaginal plugs were checked to determine Day 0 gestation. On Day 10, they were again weighed and received a single s.c. injection of urethan, 1.00 mg/g or 0.75 mg/g body weight. The pregnant mice were sacrificed on Day 18 and the implants, deaths and survivors of the fetuses were checked. Subsequently, the fetuses were taken out and their external malformation were examined. As shown in Table 1, B10.A mice had significantly higher rate of mortality than B10 mice at the dose of 1.00 mg/g of urethan. As for the external malformations (Table 2), B10.A also had higher frequency than B10 at the dose of 0.75 mg/g of urethan. These results indicate that the H-2 gene complex or its closely linked gene(s) are

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Table 1. Effect of H-2 complex on the mortality of mouse embryo following transplacental administration of urethan

Strain	Dose (mg/g)	No. of female mice with implantation	No. of implants		Deaths		Living fetuses	
			Av.	Total	No.	%	No.	%
B10	1.00	12	9.4±1.9	113	10	8.8	103	91.2
B10.A		11	8.5±1.9	93	31	33.3*	62	66.7*
B10	0.75	13	9.2±1.1	119	17	14.3	102	85.7
B10.A		11	11.0±1.3	121	17	14.0	104	86.0
B10	Control	14	9.4±2.5	132	16	12.1	116	87.9
B10.A		19	10.3±2.0	195	31	15.9	164	84.1

* $P<0.001$

Table 2. Effect of H-2 complex on the external malformations by urethan in mouse embryo

Strain	Dose (mg/g)	No. of living fetuses			Fetuses with malformation		
		Total	Male	Female	No.	%	X^2 test (p)
B10	1.00	103	52	51	25	24.2	
B10.A		62	31	31	23	37.1	N.S.*
B10	0.75	102	52	50	2	2.0	
B10.A		104	51	53	11	10.6	<0.02
B10	Control	116	65	51	0	0.0	
B10.A		164	87	77	2	1.2	N.S.

* N.S., not significant.

involved in determining susceptibility to the mortality and malformation induced by urethan.

Effect of Major Histocompatibility Gene Complex (MHC) on the Growth of Embryo-Derived Teratoma

Choji TAYA and Kazuo MORIWAKI

Mouse teratoma can be experimentally induced by the transplantation of early embryo under the kidney capsule. Previously we reported that the difference in the weight of tumors which were derived from the syngeneic transfer of 7-day-old embryo was observed among five A.H-2 congenic strains 40 days after transplantation. H-2D locus or its neighboring regions were suggested to affect the growth of the embryo-derived tumors. It has

not been clear yet whether this difference in the tumor growth is related to the degree of further malignant change in those induced teratoma or not.

In the present report, the tumors induced in a susceptible strain, A.TL, and in a resistant one, A.SW, were examined histologically. The ratio of malignant teratomas to all the developed teratomas was 18/28 in A.TL strain and 15/31 in A.SW strain. Though the former was a little higher than the latter, there was no significant difference between them. Even in in the smaller teratomas, the foci of malignant teratoma cells had been observed. These results suggest that a putative H-2 linked gene controlling the teratoma growth is not related to the rate of malignant transformation, instead to the degree of nutrient supply through the introduction of blood capillaries into the developing teratoma, because the larger teratomas were found to have a plenty of blood vessels introduced from the recipient.

Restriction Fragment Length Polymorphism of NTS rDNA in Mole Rats

Hitoshi SUZUKI, Kazuo MORIWAKI, Ryo KOMINAMI*,
Masami MURAMATSU* and Eviator NEVO**

Mole rats in Israel (*Spalax ehrenbergi*) have chromosome number polymorphism such as 2n=52, 54, 58 and 60. We attempted to estimate the genetic diversity in DNA level among those mole rat populations. Southern blot analysis with ribosomal DNA probe prepared from mouse and two restriction enzymes, EcoRI and BamHI, revealed that each individual had various sizes of major restriction fragments both digested by EcoRI and BamHI. Since the probe specificically hybridizes to an area of 3'-end of 28S rRNA gene in mice and probably in mole rat as well which is considered to be highly conserved, this length polymorphism seems to depend on sequence polymorphism of non-transcribed spacer (NTS) DNA. This fact was also confirmed by the construction of restriction maps by Southern blot analysis with several restriction enzymes. In the genomes of mole rats collected from 13 different localities in Islael, we could characterize the major types of NTS of rDNA repeating units based on the EcoRI and BamHI fragment lengths as shown in Table 1. Generally, mole rats carrying the same chromosome number had a similarly characteristic combination

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Table 1. Restriction flagment length polymorphism of rDNA in mole rats

Chro-mosomal species	Type of population	No.	Type of NTS						
			A	B	C	D	E	F	G
52	Near 52/58 hybrid zone	4	+	++			+		
	Central	4		++			+		
	Marginal	4			+		++		
54	Near 54/58 hybrid zone	4		+				++	++
	Central	4		+					
	Marginal	4		+				+	++
58	Near 58/52 hybrid zone	4				++			
	Central	4				++			
	Near 58/54 hybrid zone	4	+					+	++
60	Near 60/58 hybrid zone	4	++	+					
	Central	4			++		+		
	Marginal	4		++					
60	Isolate	2		++					

Each restriction NTS DNA types is tentatively designated by the letters.

Approximate restriction fragment sizes (kb) introduced either by EcoRI or BamHI are as follws in the parentheses: A (6.6–7.4, 8–9), B (8.6–9, 10–10.5), C (8, 10), D (7.5–8.2, 8.5–9), E (12.5–13, 9.5–10), F (12–14, 16–18), and G (16–17, 10–11).

of the types of NTS each other, suggesting that the specificities of the NTS of rDNA had been generated after the chromosomal rearrangement. Some mole rats living in near 58/54 hybrid zone, however, had neighbor's type of the combination. This phenomena is possibly explained by a gene flow from 54 population to 58.

Further Survey of T-lymphocyte Differentiation Antigens in Various Mouse Subspecies by Serological Methods

Yasuyuki KURIHARA, François BONHOMME* and Kazuo MORIWAKI

We have surveyed geographical distributions of Thy-1, Lyt-1 and Lyt-2 alleles in wild mice using serological methods. European subspecies, *Mus musculus domesticus* and *M. m. brevirostris*, exhibited Thy-1.2 antigen, but Asian ones mostly Thy-1.1. Lyt-2.2 was unique to some European mice, and Lyt-2.1 was widely distributed both in Asian and European mice. *Mus musculus* subspecies from wild populations examined so far expressed

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Table 1. Allelic distribution of T-lymphocyte differentiation antigens in various mouse subspecies

Name of species or subspecies	Allele of differentiation antigens			
	Thy-1	Lyt-1	Lyt-2	No. of mice observed
Mus musculus				
M. m. molossinus	1	2	1	39
	1	2	1w	1
	1	2	NT	6
	1	2w	1	1
	1	2w	NT	1
M. m. castaneus	1	2w	1w	10
	1	2	1w	3
M. m. musculus	1	2	1	28
M. m. domesticus	2	2	1	49
	2	2	2	2
	2	2	1, 2	6
M. m. bactrianus	1	2	—	1
	NT	2	NT	1
M. m. brevirostris	2	2	2	5
M. m. subspecies				
Seychells	1	2	1	8
	1	2	—	3
China	1	2	1	20
	1	—	1	6
	1	—	—	1
Mus spretus	1	2	—	1
Mus spicilegus	1	2	—	2
	1	1	—	2

w: showed weak reactivity
—: no reaction
NT: not tested

only Lyt-1.2. We also surveyed two closely related species, *Mus spretus* and *M. spicilegus*. Though their lymphocytic phenotypes were similar to those of Asian mice, part of *M. spicilegus* expressed Lyt-1.1 that could not be found in the survey of *M. musculus* (Table 1).

Certain Asian mice related to *M. m. castaneus* had weak reactivities to anti-Lyt-1.2 and anti-Lyt-2.1 monoclonal antibodies by complement-mediated cytotoxicity test, which was confirmed by absorption test as well. At present, we can not decide whether the weak reactivities resulted from

the less amount of expression or from the little change of the antigenic molecules on cell surface. Further experiments to solve this problem are currently in progress.

Expression of Rat Class I Antigens on the Erythrocytes of Various Strains

Tsukasa HIRASHIMA*, Takashi NATORI** and Kazuo MORIWAKI

In BI rat strain (haplotype i) RT1.E antigens can not be expressed on the erythrocytes (Heing, W. *et al.* Journal of Immunology, **128**: 402, 1982). We have surveyed this trait among 10 inbred strains of rat by using either hemagglutination method (Natori *et al.* Transplant. Proc. **13**: 1563, 1979) or flat plate micro-cytotoxicity method (Shiroishi *et al.* Microbiol. Immunol. **25**: 1327, 1981).

Each antigenic specificity of RT1 was detected by the following antisera. "1": WKAH anti-F344 absorbed with ACI. "2": F344 anti-TO. "3": K(DA) anti-BN. "4": WKAH anti-ACI (monoclonal antiserum).

As shown in Table 1, RT1 specificity "1" can not be expressed on the erythrocytes in several strains such as W/Ms, BN/fMai, ACI/Ms and NIGIII/Ms. Similar differential expression of RT1 on erythrocytes and lymphocytes has also been observed in the specificities "2" and "4". Speci-

Table 1. Reactivity of four alloantisera with erythrocytes and lymphocytes

Strain	Hemagglutination activity RT1 antigenic specificities				Cytotoxic activity			
	1	2	3	4	1	2	3	4
F344/Ms	+	−	−	−	+	−	−	−
BUF/Ms	+	−	−	−	+	−	−	−
LEJ/Ms	−	+	−	−	−	+	−	−
W/Ms	−	+	−	−	+	+	−	+
WKA/Ms	−	+	−	−	−	+	−	+
BN/fMai	−	−	+	−	+	−	+	−
ACI/Ms	−	−	−	+	+	−	−	+
ALB/Ms	−	−	−	−	+	+	−	+
WKA/Hok	−	−	−	−	−	+	−	−
HIGIII/Ms	−	−	−	−	+	+	−	−

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ficity "3" was detected only in BN/fMai strain both on erythrocytes and lymphocytes. Whether each of those antigenic specificity is coded for by RT1.A or RT1.E is not yet clear.

Meantime, it should be desirable to adopt the RT1 specificity on lymphocytes as a genetic profile of inbred strain.

Analysis of Mitochondrial DNA in Cytoplasms of Male-Sterile Rice

Saburo NAWA, Masa-aki YAMADA, Yoshio SANO and Taro FUJII

A number of cytoplasmic variations conferring male sterility in maize have been recognized and classified into several groups depending on the ability of nuclear genotypes in tester stocks to restore male fertility. On the other hand, several discrete low molecular weight DNAs in mitochondria were found in all lines (including normal cytoplasm) with corresponding different banding patterns. These patterns were shown to be correlated with several type cytoplasms defined by nuclear fertility restorer genes. Among these extrachromosomal DNAs present in mitochondria, the candidates for the male sterility were S-1 and S-2, linear episomes of 6.2 and 5.2 kb, respectively, which were present only in the S-cytoplasms. Certain cms-S strains have undergone spontaneous reversion to male fertility at a high frequency. It has been known that concomitant with the reversion of the male sterility to the fertile condition was the disappearance of the mitochondrial plasmid-like DNAs (S-1 and S-2) and changes in the mitochondrial chromosomal DNA.

In rice, cytoplasmic male sterility was obtained by Shinjo and Omura. Shinjo found that the cytoplasmic male sterility was controlled by a nuclear restoration gene *Rf* and produced the isogenic BT lines having genetic background of Taichung 65 by repeated back crossing. Yamaguchi and Kakiuchi detected two small mitochondrial DNAs, B-1 (1.5 kb) and B-2 (1.2 kb), in BT cytoplasm of male sterile rice, whereas none in normal fertile cytoplasm. Although no spontaneous reversions have been reported from BT lines of rice, Fujii obtained two revertants to male fertility by treating BT lines with EMS. We examined mt-DNAs from the revertants, where some changes of B-1 and B-2 would be expected if the small mt-DNA are the molecular components responsible for these reversions. Mitochondrial DNAs were prepared from rice lines with normal cytoplasm (*n*; *rfrf*), with

BT source of male-sterile cytoplasm (*cms*; *RfRf*) and with the revertants of fertile cytoplasm (*rev*; *rfrf*). Agarose gel electrophoresis of these preparations revealed a main high-molecular-weight DNA band. However, no additional low-molecular-weight DNA bands were detected in all cytoplasms, when young shoots were used as the source of mitochondria fraction. It was found that preparations from calluses of the male sterile cytoplasm contained two fast-migrating bands at the positions corresponding to approximate molecular weights of 1.5 and 1.1 kb, respectively. However, it became evident that these bands were detected in mitochondrial preparations from normal fertile cytoplasm and from the revertants. Both of the bands were sensitive to ribonuclease and resistant to deoxyribonuclease. The evidence that the two bands migrated at the same positions as 28 S and 18 S ribosomal RNA, respectively, suggests that they were derived from ribosomes as contaminants. After RNase treatment, additional electrophoretic bands that might be DNAs of molecular weights of B-1 and B-2 were not detected in all cytoplasms. We used the male sterile cytoplasm with the nuclear restorer gene (*cms*; *RfRf*) as the source of mt-DNA to get many seeds, on the basis of the fact in maize that the fertility elements are present in the S-cytoplasm groups regardless of nuclear background, even in the cytoplasm with the nuclear restorer gene. This may not fit the case of BT lines of rice. If so, our data suggest that concomitant with the reversion of the male sterility in BT rice to fertile condition, either by the nuclear restorer gene or by treatment with EMS, is the disappearance of the mitochondrial plasmid-like DNAs, B-1 and B-2. Further analysis of mt-DNAs of *cms*; *rfrf*, however, is necessary.

IV. DEVELOPMENTAL GENETICS AND SOMATIC CELL GENETICS

Electron Microscopic Studies on the Ultrafine Structures of *In Vitro* Differentiated Cells of *Drosophila melanogaster*

Yukiaki KURODA and Yutaka SHIMADA*

In the series of tissue culture studies of *Drosophila* embryonic cells, the tissue specificity and time specificity of the lethal gene actions have been investigated by examining the defects in cultured cells from some sex-linked recessive lethal embryos. Thus, the one-to-one correspondence has been analyzed between a differentiated character or function and a specific gene on a specific locus on the chromosome.

When undifferentiated cells dissociated from post-gastrula embryos of the wild-type strain were cultured in medium K-17 supplemented with 15% fetal calf serum and 0.1 μg/ml fetuin, they differentiated into various embryonic or larval cells such as muscle cells, epithelial cells, cellular spheres and nerve cells. Their specific morphology and function were easily characterized under a light microscope.

In the present experiment, the ultrafine structures of these differentiated cells were examined under an electron microscope. Cells dissociated from post-gastrula embryos of the Oregon-R strain of *D. melanogaster* were cultured for various days. Muscle cells, epithelial cells and nerve cells which differentiated *in vitro* were fixed with 2.5% glutaraldehyde and 1% osmic acid solution. Then ultrathin sections were prepared, stained with uranyl acetate and lead citrate, and observed under an electron microscope.

In muscle cells, the formation of typical myofibrils was observed. In syncytium of fused muscle cells, the characteristic M and Z lines in the A and I bands of sarcomeres were clearly found. In nerve cells, many neurofibrils were parallel running in nerve fibers which extended and branched during cultivation. In epithelial cells, the formation of interdigitations, gap junctions and desmosomes were observed between the membranes of adjacent cells.

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The results suggest that the ultrafine structures of these differentiated cells may be useful for analyzing the more detailed characteristic action of the specific genes during normal development.

Expression and Induction of Tissue-Specific Enzymes in Cultured Chicken Liver Cells

Yukiaki KURODA and Etsuko SUGAWARA*

To examine the effect of various chemicals on the function of living organisms, the induction of tissue-specific enzymes was used as markers. The treatment with 0.05% trypsin solution was found to give a good result to dissociate cells from livers of 6-day chickens. Collagen (Vitrogen 100) was effective in adhesion of liver cells on the surface of petri dishes. An attempt was made to induce the activity of tyrosine aminotrasferase (TAT) in these liver cells.

Dissociated liver cells were cultured in medium 199 supplemented with 10% fetal calf serum. After cultivation for 22 hours, 10 μM hydrocortisone was added to culture medium and the activity of TAT was determined. The enzyme activity rose up about three times 24 hours after addition of hydrocortisone. The addition of insulin before treatment with hydrocortisone had no effect on the induction of the enzyme activity.

The effective concentrations of hydrocortisone for induction of the TAT activity were 1–30 μM and no difference in effectiveness was found within these concentrations. The addition of 10 μM dexamethasone was also effective in induction of the TAT activity. These results indicate that this system may be used for examining the effect of various chemicals on the function of living organisms.

Mutagenic Activity of Ames Test-Negative Compounds in Cultured Chinese Hamster Cells

Yukiaki KURODA, Akiko YOKOIYAMA and Tsuneo KADA

In the last decade, various mutagens in the environment has been effectively detected by the microbial systems including the Ames tests in the presence of the microsome fraction of mammalian livers. On the other hand, not so few chemicals showing no mutagenicity in microbial systems

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were found to have the carcinogenic activity in experimental animals.

In the present experiment, several carcinogens which had no mutagenicity in the Ames test and was proposed to be tested for their mutagenicity in the International Programme on Chemical Safety (IPCS) of the World Health Organization (WHO), were examined in their mutagenicity in cultured Chinese hamster V79 cells.

Chemicals tested were hexamethylphosphoramide (HMPA), *o*-toluidine, benzene, safrole and phenobarbital as carcinogens and caprolactam and benzoin as non-carcinogens. Cells were treated with these chemicals at various concentrations for 3 hours, and their cytotoxic effects were examined by the colony-forming activity of cells. HMPA, *o*-toluidine, benzene and caprolactam had no or weak cytotoxic effects at concentrations up to 500 μg/ml. Safrole, phenobarbital and benzoin had moderate or strong cytotoxic effects on V79 cells.

Next, the activity to induce 6-thioguanine (6TG) resistant mutations of these chemicals in V79 cells was examined. Safrole and *o*-toluidine induced mutations at 200 μg/ml or 400 μg/ml without addition of S-9 Mix. HMPA and phenobarbital also induced mutations at concentration of 500 μg/ml. The mutagenicity of benzene was not or slightly detected in V79 cells, both with and without S-9 Mix. On the other hand, caprolactam and benzoin had no mutation-inducing activity without S-9 Mix.

These results indicate that four carcinogens except for benzene induced 6TG-resistant mutations, whereas two non-carcinogens had no mutagenic effect in Chinese hamster V79 cells. Thus, the correlation has been found between the tumor-producing activity in animals and the activity to induce 6TG-resistant mutations in Chinese hamster V79 cells.

Effect of Some Growth Factors on Cultured Human Diploid Cells in Clonal Culture

Yukiaki KURODA

In the previous study, the effects of epidermal growth factor (EGF) and fibroblast growth factor (FEF) on the mode of proliferation of human diploid cells have been analyzed in clonal culture. It has been found that both EGF and FGF had growth-stimulating effects on both dividing and non-dividing cells in population.

In the present experiment, effects of platelet-derived growth factor

(PDGF), fibronection and hydrocortisone on the growth pattern of human diploid cells at the sixth population doubling level were examined in clonal culture.

PDGF had a growth-stimulating effect on cells at a concentration of 0.1 unit/ml, but an inhibitory effect at higher concentrations. Fibronectin also showed a growth-stimulating effect on only dividing cells at a concentration of 10 ng/ml. Higher concentrations of fibronectin showed a decrease in its stimulating effect on the growth of cells.

Hydrocortisone was the most effective among these three growth factors. At a concentration of 0.05 μg/ml, it specifically stimulated the growth of only dividing cells in population, but no effect on non-dividing cells, resulting in 10% increase in the average cell number per colony, compared with that in control cultures. These results indicate that the action of hydrocortisone was different from that of EGF or FGF. The former was effective on only dividing cells, and the latter was effective on both dividing and non-dividing cells in population. The difference in the action of growth factors may be related to the control action of growth factors for the senescence of human diploid cells.

Histological Analysis of Embryonic Lethal Mutant in *Drosophila melanogaster*

Kiyoshi MINATO

The process of nervous hypertrophy in an embryonic lethal mutant, *Df* (1) *Notch-8*, of *Drosophila melanogaster* has been analyzed histologically. The hypertrophy of nervous tissue in this mutant appeared to be caused by more segregated neuroblast cells than those in normal embryos in the earlier period of embryonic development.

In this year, a further improvement of fixative technique was successfully made to make more accurate analysis possible. Moreover, a new incubation method of embryos was devised, in which each embryo was incubated in many column-drops of liquid paraffin made in the space of 450 μm depth between the cover-slip and the slide glass. This method makes it possible to observe very easily under the microscope the change of the external morphology of many embryos in a better orientation, without developmental arrest by lack of oxygen and, hence, to analyze quantitatively even the mutants having various lethal times and lethal features.

Using this method, the external morphology of *Notch-8* embryos in the process of neuroblast segregation was microscopically observed in ventral view for ventrally-segregating neuroblasts, but no significant difference could be detected between mutant and normal embryos. With the same method, the analyses of other embryonic-lethal mutants are now in progress.

Mode of Action of an Inhibitor for Stenotele Differentiation in *Hydra*

Toshitaka FUJISAWA

An inhibitor for stenotele differentiation has been found in *Hydra* tissue. It was examined at which stage during stenotele differentiation the factor exerts its action.

Stenotele is one of 4 nematocytes (sting cells) in *Hydra*, all of which differentiate from interstitial stem cells after 2 to 5 rounds of cell division. Commitment of a particular type of nematocyte occurs near the S/G2 boundary in the terminal cell cycle just prior to cytodifferentiation.

The tactics to determine the timing of the inhibitor action during stenotele differentiation is the following. The precursor cells are exposed to the inhibitor for certain time periods and allowed to differentiate into stenoteles. Since the cell cycle time of precursor cells (18h), the stenotele differentiation time (72h) and the maturation time of stenotele capsule (24h) are known, it is possible to reconstruct the differentiation pathway of cells which respond to the inhibitor and to detect the timing of the inhibitor action.

By using the above method it was determined that the inhibitor exerts its action for about 6h period after the S/G2 boundary in the terminal cell cycle. The inhibitor applied before the S/G2 boundary or after first 6h of G2 phase had no effect. These results appear to suggest that commitment is a process extending over about for 6h during which the process is vulnerable to the external signals and can be changeable but after which the process is no longer reversible.

Molecular Cloning of *extra organs* Locus of *Drosophila melanogaster*

Masatoshi YAMAMOTO and George L. G. MIKLOS

We tried to clone the DNA of *extra organs* gene which plays an im-

portant role in the morphogenesis especially at the process of imaginal disk development. Since the gene is known to localize on the 20A1-2 bands in the salivary chromosome, we first looked for a cloned DNA fragment which is from the very intimate bands of the *Drosophila* genome. We planned to use the DNA fragment as a probe and walk into the *eo* locus. Among already published DNA fragments, collagen-like DNA was reported by Monson *et al.*, to hybridize *in situ* at the region 19E-19F. The gift DNA from Monson was then fine mapped to determine the gene locus of collagen. In the proximal region of the X chromosome in *Drosophila* there are abundant chromosomal rearrangements and thus it is possible to make up a hemizygous deficiency or a duplication at the level of one band genetically. We made up a series of deficiencies in the region by appropriate chromosomal manipulations. By Southern hybridization it is possible to determine on which band the DNA fragment used as a probe localizes in the X. If a probe DNA comes from a band deleted as a hemizygous deficiency the intensity of hybridization of the DNA is about a half strength of the one hybridized with diploid amount of autosomal DNA which is considered as an internal control. From this series of experiments the collagen-like DNA was determined to locate at 19F6 which is one band distal from 20A1. We then used this collagen-like DNA as a probe for the walking and cloned 28 kb long DNA to the proximal and 24 kb to the distal. The DNA fragment of the most proximal was not in the chromosomal fragment of $T(X;\ Y)B154^{R}$, but within $y^{+}\ Ymal^{126}$ chromosome. In genetical examinattion, *eo* phenotype appears in 5% of the individuals whose genotype is $Df(1)A7/y^{+}\ Ymal^{126}$, but it significantly increases up to 85–90% in the flies of $Df(1)A7/\ T(X;\ Y)B154^{R}$. This indicates at least a part of *eo* gene is located between the breakpoints of $T(X;\ Y)B154$ and $y^{+}\ Ymal^{126}$. We have thus almost reached to one end portion of the *eo* gene. We are now trying to clone the DNA up to the breakpoint of $T(X;\ Y)B154$.

A Novel Method for Fate Mapping of the Genital Disk: The Region Important for the Testes Elongation and Morphogenesis

Mitsuru SAKAIZUMI and Masatoshi YAMAMOTO

The adult testes of *Drosophila melanogaster* is composed of a pair of coiled tubes approximately 2 mm in length and 0.1 mm in diameter. The adult reproductive system is derived from the gonads and the genital disk.

The somatic gonadal cells develop independently from the germ cells and perform the morphohenesis of the adult testes. The rest of reproductive system is contributed by the development of the genital disk. At the larval life however the gonads and the imaginal disk are not connected.

At 1940's, C. Stern demonstrated from the studies of organ culture that it is the pupal stage when these tissues are connected and then the larval spherical gonads start elongation and coiling. Since then no study on the testes morphogenesis has been done and it remains totally as a dark box of the *Drosophila* development.

By using the mutant *extra organs(eo)* which causes a deletion or a duplication in a region of genital disk as well as other imaginal disks, we have studied correlations between the failure of testes elongation and the anomalies in the male genitalia.

It is obvious that the testes elongation is totally dependent on the imaginal disk development because the male of complete absence from the outer-genitalia (derived from genital disk) retains under-developed spherical gonads equivalent to that of 72 hrs after puparium formation. Furthermore, if the half of the outer-genitalia is deleted one side of the paired internal genitalia is also missing and one of the gonads remains as undeveloped. The examination of about 450 *eo* individuals led us to conclude as follows;

1. There is a hierarchy in the appearance of abnormalities within the disk. This suggests the process of genital disk development namely the cell lineage. The higher abnormalities to the lower in the outer-genitalia goes posterior lateral plate=posterior clasper → anterior clasper → anterior lateral plate → penis → genital arch → anal plate. When the anal plates are missing the whole male genitalia is without a failure completely deleted. On the other hand there are many males having deletions in the posterior region of lateral plates and claspers but normal in the anal plates.

2. The region which is important for induction of testes elongation and coiling can be fate mapped at the overlapping area between the region for the posterior lobe of lateral plate and the posterior portion of the clasper. The region may well become the testicular duct of mesodermal origin and function in the induction of testes elongation.

Freezing of Silkworm Ovaries

Jun KUSUDA, Kimiharu ONIMARU, Takehico NOGUCHI
and Okitugu YAMASHITA*

Since the successfull preservation of mouse embryos at −196°C, the freezing technique has been applied to store the oocytes and embryos of several mammalian species for the purpose of the maintenance of their genetic strains economically and safely. However, such a subzero temperature preservation is not reported in insect. Here, we show that silkworm ovaries preserved in liquid nitrogen produce the mature eggs retaining the capacity of embryogenesis and post-embryonic development.

Ovaries were taken out from 2 day old fifth instar larvae of a hybrid of N106 and Cambodge which carried a dominant marker gene. They were suspended in successive concentrations (0.5 M, 1.0 M, 1.5 M) of glycerol in Grace's medium containing 15% fetal calf serum. The organs were packed into plastic semenstraws and cooled to −7°C. Straws were seeded by holding with forceps precooled in liquid nitrogen to induce iceformation. They were gradually cooled to −35°C at a rate of 1°C/min using a program freezer and the straws were finally plunged into liquid nitrogen. After freezing for 48 hr, the ovaries were thawed rapidly by agitating in a 40°C water bath (at a rate of 500°C/min) and glycerol was washed out. The treated ovaries were transplanted into the haemocael of 4 day fifth instar larvae carrying no marker genes. The larvae grew to adults normally.

Since the planted ovaries were not connected with the host oviduct, mature eggs were dissected out from adults and parthenogenetically activated by incubation at 46°C for 18 min. The activated eggs were then treated with HCl solution (specific gravity, 1.075 at 15°C) at 46°C for 5 min to prevent diapause. About 10 days after the acid treatment, larvae hatched from eggs. They grew to fifth instar and exhibited the mark of marker gene on their integuments. Thus, the silkworm ovaries can survive in freezing temperature retaining the capacity of further development.

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V. CYTOGENETICS

Decrease of C-Bands and NORs in the Black Rats from Amami and Tokunoshima

Tosohide H. YOSIDA

Large and clear heterochromatic C-bands are generally observed near the centromere of all chromosomes in the black rats (*Rattus rattus*), but the Japanese black rats (*R. rattus tanezumi*) were remarkable by showing a decrease of C-bands in some chromosome pairs (Yosida and Sagai 1975, Chromosoma **50**: 283–300). The animals collected from the plain area of Amagi and Tokunoshima islands located in southern Japan, were characterized by a remarkable decrease of C-bands in their karyotype. They had C-bands in only two of 13 acrocentric autosome pairs, and complete decrease of C-bands occurred in the other 11 acrocentric autosome pairs. On the other hand, the black rats collected from the high land of the islands showed the usual C-band type. The nucleolar organizer regions (NORs) of the black rats are generally observed in three chromosome pairs (nos. 3, 8 and 12) (Yosida 1979, Proc. Jap. Acad. **55B**: 481–586), but in the animals having decrease of C-bands collected from the island, the defficiency of the NORs was observed in pair no. 3. The rats with usual C-bands and NORs collected from the high land of Amami were suggested highly to be an ancestral type of the Japanese black rats which were migrated primarily from the continent of Asia, but those with the decrease of C-bands and NORs would be derived from the former by the diminishing mutation of the inner chromosomal organizations such as C-bands and NORs (Proc. Japan Acad. **59B**: 211–214).

Location of Ribosomal RNA Genes in the Amami Black Rats by *in situ* Hybridization Technique

Tosihide H. YOSIDA, Hitoshi SUZUKI and Takeharu KANEHISA[1)]

In the black rats, the nucleolar organizer regions (Ag-NORs) of chromosomes have been observed in chromosome pairs 3, 8, and 13 in all sub-

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species belonging to several geographical type (Yosida 1980, Cytogenetcs of the Black Rat, Univ. Tokyo Press), but those collected from Amami had only two NORs in chromosome pairs 8 and 13 described in the previous report. To know whether the NOR is really lost in the chromosome pair no. 3 in this sepcimen, we investigated the site of NOR genes in the Amami black rat by means of *in situ* molecular hybridization technique by use of the radioactive complementary r-RNA. The result obtained was as follows; in the Amami black rats the location of ribosomal 18S+28S RNA genes was found in the centromeric regions of the pair nos. 8 and 13, but in the chromosome no. 3, small silver cluster was rerely observed. This means that the copy number of r-RNA genes would be extreamely fewer in pair no. 3 than those of pair no. 8 and 13 (Proc. Japan Acad. **59B**: 215–218).

Phylogenetic Relationship among Several Karyotype forms of the Black Rat and some Other Related Species

P. B. BAVERSTOCK[1)], M. ADAMS[1)], L. R. MAXSON[1)] and T. H. YOSIDA

The black rat, *Rattus rattus*, consists of the Asian ($2n=42$), Ceylonese ($2n=40$), Oceanian ($2n=38$) and Mauritius ($2n=42$ by fission) types. The Asian type was divided into two types; high and low C-bandings. As the related species to the *R. rattus*, *R. norvegicus*, *R. losea* and *R. exulans* were used. We used isozyme electrophoresis and microcomplement fixation to elucidate the genetic distance and phylogenetic relationship among each of the various karyotypic forms of *R. rattus* and other related species. The results showed that (1) the Mauritius type black rat was genetically very similar to the Oceanian form, suggesting that this island population has undergone very rapid chromosomal evolution; (2) the Ceylonese type was genetically distinct from the Oceanian type; the genetic difference was probably insufficient, however, to prevent future introgression; (3) the level of genetic differentiation occurring between the Asian type on the one hand and Oceanian, Ceylonese and Mauritius types on the other hand was suggested that these two groups were either full species or incipient species; (4) in contrast to data from amino acid composion of transferrin and from restriction endonuclease digests of mtDNA, the present data suggested that the various karyotype froms of *R. rattus* were phylogenetically more

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closely related to each other than any is to *R. norvegicus* and others, and that they are related by a series the Asian, Ceylonese and Oceanian types; (5) the *R. rattus*/*R. norvegicus* divergence would have occurred 2～8 million years ago, whereas various chromosome types of *R. rattus* diverged over the last 4 million years (Genetics **105**: 965–983).

Frequency of Chromosome Translocations in the Black and Norway rats after γ-Irradiation

Toshihide H. YOSIDA

Polymorphism or geographical variations due to chromosome mutations were often observed in the natural population of the black rat (*Rattus rattus*), while in the Norway rat (*R. norvegicus*) it was very few. On the difference of the chromosomal divergence in these two species, the present author (Yosida 1981, Environmental Mutagen and Carcinogen, Univ. Tokyo Press) has suggested that it should be dependent on circumstances in which both species were encountered during the migration from their native land to the other parts. Radioactivity exposed from the earth or cosmic rays seemed to be one of important facters in the circumstances. Another explanation to the above event is that the black rat is more sensitive to mutagens than the Norway rat. To solve this problem a rate of chromosomal mutation in the black and Norway rats was examined by irradiation with γ-ray. The 400 to 750R γ-rays from Cs^{137} were exposured to males of the black and Norway rats and they were mated to the non-irradiated females. The rate of chromosome mutations in offspring thus obtained was just the same as 0.02 in both species. This result suggests strongly that the black rat with a higher cocurrence of chromosome mutation in the natural population is due to the heavier exposure from the natural radiation during migtation than in the Norway rat (Proc. Jap. Acad. **59B**: 263–266).

A Black Rat with Chromosome Alterations Born after γ-Irradiations

Toshihide H. YOSIDA

In the previous paper the same frequency of the chromosome alterations in the black and Norway rats in the F_1 generation born after γ-irradiation

has been reported. In that experiment we found that only one Ceylonese type black rat (*Rattus rattus kandianus*) among 50 F_1 offspring thus obtained showed chromosomal alterations. The karyotype of the normal Ceylonese type black rat was characterized by having 40 chromosomes (20 pairs), among which 11 autosome pairs were acro- or subtelocentrics, one autosome pair (M_2) was a large metacentric which was resulted from the translocation between acrocentric pair nos. 11 and 12. The remaining 7 autosome pairs were small metacentrics and the X and Y were long and short acrocentrics. One male black rat born after mating between irradiated male and non-irradiated female had the following abnormalities; a deletion at about one fourth of long arm of pair no. 1 was found, and the broken end was translocated to the terminal side of the no. 5 chromosome. A pericentric inversion had been taken in one of the pair no. 9. The another complicated chromosome alteration was found to occur between M_2 (11/12) and no. 13. Namely, the Robertsonian fission occurred in the metacentric M_2, and no. 12 chromosome thus formed was translocated again to the no. 13 chromosome. The male black rat with such chromosome alterations appeared to be healthy and grew into the adult stage. The external sexual organs of the male seemd to be normal, but it was sterile (Proc. Japan Acad. **59B**: 308–311).

A Linkage Test on the Bared Gene to the Inversion no. 1

Tosihide H. YOSIDA

As previously reported (Yosida 1980) an LEM strain rat with an inversion no. 1 was established from the spontaneous chromosome mutants occuured in an LEW strain rat. In the fifth inbreeding generation of the LEM strain rat, a bared (*ba*) mutant appeared spontaneously (Yosida 1981). Based on the mating between the mutant and the normal haired LEM strain rats, the bared character was shown to be due to a simple and recessive mutant gene. To know whether or not the *ba* gene is located on the inversion no. 1 chromosome, the segregation ratio in the F_2 generation from hybrids between the bared rat with inversion no. 1 and the normal Wistar strain rat (WM) without inversion was investigated. All F_1 rats thus obtained had the normal hair coat, but their karyotypes showed without exception an inversion/normal heterzygous pair no. 1 as expected. Among 55 F_2 offspring obtained from matings between the F_1 hybrids described above, 16

rats were bared, while the remaining 29 rats were normally haired. The segregation ratio was not significantly different from the expected value as a simple and recessive mutant gene. A relation between the segregation ratio of karyotypes and that of the bared rats was examined in 21 F_2 offspring. Three among them were bared, but the remaining 18 rats were normal. The ratio of the bared to the normal rats was not significantly different from the expected one. The karyotype of the bared rats was all characterized by having the inversion and normal heteromorphic pair no. 1. On this basis it was strongly suggested that the bared gene might not be linked to the inversion pair no. 1, but to any other chromosome pairs (Proc. Japan Acad. **59B**: 531–354).

Chromosome Translocations Occurred in Offspring of the Norway Rats after γ-Irradiation

Tosihide H. YOSIDA

It is previously reported that the frequrncy of the Norway rats with the chromosome translocation in the offspring born after mating between irradiated male and non-irradiated females was 0.02, which was just the same as that obtained in the black rats. In that experiment 400 to 750R of γ-rays from Cs^{137} were irradiated to the males of Norway rat and they were mated to the non-irradiated females. By such matings 273 offspring from 55 litters were obtained. Among them 6 individuals had chromosome translocations. For the present study ACI/N, ALB, BUF, F-344, LET, NIG-III, WM and WKS strains were used. On the induction of translocations, there was no strain difference of the rat. Ten autosome pairs, such as nos. 1, 3, 4, 7, 8, 9, 11, 12, 14, and 18 and the Y chromosome were involved in the translocation. The frequency was calculated from the chromosome length and found that it was not different significantly from the difference of the chromosome length or type. It occurred ar random through the length of chromosomes. The translocatoon of the chromosomes occurred in the offspring in the rat after γ-irradiation seemed to be always reciprocal (Proc. Jap. Acad. **60B**: 46–49).

Translocation between Autosome nos. 9 and 18 Occurred in a F-344 Rat after γ-Irradiation and its Genetics

Tosihide H. YOSIDA

In the previous report it was described that 6 rats born after γ-irradiation had various types of translocation between autosomes and also the autosome and sex chromosome. Among them the translocation between autosomes 9 and 18 was found in a F-344 male. In the normal karyotype of the rat no. 9 was characterized by acrocentric homologous pair and no. 18 by metacentric homologous pair. In the one male rat born after γ-irradiation, however, one of the no. 9 chromosomes was remarkable by the large submetacentric, and one of no. 18 chromosomes by small acrocentric. This karyotype was developed by following two events; the fission of the pair no. 18 at the centromeric region, and translocation of the one arm of the no. 18 to the centromeric side of pair no. 9 was taken place. This relation was clearly shown by G-banding staining. The male rat with 9/18 translocation was mated to normal F-344 females and 38 F_1 offspring were obtained. They were segregated to 19 normal and 19 translocation heterozygotes, just we expected. To obtain the F_2 offspring, the male and female translocation heterozygotes were mated and 63 F_2 offspring were obtained. They were segregated only to two types, such as 13 normal rats and 50 translocation heterozygotes. The rats with the translocation homozygous pair were never obtained in the F_2 and thereafter. The 9/18 translocation homozygotes were suggested to be lethal (Proc. Japan Acad. **60B**: 50–53).

4/7 Translocation Occurred in a WM Strain Rat after γ-Irradiation and its Transmission to Offspring

Tosihide H. YOSIDA

Among several translocations occurred in the Norwwy rats born after mating between γ-irradiated males and a non-irradiated females, a translocation between pair nos. 4 and 7 was found in a male WM strain rat. About two third in the distal part of one no. 7 chromosome were deleted and the broken end was translocated to the terminal region of one of the pair no. 4. By such a translocation, one of the pair no. 4 was of an extraordinary long acrocentric, while one of the pair no. 7 was like a small dot.

By mating between the male with translocation and the 5 normal females of the WM strain rats, 14 offspring were obtained. The mean litter size was 2.8 ± 1.3 which were smaller than the normal litter size in this strain. Among 14 offspring, 7 rats (3♀ : 4♂) were the hetrrozygotes having the 4/7 translocation and the normal partner, but the remaining 7(2♀ : 5♂) showed the normal pairs of nos. 4 and 7. Among the 5 males, however, 4 were the normal XY-sex chromosome mechanism, but the remaining one was characterized by having 43 chromosomes including XYY-sex chromosomes. This male showed the normal external feature, but it is unknown at present whether or not, the male is a fertile. Such an abnormal sex chromosome should be due to the non-disjunction of the Y chromosome in the male parent with 4/7 translocation (Proc. Japan Acad. **60B**: 88–91).

11/Y Translocation in the NIG-III Strain Rat Occurred after γ-Irradiation and its Genetics

Tosihide H. YOSIDA

A remarkable case of the translocation between the autosome no. 11 and the Y chromosome was found in a male of the NIG-III strain rats born after mating between the γ-ray irradiated male and the non-irradiated female. A large part of the Y chromosome remaining the centromere was translocated to the short arm of the no. 11 chromosome and the remaining Y with the centromere was deformed as an extremely small element like a dot. By mating between the 11/Y translocation male and the normal female, 133 rats (73♀ : 60♂) were obtained in the F_1 to F_2 generations. Among 73 females, 62 were of the normal female karyotype with 42 chromosomes including the normal pair no. 11 and two Xs. Ten females, however, had 41 chromosomes, among which one had the normal pair of no. 11, but a single X, and the remaining 9 females showed the translocation heterozygous no. 11 and a single X. Remaining one female had 42 chromosomes, but its karyotype was conspicuous by having a translocation heterozygous pair no. 11 and two Xs. Eventhouth these 10 had the large part of the Y element by the transloction, their sex characters were always of femaleness because the dot-Y element was not included.

Among 60 males thus obtained, 46 had the translocation heterozygous pair no. 11 and X and dot-Y sex elements similar to the male parent. The other 13 males, however, had 43 chromosomes, among which 40 were normal

chromosomes including normal pair no. 11, but the sex chromosomes were remarkable by having two Xs and one dot-Y. The occurrence of the XO female and XX/dot-Y males was considerably high in their frequencies and they were found at almost identical number (10♀ : 13♂) (Porc. Japan Acad. **60B**: 125–128).

1/12 Translocation Occurred in a WKS-Strain Rat after γ-Irradiation and its Transmission into Offspring

Tosihide H. YOSIDA

A new translocation between pair nos. 1 and 12 occurred in one male of the WKS-strain rats born after mating between the irradiated male and non-irradiated females. The translocation between these two chromosomes occurred spontaneously in the Lewis strain rat has been reported by the preswnt author (Yosida 1980). In that case, the long arm of the no. 12 was broken and the broken end was translocated to the short arm of the pair no. 1. In the present case, however, the long arm of the pair no. 1 was broken and the broken end was translocated to the short arm of the pair no. 12. Thus, one of the pair no. 1 became shorter than the normal partner, while one of the pair no. 12 deformed to meta- or submetacentrics. Although the reciplocal translocation between these two chromosomes was difficult to demonstrate by the conventional or G-banding staining, it was clearly shown by the tranlocation of the nucleolar organizer region (NOR).

The translocation male was mated to the normal females of the same strain rats. The 23 F_1 hybrids (10♀ : 13♂) from 4 litters thus obtained were segregated to 8 normal homozygotes (5♀ : 3♂) and 15 translocation heterozygotes (5♀ : 10♂). The segregation ratio was not significantly different from the expected one. The 32 offspring (17♀ : 15♂) in total 9 litters were obtained by mating between female and male heterozygotes. They were segregated to 12 (4♀ : 8♂) normals, 18 translocation heterozygotes (12♀ : 6♂) and 2 tranlocation homozygotes (1♀ : 1♂). The segregation ratio was significantly different from the expected one (Proc. Japan Acad. **60B**: 227–230).

A Tanuki or Japanese Raccoon Dog with 40 Chromosomes Including Two B-Chromosomes

Tosihide H. YOSIDA, Masayasu Y. WADA[1] and Oskar G. WARD[2]

The chromosome number of the tanuki or Japanese raccoon dog (*Nyctereutes procyonoides vivverinus*) has been described by several investigators to be $2n=42$. Recently one specimen was caught in Amagi, Shizuoka-ken, Japan, and its karyotype was analysed by conventional, G- and C-banding stainings. By use of such techniques, we found that this specimen was remarkable by having $2n=40$, including two supernumerary or B-chromosomes. The karyotype of this specimen was consisted of 13 meta- or submetacentric autosome pairs (nos. 1 to 13), 5 acrocentric autosome pairs (nos. 14 to 18) and a metacentric X and a small Y. In addition to the above chromosomes, two acrocentric B-chromosomes were included in its karyotype. They were dissimilar in the shape and stained heavily through their whole length by the C-band staining. All autosome pairs and the X were differentiated from each other by their G-banding characteristics, but B's were distinguishable from the other autosomes by absence of the clear G-bandings. Thus, the karyotype of this raccoon dog was determined to consist of 36 autosomes, two B-chromosomes and an XY complement. The previous reports that the raccoon dog had 42 chromosomes thus would be due to failure in identification of B-chromosomes among autosomes (Proc. Japan Acad. **59B**: 267–270).

Somatic Variation of B-Chromosomes in Two Japanese Raccoon Dogs and Robertsonian Fission of no. 8 Chromosome

Tosihide H. YOSIDA, Masayasu WADA[1], Oskar G. WARD[2] and Doris H. WURSTER-HILL[3]

The karyotype of one Japanese raccoon dog (*Nyctereutes procyonoides viverrinus*) with two B-chromosomes has been reported in the previous report. This specimen (RD-8301) captured in Amagi, Shizuoka-ken,

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Japan, was characterized by having 40 chromosomes, among which 36 (18 pairs) were autosomes, two were X and Y chromosomes and the remaining two were B-chromosomes. Recently, we analysed cytogenetically on the other two raccoon dogs (RD-8302 and RD-8303) newly captured from the same place as the above, which were remarkable by having a wide somatic variation of the number of B-chromosomes. We also found a Robertsonian fission in one of the autosome no. 8 in these two specimens.

The basic karyotype of the RD-8302 was the same as the previous one (RD-8301), but the centromeric fission was observed in one of the chromosome pair no. 8. By such fission, an increase of two new acrocentrics and decrease of one metacentric no. 8 occurred in this specimen. The another remarkable karyotype feature of this specimen was the presence of wide numerical variation of the B's in somatic cells. Among 100 somatic cells analysed 44% cells had 41 chromosomes including 2 B's, 21% cells had 44 chromosomes having 5 B's. Each with 43 (4 B's) and 42 (3 B's) were at 18% and 12% respectively. Those with 1B and 6B's were also counted at 4 and 1%, respectively. The karyotype of the RD-8303 was quite similar to the above specimen due to having the centromeric fission of one of pair no. 8. This animal, however, was remarkable in having only two cell types due to the variation of B's. Among 96 cells counted, 79 cells (82%) had 43 chromosomes including 4B's, but the remaining 17 cells (18%) had 42 chromosomes including 3B's. Cells with the other variation did not be observed. Distribution of B's was examined in a specimen, RD-8301, previosuly reported. Among 88 cells examined, 85 cells (96.5%) showed exactly 40 chromosomes including 2B's, just as descrived previously, but one cell had one B and the remaining two cells had 3B's (Proc. Japan Acad. **60B**: 17–20).

Robertsonian Fission of the no. 6 Autosome Newly Found in a Japanese Raccoon Dog Collected in Aichi-Ken

Tosihide H. YOSIDA and Masayasu Y. WADA[1)]

As described in the previous paper, among three Japanese raccoon dogs, *Nyctereutes procyonoides viverrinus*, collected in Shizuoka-ken, Japan, one did not have any Robertsonian fission, but the other two were characterized by having the fission of no. 8 chromosome. In one specimen (RD-8409)

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collected from Aichi-ken, Japan, we found a new Robertsonian fission occurred in the no. 6 chromosome. This individual also showed a bimodal distribution in two and three B-chromosomes in the somatic cells. The Robertsonian fission of pair no. 6 was clearly demonstrated by G-banding technique. Namely one partner of the pair no. 6 was normal submetacentric, but the other one was broken at the centromeric region and two new acrocentrics were formed. The no. 8 chromosomes of this individual were normal submetacentrics. The two acrocentrics formed by the fission of no. 6 had clear C-bands like the other normal metacentrics.

In the present specimen, two and three B-chromosomes were observed in most cells. Among 122 cells counted, 64 (52.4%) cells had two B's, 56 (45.9%) cells had three B's, and the remaining only two cells had one B-element. Thus, the specimen, RD-8409, was mosaic in the somatic cells due to the different number of the B-chromosomes (Proc. Japan Acad. **60B**: 289–292).

A Japanese Raccoon Dog with Three Robertsonian Fissions in nos. 5, 8 and 11, Found Newly in Kanagawa-Ken

Tosihide H. YOSIDA and Masayasu, Y. WADA[1)]

Two types of the Robertsonian fission occurring in no. 6 and no. 8 chromosomes in the Japanese raccoon dog, *Nyctereutes Procynoides viverrinus*, have been described in the previous reports. A specimen (RD-8410) collected from the suburb of Yokohama, Kanagawa-ken, Japan, however, was characterized by having the Robertsonian fission in three chromosomes, nos. 5, 8 and 11. This specimen had also three and four B-chromosomes in somatic cells.

The Robertsonian fission of the no. 8 chromosome was similar to the other specimens described previously. In the present specimens, however, other new Robertsonian fission was found in pair nos. 5 and 11. Each one of these metacentrics were broken at the centromeric region and the six acrocentrics appeared newly, while three metacentrics decreased from the basic karyotype. By such events the total chromosome numbers except B's were increased to 41. All 119 cells so far examined had the same three Robertsonian fissions as described above. It is interested that all the

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acrocentrics newly formed by fission of metacentrics had a clear and large C-bands similar to the other metacentrics.

The present material was also characterized by somatic variation of B-chromosomes. Among 119 cells so far analysed, 71 cells (59.6%) had three B's, 43 cells (36.1%) had four B's, and the remaining 5 cells (4.3%) had two B's, All B-chromosomes found in this specimens were acrocentrics as found in the other Japanese raccoon dogs, and all these B's were heavily stained through their whole length by the C-band staining (Proc. Japan Acad. **60B**: 293–296).

Variation of B-Chromosomes in Four Youngs from One Litter Collected in Saitama-Ken

Tosihide H. YOSIDA and Masayasu Y. WADA[1)]

The karyotypes of 5 specimens of the Japanese raccoon dog, *Nyctereutes procyonoides viverrinus*, collected from several places of Japan, were remarkable by having several numbers of B-chromosomes and also various types of the Robertsonian fission. These specimens, however, had not any relation from each other. We newly obtained four young specimens from one litter of the Japanese raccoon dog from Saitama-ken. All four specimens, RD-8404, RD-8405, RD-8406 and RD-8407, had the same basic chromosome number and karyotype; $2n=38$ consisting of 36 autosomes and XX- or XY-sex chromosomes. The Robertsonian fission of autosomes was not observed in the all four specimens.

All four specimens had the B-chromosomes as similar to the other Japanese raccoon dogs observed previously. Interesting in the present materials, however, was that among four spcimens, two (RD-8404 and RD-8406) showed a modal distribution in 2B's, but the other two (RD-8405 and 8407) in 3B's. In RD-8404, 2B's were found in 97 (95.1%) among 102 cells counted. Only 4 (3.9%) and 2 (1.9%) cells had one B and three B's, respectively. In the case of RD-8406, 2B's were observed in 84 (80.0%) among 105 cells counted, but cells with 3B's were observed at higher frequency (18.1%) than in the above specimen. In the case of specimens with 3B's, the RD-8405 had the 3B's at 85.3%, 2B's at 12.7% and one B at 2.0%. In the RD-8407, the variation was slightly different from the above specimen.

1) Amagi Wild Boar Park, Agagi, Shizuoka-ken.

Among 110 cells counted, 3B's were observed in 64.5%, and 4B's were at 30.0%. The cells with 2B's and one B were at 4.5 and 1.0%, respectively. Based on the above investigations, it is suggested that the differece of the number of B's in four youngs born from the same parents seems to be due to the random segregation of B's in the meiosis of the parents. On the somatic variation of the B's it is suggested to be derived from the non-disjunction in somatic cells in the course of the ontogeny (Proc. Japan Acad. **60B**: 297–300).

B-Chromosomes and Robertsonian Fission in 13 Japanese Raccoon Dogs, with a Note on Somatic Variation of the Number of B's

Tosihide H. YOSIDA and Masayasu Y. WADA[1)]

In the previous reports the karyotypes of 9 Japanese raccoon dogs (*Nyctereutes procyonoides viverrinus*) were described. All these specimens had B-chromosomes in their karyotypes ranging from 2 to 4 showing somatic variations. On the other hand, the Robertsonian fission of some metacentric autosomes has been found in 4 specimens among them. In adition to these 9 animals, we analysed 4 specimens newly collected from Shizuoka-ken and Aichi-ken. Based on these 13 Japanese raccoon dogs, the frequency of the B-chromosomes and the Robertsonian fission, with special interest to the somatic distribution of B's in 1,732 cells.

Among 4 specimens newly collected, 3 (RD-8401, RD-8402 and RD-8403) were collected from Shizuoka-ken and one from Aichi-ken. Among these 3 specimens, the Robertsonian fission was found in only one specimens (RD-8402), but in the other 3 the basic karyotype was observed. These all specimens had the B-chromosomes showing some somatic variation. Model occurrence of 3B's was observed in two specimens (RD-8402 and RD-8408) and the other two (RD-8401 and RD-8403) had 4B's.

Among 13 specimens collected in Japan, 5 had the Robertsonian fission; three specimens in no. 8, one in no. 6 and the remaining one in three chromosomes, nos, 5, 8 and 11. Based on these results, it can be said that about 38.5% of the Japanese raccoon dogs have the Robertsonian fission. All these specimens so far examined had the B-chromosomes of two to four,

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although some somatic variation was observed in all of them. Among 13 specimens, model occurrence of 2B's were observed in 5 specimens, 3B's in the other 5 specimens, and the remaining 3 specimens had 4B's. From the above 13 specimens the somatic variation was examined in 1,372 cells in total. Among them, cells with 2, 3 and 4B's were frequently observed at 31.6, 36.2 and 23.3%, respectively. The other cells with 1, 5, 6 and 7B's were extreamly fewer, such as 2.0, 5.0, 0.7 and 1.2%, respectively. The cells without B's and those with more than 8B's were not observed. In the Japanese raccoon dogs, the individuals with few B's would be preferentially selected than those without B's and also more number of B's (Proc. Japan Acad. **60B**: 301–305).

Karyotype of an Indian Spiny Mouse with 24 Chromosomes

Parasana KUMARI,[1] Nimmakayalu V. ASWATHANARAYANA[1] and Tosihide H. YOSIDA

The Indian spiny mouse, *Mus platythrix*, has a characteristic karyotype consisting of 26 acrocentric chromosomes as described by several investigators. Yosida (1979, 1980), one of the present authors, has described that the karyotype of this species was derived from that of the house mouse, *M. musculus*, by tandem fusion of several pairs of the autosomes. He suggested that the chromosome evolution might have occurred sequentially by the tandem fusion (Yosida 1983). He also suggested that specimens with fewer chromosome numbers than $2n=26$ would be found somewhere in India by sequential fusion of chromosomes in this species. As predicted by him, we discoverd a male specimen with a reduced chromosome number of 24, characterized by all acrocentrics.

Among 27 specimens collected in Mysore, 26 showed the usual diploid number ($2n=26$), with all acrocentric chromosomes. One male, howefer, had 24 chromosomes. Among 12 autosome pairs (no. 1 to 12) designated in the original karyotype, one of the smaller pairs, probablly no. 11 appeared to be missing in the present material. This pair might have translocated tandemly to any one of the other autosome pairs as in the other large acrocentrics, although it was not identified. Interesting is the fact that the Y chromosome in this specimen was considerably smaller than the other specimens with $2n=26$. It seem that in the course of development

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of 24 chromosomes, the deletion in the Y element would have been taken place (Proc. Japan Acad. **60B**: 85–87).

Chromosomes of the Honshu Sika Deer, *Cervus nippon centralis*

Masayasu Y. WADA[1] and Tosihide H. YOSIDA

Karyotypes of the Ezo sika deer (*Cervus nippon yesoensis*), Kuyshu sika deer (*C. nippon nippon*) and Manchurian sika deer (*C. nippon nortulorum*) have been described some investigators (Makino and Muramoto 1966, Gustavsson and Sundt 1968, 1969, Miyake *et al.* 1982). On the chromosome complement of the Honshu skia deer it has not yet been reported so far. The present report deals with the karyotyp of Giemsa, G- and C-band stainings of the Honshu sika deer. Chromosome number of this subspecies was $2n=68$ consisting of 32 acrocentric and one metacentric autosome pairs, and an acrocentric X and a submetacentric small Y. They were identified by their G-banding chractristics. All acrocentric autosomes and the X chromosome had clear C-bands at their centromeric regions, but not in the matacentic autosome. Small satellites were observed at the distal ends of pair nos. 1 and 2. Karyotype of the Honshu sika deer was compared with those of Ezo, Kyushu and Manchurian sika deer which were classified as the different subspecies from the Honshu sika deer. The karyotypes of the Ezo, Kyushu and Manchurian sika deer were similar to the present material by having 68 chromosomes. Although the small satellite was observed in the pair nos. 1 and 2 in the Honshu sika deer, it was not shown in the Ezo and Kyushu sika deer. In the Manchurian sika deer it was observed only in the autosome pair no. 1. One of the present authors (Yosida 1980) has described that the differentiation of the nucleolar organizer is important to the differentiation of species. It is well known that the nucleolar organizer is located at the satellite region. The sika deer would be interesting materials to know the above problems (La Kromosome II, 35–36: 1117–1121).

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A Simple Technique for Chromosome Observations from the Small Mammals and Birds, and Karyotypes of Some Mammals and a Bird

Masayasu Y. WADA[1] and Tosihide H. YOSIDA

The present paper deals with a simple and easy technique for observation of chromosomes in small mammals and a bird and their karyotypes. A small amount of blood was collected by the claw-cutting method, and the whole blood was cultured by our routine method for the chromosome observation. Two small mammals, the masked palm civet (*Paguma lorvata*) and the common palm civet (*Paradoxurus hermaphroditus*) and domestic fowl (*Gallus gallus domesticus*) were used in the present study. The blood (0.2–0.3 ml) of these animals was collected by cutting the base of the claw, and the whole blood was cultured *in vitro* by use of the culture medium (MEM) with PHA. Sixty six hours after culture the slides were made for chromosome observation. Chromosome number of the masked palm civet was $2n=44$ and the karyotype was consisted of 9 subtelocentric autosome pairs, 8 acrocentric autosome pairs and 4 small meta or bsumetacentric autosome pairs, and a large metacentric X and the smallest Y. The common palm civet had $2n=42$, consisted of 10 subtelocentric autosome pairs, 5 acrocentric autosome pairs, 5 meta or submetacentric autosome pairs and the X (large metacentric) and Y (small dot like) elements. The chromosome number of the domestic fowl was 78, among which no. 1, 2, 8 and 9 autosome pairs were submetacentrics, no. 3, 6, 7, and 10 to 39 autosome pairs were acrocentrics. Sex chromosome (Z/Z) was shown to be no. 5 metacentrics (La Kromosomo II-32: 971–976).

A Simple Technique for Identification of Sex-Chromosome (W) in the Bird

Masayasu Y. WADA[1] and Tosihide H. YOSIDA

A simple and applicable method for the identification of sex in the bird was developed by use of direct air drying preparation. The chromosome preparations were made from the feather pulp by the following technique. (1) Mitotic arrest. In young domestic fowl, 0.025% solution of demecolcine (or colchicine) was injected into the peritoneal cavity (0.2 ml per one in-

[1] Amagi Wild Boar Park, Amagi, Shizuoka-ken.

dividual of the 7 day young chick). The injection was made 1 hr before harvesting the feather. The feather was plicked for chromosome observations. (2) Slide preparation. The base of feather was cut in the petri dish into about 1 mm^3, and immediately transferred to 0.075 M KCl solutopn for 50 min at room temperature. Two or three pieces of the cut pulp were removed from the hypotonic solution and placed on a clearn slide. Then, the materials were minced into small pieces and fixed on the slide by applying the method of Imai *et al.* (1977; Chromosoma **59**: 341–393). The slide was air-dried for one day. (3) Staining. The completely air-dried slides were stained with 3% Giemsa solution for 10 min. For C-bandings of the slide, the Giemsa stain was removed from the slide by immersion into 50% acetic acid. Then, the trypsin C-banding method was applied (Wang and Shoffner 1974; Chromosoma **47**: 61–69). By the above thechnique the metaphase chromosomes became generally vaguely by the long treatment with trypsine, but only the W-chromosome was stained darkly and conspicously. In the brid the individual with the dark stained chromosome was determined to be female (WZ-type) (Proc. Japan Acad. **59B**: 223–226).

Identification of the Sex Chromosome in a Sex Reversed Peacock

Tosihide H. YOSIDA, Masayasu Y. WADA, Yohei SAKAI
and Norio KONDO

The peacock is a typical sexual dimorphism bird. The upper tail covers are beautiful and long in the mail, while they are not in the female. We, however, encountered an intereting case that a peacock, which was recognized primarily as a female in the secondary sexual characteristics, has changed into a male character after full-grown and the upper tail covers have begun to grow just as those of the male. Due to such a change of the secondary sexual feature, it was difficult to determine whether or not, this peacock was a real female. It has been well known that the bird is characterized by having a sex determining mechanism as the female dimorphism or the ZW-type.

Sex chromosome (W) of the bird can easily be demonstrated by our simple technique by use of the feather pulp described in the previous paper. By applying this method, the W-chromosome of the sex reversed peacock could cleary be demonstrated. By use of this technique, we found that the sex reversed bird was primarily a female, because the W-chromosome was

exactly included. The chromosoe constitution of the bird was also analysed in cultured cells from the biopsy skin by applying the serial conventional Giemsa, G- and C-band staining methods, and confermed that the sex reversed peacock had 32 autosome pairs and ZW-sex chromosome complement (Proc. Japan Acad. **59B**: 304–307).

Karyotypes of Four Dragonet Fish Species

Makoto MUROFUSHI[1], Shohei NISHIKAWA[2] and Tosihide H. YOSIDA

The karyotypes of four dragonet fish species, *Callionymus punctatus*, *C. beniteguri*, *C. ornatipinnis* and *C. doryssus*, were examined. The chromosome number in the male and the female of *C. punctatus* was $2n=38$, all of which were acrocentrics. The sex chromosome in this species was an usual X-Y complement, although it was not identified from the other autosomes. The chromosome numbers of *C. beniteguri* and *C. ornatipinnis* were $2n=38$ in the female and $2n=37$ in the male. The sex chromosome mechanism of these two species was XX-Y. All autosomes and X-chromosomes were subtelocentrics, but the Y-chromosome was large metacentric. On the other hand, the chromosome number of *C. doryssus* was $2n=32$ in the male, among which one pair was large metacentric and the remaining fifteen pairs were acrocentric. The sex chromosome was not identified in this species, because the female specimens were not examined. The secondary constriction was found in one chromosome pair of all four species; in the pair no. 1 of *C. beniteguri*, in the pair no. 2 of *C. doryssus*, and in the pair no. 10 of *C. ornatipinnis* and *C. punctatus*. Position of the secondary constriction of the pair no. 10 in the latter two was different from each other (La Kromosome II-34: 1079–1084).

Karyotypes of Two Species in the Sandperch Fishes

Makoto MUROFUSHI[1], Shohei NISHIKAWA[2] and Tosihide H. YOSIDA

The karyotypes of the species in the sandperch fishes (*Parapercis pulchella* and *P. sexfasciata*) were examined. The diploid chromosome number in *P. pulchella* was $2n=42$, while *P. sexfasciata*, $2n=26$. The karyotype of the formaer species was consisted of 4 metacentric pairs (nos. 1 to 4) and

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17 acrocentric pairs (nos. 5 to 21), but in the latter species 11 metacentric pairs (nos. 1 to 11), one submetacentric pair (no. 12) and one acrocentric pair (no. 13). The number of chromosome arms in these two species was 50. The secondary constriction was found in pair no. 6 in *P. pulchella* and pair no. 4 in *P. sexfasciata*. One hetcromorphic pair (no. 12) and one small metacentric pair (no. 11) like as a minute was found in the latter species. The sex chromosome could not be identified in both species. (La kromosome II-35: 1122–1125).

Multiple Sex Chromosome Mechanism (XX-Y) Newly Found in the Snake Eel, *Muraenichithys gymnotus*

Makoto MUROFUSHI[1] and Tosihide H. YOSIDA

The multiple sex chromosomes (XX-Y) in the fish have been found in a single filefish species (Murofushi *et al.* 1980) and two dragonet fish species (Murofushi *et al.* 198*fl*). A similar case of the multiple sex-chromosome has newly been found in one species of the snake eel, *Muraenichthys gymnotus*. Six specimens including 3 males and 3 females of the snake eel were used in this study. They were collected from two-hundred meter depth of the Suruga Bay, Shizuoka-ken, Japan.

The chromosome number of the female snake eels was $2n=48$, consisting of subtelocentric pairs and 22 acrocentric pairs, while in the males it was $2n=47$. In the male cells one large metacentric was always observed. Judging from the multiple sex-chromosome mechanism in the other fish species found in the filefish and dragonet fish, the large metacentric element was suggested to be the Y-chromosome. Thus, the sex-chromosome mechanism in this species is inferred to be the multiple X_1X_2-Y. (Proc. Japan. Acad **60B**: 21–23).

Comparative Study on Two Species of Snipefishes

Makoto MUROFUSHI[1], Shohei NISHIKAWA[2] and Tosihide H. YOSIDA

The karyotypes of two species in the snipefish, *Macrorhamphosus sagifue* and *M. japonicus*, were comparatively examined. Two specimens in the

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former species and four specimens in the latter species were collected from two hundred meter depth of the Suruga Bay, Shizuoka-ken, Japan. The diploid chromosome number of these two species of the snipefishes was forty-eight ($2n=48$), all of which were acrocentrics ranging from large to small in the size. The secondary constriction was found near the centromere of the pair no. 9 in the both species. The idiograms of these two species and the length of each chromosome pair were very similar from each other.

These two snipefish species were separated by the difference of ratio in body size and length of spine, but the karyotypes of *M. sagifue* could not be distinguished from that of *M. japonicus*. Okada and Suzuki (1951) asserted that these two of the snipefishes should be integrated to one species as *M. scolopax*. Based on the present karyological data, the opinion of the latter authors seems to be reasonable (Jap. Jour. Genet. **59**: 155–158).

Karyotypes of the Red Swamp Crayfish and the Japanese Lobster

Makoto MUROFUSHI[1], Yosihaki DEGUCHI[2] and Tosihide H. YOSIDA

The karyological studies on Crustacea are insufficient and most of the studies have been carried out by the classical technique by several investigators. The chromosomes of the red swamp crayfish, *Procambarus clarkii*, and the Japanese lobster, *Nephrops japonicus*, have also been observed by Niiyama (1934, 1939) by that technique. We obtained the red swamp crayfish from the paddy field in Mishima, Shizuoka-ken and the Japanese lobster from Suruga bay, Shizuoka-ken. The chromosomes were observed by an air-drying method modified from our routine technique for fishes (Murofushi and Yosida 1979).

The diploid chromosome number in male and female specimens of the red swamp crayfish was $2n=188$, consisting of 42 meta- or submetacentric and 52 subtelo- or acrocentric pairs. The diploid number of male and female specimens of the Japanese lobster was $2n=136$, among which 46 pairs were of meta- or submetacentrics and 22 pairs were of acro- or subtelocentrics. Niiyama (1934 and 1939) has reported that the chromosome number of the former spesies was $2n=192$ and that of the latter species, $2n=164$. The difference seen in these two studies are probably due to the

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difference of the method utilized in the karyotype analysis (Proc. Japan Acad. **60B**: 306–309).

Karyotype of the Small Free-Living Nematode, *Caenorhabditis elegans*

Tosihide H. YOSIDA, Tamiko SADAIE and Yoshito SADAIE

The small free-living nematode, *Caenorhabditis elegans*, has recently been widely utilized as an attractive organism in molecular biology. In the adult hermaphrodites, only about 800 somatic cells are included in one individual and their cell lineage can easily be recognized in the body. The haploid DNA of this worm was to be only about 20 times as large as that of the genome of *E. coli*. It is also known to have six linkage groups. The karyotype of this worm by the light microscope, however, has not yet been demonstrated so far. By use of the drying technique, we succeeded to observe the chromosomes of this small nematode in somatic and germ cells. The number of the diploid chromosomes was determined to be twelve and they were classified into six homologous pairs by G-band staining. Six chromosome pairs were similar in its size. The smallest chromosome was about 0.7 time as large as the largest one. In the present comunication we could not assign the number of the linkage group and also recognize the X-chromosome from the autosomes. A series of the first meiotic chromosomes was also successfully demonstrated. All of these cells showed to habe six tetrads (Proc. Japan Acad. **60B**: 54–57).

Inhibition of Sister Chromatid Exchanges in Bloom Syndrome Cells with Cycloheximide

Yukimasa SHIRAISHI[1], Tosihide H. YOSIDA and Avery A. SANDBERG[2]

Effects of cycloheximide (CH) and deoxycytidine (dC) on the frequency of sister chromatid exchanges (SCEs) in normal and Bloom syndrome (BC) cells labeled with BrdU during first, second and third cell cycles were evaluated, using endomitotic and three-way differentiation analyses. When CH at 0.2 and 2.0 μg/m*l* was added to normal and BS cultures of BrdU labeled endomitoses, the rate of single SCEs was significantly decreased in

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BS cells, though the rate of reduction in single SCE was slight in normal cells. No significant change was detected in the twin SCE rate. In BS cells, treatment with CH at 0.2 and 2.0 μg/m*l* produced significant reductions in SCE in both the second (SCE_2) and third (SCE_3) cell cycles—sometimes reaching normal level. Treatment with dC at 13 and 26 μg/m*l* resulted in almost no significant changes in rates of SCE during first, second and third cell cycles. When CH was added to BrdU labeled normal and BS cell cultures, the cell growth rates improved from 35 to 70 per cent over the control level in the BS cells, though in normal cells CH resulted in a lower cell growth rate in a dose dependent manner. Deoxycytidine did not noticeably affect the cell growth rates in BrdU labeled normal and BS cultures. The finding that the reduction of BrdU-induced SCE in BS is paralleled by cell growth improvement is of special interest (Cancer Genet. & Cytogenet. in press).

Chromosome Observations on Tropical Ants from Western Malaysia. II

Hirotami T. IMAI, William L. BROWN Jr.[1], Masao KUBOTA[2], Hoi-Sen YONG[3] and Yow Pong THO[4]

Imai and Kubota went to West Malaysia in 1982 and 1983 for a karyological survey of tropical ants. The project was performed in cooperation with H. S. Yong and Y. P. Tho. The identification of species was made by M. Kubota and W. L. Brown, though it remains unsettled yet in some ant groups, e.g., *Pheidole* and *Camponotus*. We already described chromosomes of 73 ant species collected in 1982 (Ann. Rep. Natl. Inst. Genetics, **32**: 71–73). The present paper reports chromosomes of 86 species (39 genera, 6 subfamilies) collected in 1983. A total of 119 colonies, labelled as HI83-(1-119), were collected from the following localities: campus of Forest Research Institute (HI83–1–42), Jeram Toi (HI83–43–46, 49), Pasoh Forest (HI83–47–74, 103), Gombak Station of the University of Malaya (HI83–76–83, 96, 109–115), Genting Highlands (HI83–84–95), Ulu Gombak

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Table 1. Chromosome numbers of Malaysian ants. II

Taxa (Colony number)	Chrom. number (n) 2n
PONERINAE	
Platythyrea quadridenta (HI83–22, 50)	(9) 18
P. tricuspidata (HI83–116)	96
*Gnamptogenys menadensis** (HI83–61)	42
G. binghami (HI83–21)	(22)
*Leptogenys diminuta=sp. 1** (HI38–14, 15, 86)	38
*L. myops=sp, 2** (HI83–113)	(24)
L. sp. 3 (HI83–109)	48
L. iridescens (HI83–47, 65)	46
L. borneensis (HI83–66)	46
*Diacamma sp. 1** (HI83–16, 99, 106)	44
Amblyopone reclinata (HI83–20)	38
Pachycondyla "*n. sp. A* of Brown" *=Mesoponera sp. 1* (HI83–63, 71)	(18) 36
P. leeuwenhoeki	(8) 16
*P. rubra**=*Mesoponera sp. 2* (HI83–3, 11, 12) Robertsonian polymorphism	38, 40
P. tridentata (HI83–51)	28
Ponera japonica (HI83–94)	12
Hypoponera pruinosa (HI83–110)	(12) 24
H. sp. 2 (HI83–35)	38
H. sp. 3 (HI83–45)	36
Cryptopone testacea (HI83–54)	(9) 18
*Odontomachus simillimus =sp. 1** (HI83–17, 18)	44
*O. rixosus=sp. 2** (HI83–38)	(15) 30
O. latidens (HI83–25)	(15)
PSEUDOMYRMECINAE	
Tetraponera sp. 2 (HI83–97)	42
DORYLINAE	
Aenictus laeviceps? (HI83–103)	22
MYRMICINAE	
*Aphaenogaster beccarii** (HI83–55)	30
*Pheidole sp. 1** (HI83–101)	(10)
P. sp. 7 (HI83–7)	16
P. sp. 11 (HI83–53)	22
P. sp. 12 (HI83–44)	(10) 20
P. sp. 13 (HI83–102)	38
P. sp. 14 (HI83–32)	20
P. sp. 15 (HI83–6)	20
P. sp. 16 (HI83–104)	(9) 18
P. sp. 17 (HI83–27) Translocation polymorphism	20, 21
P. sp. 18 (HI83–8, 88)	20
P. sp. 19 (HI83–9, 90)	20
P. sp. 20 (HI83–58, 60, 108)	(18) 36
P. megacephala (HI83–82)	20
*Crematogaster sp. 1** (HI83–59)	26
*C. sp. 4** (HI83–5, 49)	24
C. sp. 5 (HI83–4)	26
C. sp. 6 (HI83–72)	26
*Monomorium sp. 2** (HI83–85)	22
M. sp. 3 (HI83–96)	(11) 22
M. sp. 4 (HI83–105)	(11)
Lophomyrmex bedoti (HI83–73, 75)	38
Vollenhovia sp. 1 (HI83–46, 69) Robertsonian polymorphism	49, 50
V. sp. 2 (HI83–93, 95) Robertsonian polymorphism	33, 34
Acanthomyrmex sp. 3 (HI83–10, 98, 114)	(11) 22
Pristomyrmex sp. 2 (HI83–77)	(14)

Table 1. Continued

Taxa (Colony number)	Chrom. number (n) 2n
Lordomyrma sp. 1 (HI83–37)	(11) 22
*Myrmicaria sp. 2** (HI83–87)	44
M. sp. 4 (HI83–43)	(23) 46
Tetramorium pnyxis (HI83–56)	20
T. eleates (HI83–30)	28
T. seneb ? (HI83–29)	20
*Smithistruma taipingensis** (HI83–111)	(12) 24
Strumigenys godeffroyi (HI83–31)	40
Dacetinops concinna (HI83–115)	16
Eurhopalothrix n. sp. (*procera* group) Brown (in press)	18
DOLICHODERINAE	
*Dolichoderus bituberculatus** (HI83–83) B-chromosome polymorphism	30, 31, 32, 33
Iridomyrmex cordatus (HI83–74)	16
*Technomyrmex sp. 1** (HI83–118)	30
FORMICINAE	
*Anoplolepis longipes** (HI83–1, 2, 117)	34
*Acropyga acutiventris** (HI83–28, 119) Robertsonian polymorphism	28, 29
Plagiolepis sp. 1 (HI83–62)	18
Pseudolasius sp. 1 (HI83–57, 70)	(8)
P. sp. 2 (HI83–67, 68) B-chromosome polymorphism	(15, 17, 19) 30
*Camponotus festinus** (HI83–24, 52)	(19) 38
*C. sp. 2** (HI83–19)	40
C. sp. 7 (HI83–107)	(20)
C. sp. 8 (HI83–23) Robertsonian polymorphism	38, 39
C. sp. 9 (HI83–80, 81)	40
C. sp. 10 (HI83–41, 42) Robertsonian polymorphism	(13, 14)
C. sp. 11 (HI83–84)	52
C. sp. 12 (HI83–100)	18
C. sp. 13 (HI83–78)	18
Polyrhachis illaudata (HI83–36)	(18)
P. hector (HI83–76, 112)	(21) 42
P. rastellata (HI83–39, 40, 79)	42
Echinopla sp. 1 (HI83–48, 64)	(12) 24
*Paratrechina sp. 4** (HI83–34)	16
P. sp. 6 (HI83–92)	30
P. sp. 7 (HI83–33)	16
*Prenolepis jerdoni** (HI83–26, 91) B-chromosome polymorphism	(16, 20, 25, 27) 30, 31, 32, 34

N.B. The species with asterisks were observed also in the survey of 1982.

(HI83–97–108), and Templer Park (HI83–116–119). Among 119 colonies, we were able to observe chromosome spreads in all samples except HI83–13, 46, 57, 63, 79, 89, and 119. The results are summarized in Table 1. In addition, two species collected at Sungey Menyala Reserve, Negeri Sembilan, in 1981 by Brown and Pong (*Pachycondyla leeuwenhoeki* and *Eurhopalothrix*

n. sp.), were karyotyped separately by Brown; these have been added to Table 1.

Modes of Inheritance of X-Y Dissociation in Inter-Subspecies Hybrids between BALB/c Mice and *Mus musculus molossinus*

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Genetic analysis of the high frequency of X-Y chromosome dissociation found in primary spermatocytes of F_1 hybrids between Japanese wild mice (*Mus musculus molossinus*) and inbred laboratory mice (BALB/c) was attempted. The frequency of X-Y dissociation (X//Y) in both BALB/c and *M. m. molossinus* was lower than 30% (Low X//Y), while the value was more than 70% (High X//Y) in their F_1 hybrids. Two types of progeny (High X//Y and Low X//Y) appeared in the backcross between BALB/c and High X//Y males, although the frequency of Low X//Y progeny decreased with increasing numbers of backcross generations (26.5% at N_2, 13.2% at N_3, 5.3% at N_4, and 0% at N_5). Low X//Y sires produced only Low X//Y mice. We hypothesize that at least one heritable factor which is responsible for the end-to-end association of the sex chromosomes (temporarily symbolized as *Sxa*) is located on the common part of the X and Y chromosomes. The *Sxa* allele of BALB/c is Sxa^a and that of *M. m. molossinus* is Sxa^b. The genotype expected in High X//Y males is Sxa^a/Sxa^b and in Low X//Y males and their parental stocks either Sxa^a/Sxa^a or Sxa^b/Sax^b. The repeated segregation of Low X//Y progeny from High X//Y sires is interpreted simply by assuming that crossing-over has occurred between the X and Y chromosomes. The gradual decrease in the recombinant type mice (Low X//Y) during sequential backcross suggests the presence of some autosomal factors that suppress the crossing-over of the sex chromosomes and that do not seem to function in the inter-subspecies hybrids. For details see Cytogenet. Cell Genet. **35**: 209–215 (1983).

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Rates of Mammalian Karyotype Evolution by the Karyograph Method

Hirotami T. IMAI, Takeo MARUYAMA and Ross H. CROZIER[1)]

Using the karyograph method devised by Imai and Crozier (1980), we attempted to estimate the rate of mammalian karyotype evolution. Denoting the chromosome number and arm number of the mean haploid karyotype ($\overline{K_i}$) in a given mammalian taxon by $\bar{n}_i$ and $\overline{AN}_i$, respectively, the karyotype can be represented at a point $\bar{K}_i(\overline{AN}_i, \bar{n}_i)$ on a two-dimensional lattice (karyograph), where the arm number is plotted against the chromosome number. If we assume that $\bar{K}_0(\overline{AN}_0, \bar{n}_0)$ is the karyotype of primordial mammals, it is possible to define three types of karyological distance between the K_0 and K_i. Namely, the distance defined by the difference in the arm numbers ($\bar{D}_{AN}=\overline{AN}_i-AN_0$), the distance by the chromosome numbers ($\bar{D}_n=n_i-n_0$), and the karyonumber distance ($\bar{D}_{KN}=\bar{D}_{AN}+\bar{D}_n$). Imai and Crozier (1980) found that mammalian karyotypes tend to evolve, in a statistical sense, orthodirectionally toward increasing chromosome number and/or arm number mainly by centric fission and by pericentric inversion changing acrocentrics (Ā) to metacentrics ($\overline{\text{M}}$) (p.i. ($\overline{\text{AM}}$)). The distances $\bar{D}_n$ and $\bar{D}_{AN}$ are, therefore, the total numbers of centric fissions and of p.i. ($\overline{\text{AM}}$) accumulated in the $\bar{K}_i$ since the karyotype diverged from the K_0. We assumed that mammalian karyotype evolution began from the karyotype $K_0=3\overline{\text{M}}$, which is the lowest-numbered karyotype among extant mammals, about 100 million yr ago; i.e., the common ancestor has $K_0(AN_0=6,\ n_0=3)$ and the divergence time (t) is 100 million yr. Now, the mean rate of arm number change ($\bar{V}_{AN}$), chromosome number change ($\bar{V}$), and karyonumber change ($\bar{V}_{KN}$) is given as $\bar{V}_{AN}=\bar{D}_{AN}/t$, $V_n=\bar{D}_n/t$, and $\bar{V}_{KN}=\bar{V}_{AN}+\bar{V}_n$. We estimated the rate of Karyotype evolution using 1,070 mammalian species, and obtained $\bar{V}_n=0.19$, $\bar{V}_{AN}=0.27$, and $\bar{V}_{KN}=0.46$. In other words, the chromosome number (n), arm number (AN), and karyonumber ($KN=n+AN$) in mammals change, respectively, at a rate of about one in every 5.3, 3.7, and 2.2 million yr. For details see Am. Nat. **121**: 477–488 (1983).

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Cytogenetic Analysis of Recombination in Males of *Drosophila ananassae*

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Cytogenetic studies of recombination in males of *Drosophila ananassae* were carried out by examining F_1 males derived from the mating of marker females, *b se*; *bri ru* of the BS stock, with males of two wild strains, TNG and L8. The male recombination values in both sections *b-se* (chromosome 2) and *bri-ru* (chromosome 3) are high in TNG F_1 but extremely low in L8 F_1. We demonstrate the presence of chiasmata in TNG F_1 meles at a frequency capable of accounting for the observed recombination values. A unique series of "iso-site aberrations" was also observed in TNG F_1 males. Because of a parallelism in the distribution pattern between the chiasmata and the iso-site aberrRtions, we propose that recombination in males of *D. ananassae* is meiotic in origin and that the iso-site aberrations are related to chiasma formation. For details see Chromosoma (Berl.) **88**: 286–292 (1983).

Quantitative Analysis of Karyotype Alteration and Species Differentiation in Mammals

Hirotami T. IMAI

A two-dimensional graph (species-karyotype graph or $s-k_G$ graph) was devised for analyzing the role of karyotype alteration in speciation. When the number of species and dissimilar karyotypes involved in a genus are denoted as s and k_G, the genus can be represented as a point (s, k_G) on the graph. If karyotype alteration always initiates speciation as suggested by the stasipatric speciation model, the genus will fall in the area $k_G/s \geqq 1$. On the other hand, if speciation occurs without karyotype alteration and karyotype substitution procceds subsequently in each of the resulting species, the genus will fall in the area $k_G/s \leqq 1$. Among the 235 mammalian genera examined, the genera distributed in the area $k_G/s>1$, $k_G/s<1$ and $k_G/s=1$ were 21 (8.9%), 57 (24.3%) and 157 (66.8%). This result suggests that speciation without karyotype alteration predominates in mammals. Based on the distribution patterns of mammalian genera on the $s-k_G$ graph, an

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overall tendency of the life cycle of mammalian genera to follow this sequence was suggested: $k_G/s=1$, $k_G=s=1 \overset{1)}{\rightarrow} k_G/s<1$, $k_G=1$, $s>1 \overset{2)}{\rightarrow} k_G/s<1$, $s>k_G>1 \overset{3)}{\rightarrow} k_G/s=1$, $k_G=s>1 \overset{4)}{\rightarrow} k_G/s=1$, $k_G=s=1$, that is, 1) speciation without karyotype alteration, 2) substitution of karyotypes in each resulting species, 3) climax of species differentiation, and 4) degeneration of genus by extinction of species. The parapatric distribution of karyotypically distinct populations are interpretable under the model as a transitional step in karyotype substitution. For details aee Evolution **37**: 1154–1161 (1983).

Genetic Studies on Spontaneous Sister Chromatid Exchanges in Spermatogenesis of *Drosophila melanogaster*

Masatoshi YAMAMOTO

Population cytogenetics has demonstrated that the DNA content of an organism is not fixed. Especially the content of heterochromatin (C-band) or satellite DNAs seems to be most variable and it is known as C-band polymorphism. The variation of heterochromatin content causes alterations in the frequency of recombination over and above genic regulations. Changing the amount of DNA can thus bring biologically important consequences. The mechanism how the DNA content is altered is however yet to be solved.

The postulated mechanisms are (1) unequal meiotic recombination and (2) unequal sister chromatid exchanges (USCE). The former should be less likely the mechanism because the heterochromatin hardly undergoes recombination in itself and further it does not explain the polymorphism of the Y chromosome and the B chromosome which often exists alone without their homologs in a genome. The latter is an quite attractive hypothesis and has recently been shown to occur spontaneously in a totally heterochromatic ring Y chromosome in *D. melanogaster*. Nevertheless the occurrence of spontaneous SCE's in the germ line is still a subject of cytogenetical debates.

The question of the function of SCE has been set aside so far although extensive studies on the mechanism and the increase of frequency depending on the dose of chemical mutagens have been carried out.

Here I determined genetically the frequency of spontaneous SCE in the spermatogenesis of *D. melanogaster* in order to test the hypothesis that the USCE

Table 1. The progenies obtained from the cross $zw^{+R61e19}$♂ × *C*(1)*DX*, *ywf*♀

Phenotype	No. of Flies	No. of Harem
z (lemon yellow) ♂	154663	
mosaic (lemon/wild) ♂	5	5
z^+ (wild type) ♂	21	2
ywf ♂	12	8
ywf ♀	132878	
z ♀	1	1
Super females	44	40
Total	287624	

is a mechanism in changing the content of chromosomal DNA. The fly strain I used is $zw^{+R61e19}$ which carries a mutant allele of *zeste*(*z*) and a partially duplicated white locus. zw^+ males show wild type eye colour even though the genotype is *z*. On the other hand $zw^{+R61e19}$ which is duplicated in a subsegment of the white locus shows lemon yellow eye colour in the male.

A single male of $zw^{+R61e19}$ was crossed to 10 to 15 $\overline{XX}$, *ywf* females and every other two days they were transferred into a new bottle. The male progenies coming out of the cross are all carrying the paternal genotype. The important point of this experiment is that the $zw^{+R61e19}$ X chromosome never go through the female genome. If the DNA content at the duplicated region of the white locus is altered it must be happened in the male genome, namely in the single X chromosome. A change in the DNA content at the specific locus can be easily monitored by the appearance of wild type males showing brick red eye colour. The experimental crosses were repeated 480 times and 287,624 flies were checked the phenotype individually. The results are shown in the table 1.

Among the total of 154,701 males 21 z^+ males which should be considered to be lost a part of the duplicated white locus are included. These males could be derived from a gene mutation at the *z* locus or the spontaneous partial deletion at the white locus, but the frequency 1.4×10^{-4} is too high if it were the case. Furthermore the z^+ males were confirmed not to have a mutation at the zests locus. These findings strongly suggest that *spontaneous unequal* sister chomatid exchanges (USCE) happened so that the region was deleted out from the $zw^{+R61e19}$. In addition, it is clear that the USCE's occured in the spermatogonial mitosis not spermatocytes because they came out as clusters from 2 harems of the cross.

The evidence showing that spontaneous USCE's occurs at mitotic divisions was also indicated from the mosaic (lemon yellow eye colour/wild type eye colour) males.

It should be stressed that the frequency 1.4×10^{-4} was measured in a very small euchromatic duplication. It can be thus concluded that in the heterochromatin where highly repeated DNA is abundant, the USCE's must happen at the significantly higher rates. The molecular change associated with this USCE is now under investigations.

VI. MUTATION AND MUTAGENESIS IN ANIMALS

Induction of Specific-Locus Mutations by High Temperature Exposures in Newly Laid Eggs of the Silkworm

Akio MURAKAMI

Some sixty years ago, R. Goldschmidt (1927) reported that the frequency of mutations in *Drosophila* increased by high temperature exposure. Since then, there were numerous papers on the mutation induction by low or high temperature treatments in various organisms. In the silkworm, experimental induction of various abnormalities including androgenic and parthenogenic individuals as well as polyploids was attempted firstly by Tanaka and his students. They treated eggs and pupae with low and high temperatures. Their findings were confirmed and extended by Tazima (1939, 1947).

In the silkworm, it was established that spontaneous mutation frequencies at some loci were higher than those at other loci, and were markedly dependent on seasons when experiment was carried out. This suggests that the temperature is one of the effective factors to increase the mutation frequency. To examine this supposition, the present experiment was carried out by treating newly laid eggs with various temperatures, 2.5°, 10°, 20°, 25° (as the control), 30°, 35°, 37°, and 40°C. Newly emerged virgin F_1 hybrid moths from the cross between *C108* female and *Aojuku* male were stored in a refrigerator at 5°C for 24 hrs. Those females were then mated to marker males homozygous for *pe* and *re* egg-colour genes for 4 to 5 hrs at 25°C, separated each other and then allowed to lay eggs for 20 min. The eggs collected were immediately incubated at various temperatures for 90 min. During this period eggs proceeded their development from the very late meiosis I of oocytes to completion of meiosis II or just before syngamy.

The F_1 offsprings or eggs from all those crosses were expected to be black wild-type eggs except for eggs after treatment with high temperatures at 35° to 40°C. In fact, no significant increase of abnormalities was observed in the experimental groups exposed at 2.5° to 25°C, but an increase of abnormalities at the *pe* locus was recovered in ocoytes (and/or eggs) after

treatment with relatively high temperatures at 30°~40°C.

Surprisingly a significant increase in mosaics of black (*re*$^+$) and red (*re*) serosa cells and a few recessives at the *re* locus were detected, besides the markedly high frequency of abnormalities at the *pe*$^+$ locus of either mosaic or recessive individual, when the eggs were treated with high temperatures at 37° and 40°C. The occurrence of both mosaic and recessive abnormalities at the *re*$^+$ locus in consequence of the androgenic development could hardly be interpreted, because the *pe* gene is epistatic to the *re* gene. The plausible interpretation for this finding was that these abnormalities may be produced by gene mutations and chromosomal aberrations induced with high temperature exposures, as reported in other organisms. Thus, it can be assumed from the present observation that, in silkworm females, the high temperatures are one of important factors to increase egg-colour specific-locus mutations and that the deflection of frequency in spontaneous as well as artificially-induced mutations at different seasons may be partly dependent on the temperature during experiments. This suggests that high temperatures accelerated chemical reactions which produced the high frequency of mutations.

At present, the mechanism responsible for the marked increase and/or deflection of spontaneous mutations at the *pe*$^+$ locus as compared with the *re*$^+$ locus is not clear, but it is likely to assume that, under the high temperature condition, the occurrence of the mosaics of black (*pe*$^+$) and yellowish-white (*pe*) serosa cells may originate from the abnormal development of the androgenic nucleus in one part of the egg and the normal development of fertilized zygotes in the other part. Similarly, the recessive abnormalities at this locus would have a tendency to arise from dispermic androgenic development. It could not exclude a possibility, however, that a different location of genes on the chromosome may be also related to a differential mutability: the *pe* gene on the proximal portion (5–0.0) and the *re* gene on the middle portion (5–31.7).

A New Egg Colour Mutant, Light Brown Egg with Black Eyes in the Silkworm

Akio MURAKAMI and Yosoji FUKASE

In the course of studies on the chemically-induced mutagenesis with silkworms, a number of recessive visible complete and mosaic mutants

have been recovered by the specific-locus method using egg-colour genes, *pe* and *re*. Among them a mosaic mutant composing of light brown and normal black serosa cells was detected in oocytes after treatment of pupae with a mycotoxin Aflatoxin B_1. To establish a complete mutant from this mosaic one, the mosaic mutant was first crossed to the double recessive marker stock homozygous for *pe* and *re* loci and consequently about twenty eggs for light brown were obtained in addition to a number of black and yellowish-white eggs. In the next step, the female mutants showing the light brown egg were mated to a tester male homozygous for *re* gene to discard the marker chromosome having both *pe* and *re* genes and in consequence black and red eggs were obtained. To obtain the homozygous individuals for the mutant factor, crosses between those black eggs were carried out and the offspring black, red and light brown eggs were obtained instead of yellowish-white eggs.

It is of interest to note that the pigmentation of compound eyes in the insect with the light brown eggs was hardly distinguished from that of the wild type black (+) eggs, but clearly different from the yellowish-white (*pe*) eggs. The intensity of the colour of serosa cells in the light brown eggs showed yellowish-white until the first 72 hrs after oviposition, like as that in the *pe* mutant eggs, but the pigmentation is gradually changed into light brown cloor peculiar to this new mutant phenotype by the early phase of diapause period. When a female of the light brown egg was crossed to a male of the wild-type black egg, the offspring all had light brown eggs with black eyes, and the same result was obtained in the reciprocal cross. This indicates that the mutant is recessive to the wild-type gene (pe^+). When the silkworm with light brown eggs was mated to a *pe* silkworm, all offspring in the F_1 generation, had a phenotype of the mutant, regardless of the cross system. This result shows that the factor for light brown was epistatic to the *pe*. Such being the case, we named the mutant, light brown eggs with black eyes, pe^{bw}, as one member of multiple allelomorph at the pe^+ gene locus on the proximal region of the 5th chromosome (5-0.0). Although the pigmentation of the *pe* mutant ganglia was pale pink, that of the light brown (pe^{bw}) egg was violet, compared with that of deep violet in the wild-type.

In addition to the pe^{bw} mutant, we detected several varieties of the yellowish-white eggs with different intensities of the colour. Probably, these varieties at the *pe* locus could be explained on the basis of a single gene

variation. These facts indicate that these several varieties may be the multiple allelomorph of the pe^+ gene and show various intensities of the colour of the serosa and genglion cells. Such variant alleles of the pe^+ gene would arise as gene mutations rather than chromosomal aberrations. If so, it could be said that Aflatoxin B_1 was able to induce so called "gene mutations" as well as chromosomal aberrations in germ cells of the silkwork.

For reference, a white egg lethal pe^l has long been regarded as one of variants of the pe^+ gene. However, recently, viable yellowish-white eggs was detected with a low frequency in this lethal suggesting that both the lethal and pink eye loci with yellowish-white eggs may be close together with each other. Thus the allelomorph at the *pe* locus may have a polymorphic function controlling the pigmentation of serosa and ganglion cells as well as of compound eyes.

Effects of Tritium on the Induction of Recessive Visible Mutations in Primordial Germ-Cells of Silkworm Embryos: The Nature of β-Ray-Induced Mutants

Akio MURAKAMI

Environmental contaminations with internal β-emitters arise in various nuclear operations including nuclear fusion devices. Concequently, an interest is increasing on the possible mutagenicity as well as other biological effects of β-radiation which were naturally or accidentally accumulated in the body or the tissues with low doses and low dose-rates. In this connection, the primordial germ-cell systems using diapause silkworm embryos have an advantage in detecting genetical effects by determining incorporated radionucleides in the above-mentioned exposure condition. Evaluation of the relative mutagenic effectiveness or the RBE of β-rays has been long carried out by Tazima (Res. Rept. to the Ministry of Education, Science and Culture of Japan, "The Fundamental Studies on the Biomedical Effects of Tritium" [1978]). He reported that the effect of β-rays was *ca.* two times more effective than the standard radiation. This RBE value in embryonic germ-cells indicates that β-ray-induced mutants includes genetic lesions other than gene mutations. In the present study, the progeny tests were carried out to make clear the nature of β-ray-induced mutants in primordial germ-cells using [6-^{3}H]thymidine (6T) as a model β-emitter.

The mid-stage female pupae of the wild-type *C108* strain were treated with 25 μCi/capita of 6T and mated to another wild-type *Aojuku* strain males to allow oviposition of radioactive eggs. The duration of incubation or diapause periods was about 7 months. After hatching, offsprings were grown and then mated to the marker stock moths homozygous for egg-colour genes, *pe* and *re*, to detect the recessive visible mutations. We obtained almost complete mutations, but rarely mosaic mutations.

The procedure for the progeny test was as follows. First yellowish-white egg mutants in the pe^+ locus, for example, were crossed with the tester strain homozygous for the *re* gene to obtain heterozygous chromosomes (black eggs) carrying the newly induced mutation and/or to discard the *pe re* tester chromosome. In the next step, the matings between the black eggs were carried out to obtain the homozygous chromosomes for the mutated locus and the two expected eggs, black and red, in the next generation. Lastly, the yellowish-white eggs as well as other phenotypic eggs were tested for their viabilities and/or hatchabilities. Among 32 mutants examined for both pe^+ and re^+ loci, 15 were found to be lethals, indicating about half of them might have chromosomal aberrations. However, it should be emphasized that the total number of mutants subjected to the test was up to several thousands and that the majority of them (*ca.* 90%) died before the progeny test at the stage of embryos. This clearly indicates that a large number of the mutants detected might be caused by chromosomal damages.

The present observation is in agreement with the implications deduced from the RBE value (*ca.* 2) of β-rays obtained by the specific locus test in embryonic germ-cells. It has been long established that chromosomal damages induced in pre-meiotic stage cells could hardly be transmitted to the next generation. But the present finding may contrast with such a view. Further studies are required to confirm these possibilities and also to elucidata the relative ability of β-rays to induce gene mutations and chromosomal aberrations in primordial germ-line cells of the silkworm.

A New Type Maternal Inheritance in the Silkworm

Akio MURAKAMI

The first example of maternal inheritance in the silkworm was reported by Toyama (1913). In this insect, there are a number of egg-color mutants,

w$_1$, *b*$_1$, *b*$_2$, *pe*, *re*, and others. The first three mutants showed the maternal inheritance or determination: the phenotypic expression of the individual's own genotype was delayed and segregation occurred in the F_3 generation. This unusual phenomenon was simply interpreted on the basis of the egg-cytoplasmic contribution by mother genotype regardless of either dominant or recessive trait of her genotypes. Besides those egg-color mutants, the last two *pe* (pink eyed white egg), *re* (red egg) and other egg-color mutants showed the normal mendelian inheritance. Accordingly, these *pe* and *re* genes have been used for the specific-locus method to detect the mutations.

In the series of chemically- and physically-induced mutation experiments, numerous mutants at these gene loci have been detected and/or preserved in our laboratory. However, the intensity of the color of the serosa cells in those mutants has a marked variation. For example, mutants at the *re* locus have a broad color spectrum varying from yellowish-orange which is almost indistinguishable from the yellow of the *pe* mutant to bright red. Since it is very difficult to designate accurately each mutant with a pertinent term, for convenience each mutant was classified by a wave length unit adopted from the interference color chart for NIKON polarizing microscope as follows: *re* (1100–1150mμ), *re* (1050–1100), *re* (1000–1050), *re* (975–1000), *re* (450–500), *etc.*

The bright red egg, mutant *re* (1100–1150 μm), kept its phenotype throughout subsequent generations when the mother is such a mutant. Several mutants analysed so far are viable in the homozygous state, suggesting that they may be caused by gene mutations rather than chromosomal deficiencies. When one of these mutants, *re* (1100–1150) was mated to the black wildtype (*re*$^+$) the F_1 offspring had all the black egg color regardless of the mutants used, confirming that the mutant behaved as a regular mendelian recessive mutant to its corresponding black wild-type allele. Similar situations were observed in other *re* mutants. Consequently, each mutant may occupy the same locus of the *re* gene (5–31.7). In the F_2 a segregation occurs within a single batch with a ratio of three black eggs to one red eggs. When a *re* (1100–1150 mμ) female was matted to a *re* (450–500 mμ) all offspring in the next generation showed a phenotype of the *re* (1100–1150) mutant, for example. In the reciprocal cross, the offspring showed a phenotype of the *re* (450–500) mutant. Similar situations were observed in the crosses between other *re* egg mutants. Thus, this phenomenon may be limitted to be the allele to the gene locus for the

phenotype of red eggs. The analysis of the behaviors of each mutant for further generations was not yet completed however, it appears that the trait has a tendency to behave as the maternal inheritance independent of its wild-type allele. It is also suggested that the factor controlling these phenotypes is genetically equistatic each other. It is necessary to further analyse what constitutes an essenstial factor for the phenomenon, to draw a final picture for the present finding which may throw light on the mechansim(s) responsible for the unusual maternal inheritance.

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN MICROORGANISMS AND PLANTS

Induction of Prophages in Spores of *Bacillus subtilis* by Ultraviolet Irradiation from Synchrotoron Orbital Radiation

Yoshito SADAIE and Tsuneo KADA

Recently vacuum UV has become available from synchrotron orbital radiation and can be used for studying the effect of vacuum UV on living organisms. Because vacuum UV is available only in a high vaccum, bacterial spores are appropriate materials for studying its effect on living cell. We examined the induction of prophages (SPO2) from lysogenic spores of *Bacillus subtilis* by SR UV (160 nm to 240 nm) and found that SR UV at around 220 nm was most effective in the inactivation of spores and prophage induction. SR UV at around 220 nm might produce spore specific photo products in DNA as the conformation of spore DNA is different from that of vegetative cells and UV irradiation produces a unique photoproduct from the thymine residues in spores (J. Radiat. Res. **25**: 170–173, 1984).

Detection of Natural and Environmental Bioantimutagens and their Mode of Actions

Tsuneo KADA and Tadashi INOUE

In the past few years, we found a number of new natural antimutagens working against radiation- and chemically induced mutations in bacteria. They include certain biometal compounds such as cobalt (Proc. Japan Acad. Ser. B, **5**: 234, 1978; Mutation Res. **91**: 41, 1981) and germanium (Int. J. Radiat. Biol. **42**: 653, 1982; Mutation Res. **125**: 145, 1984), ingredients of plants such as cinnamaldehyde (Agric. Biol. Chem. **48**: 1905, 1984; Mutation Res. **107**: 219, 1983), coumarin and umbelliferone (Mutation Res. **117**: 135, 1983), protoanemonin (Mutation Res. **116**: 317, 1983) and enmein (Agric. Biol. Chem. **48**: 1647, 1984). Form animal origins, placenta tissues of mammals were shown to possess strong antimutagenicity against gamma-ray-induced mutations (J. Rad. Res. **22**: 297, 1981).

The action spectra of certain antimutagens such as cobaltous chloride are very wide and covers those of not only UV, gamma-ray and MNNG but also a frame-shift mutagen (Trp-P-1) (Mutation Res. **95**: 145, 1982) indicated that the antimutagen might be working not at specific mutagenic sites but as stimulator of error-free repair of non-specific DNA lesions.

Modification of the Activities of *recA* Protein by an Antimutagenic Metal Compound, Cobaltous Chrolide

Tadashi INOUE and Tsuneo KADA

In addition to a large number of mutagenic agents in our environment, we also have various compounds which show antimutagenic activities in certain mutation assay-systems using bacteria and mammalian cells cultured *in vitro*. From the view points of both human health and fundamental genetics, it is of interest to study the mechanisms by which the antimutagenic compounds exhibit their activity. One of the potent antimutagenic compounds we have so far found is cobaltous chrolide. This antimutgen is effective in both radiation- and chemically induced mutagenesis in *Escherichia coli* as well as in spontaneous mutagenesis in *Bacillus subtilis*. In order to elucidate the mode of action of this antimutagen, we examined its effects on the purified *recA* rpotein *in vitro*. The *recA* protein of *E. coli* is the product of the *recA* gene on which various cellular functions including mutagenesis, recombination and repair profoundly depend. It promotes *in vitro* the homologous pairing of double-stranded DNA and linear single-stranded DNA, thereby forming a three-stranded joint molecule called D-loop. The protein also catalyzes the hydrolysis of ATP which depends upon single-stranded DNA or replicative form I DNA. Experimental results showed that both D-loop formation and replicative form I DNA-dependent ATPase activity was stimulated whereas the single-stranded DNA-dependent ATPase activity was partially inhibited by the antimutagenic metal compound, suggesting that the antimutagenicity of this metal compound is ascribed to the elevated activity of recombinational repair which is thought to be error-free. This is also supported by the fact that the survival of mutagen-treated bacteria was enhanced by the posttreatment with the antimutagen.

This work was done in collaboration with Drs. T. Shibata, O. Makino, T. Ando (Ricken Institute, Wako) and T. Ohta (Institute of Environmental

Toxicology, Tokyo).

Restorative Effects of the Human Placenta Extracts on Gamma-ray-irradiated Mice

Tsuneo KADA and Hideo TEZUKA

Effects of the human placenta extracts were observed on mice which were lethally exposed to gamma-ray. Placental tissues obtained from thirty individuals were homogenized and mixed. The mixture was deproteinized by treating with pronase and heating in boiling water, followed by centrifugation. The clear supernatant solution was stocked at −80°C. We previously reported that such an extract showed an antimutagenic activity on gamma-ray-induced reverse mutations in *E. coli* B/r WP2 *trp* (J. Radiat. Res. **22**, 297, 1981).

Male $B_6C_3F_1$ mice of 9 weeks old were purchased from Japan Charles River Co. Ltd. and kept in this laboratory for a week before use. Whole-body irradiation was carried out with 10 Gy of gamma-ray from a ^{137}Cs source at a dose-rate of 0.5 Gy/min. Within 40 minutes after irradiation, 0.3 m*l* or 0.6 m*l* of the placenta extract was injected i.p. into each mouse. For the control mice the corresponding amount of physiological saline was given. All the mice were housed in cages kept in an air-conditionned room

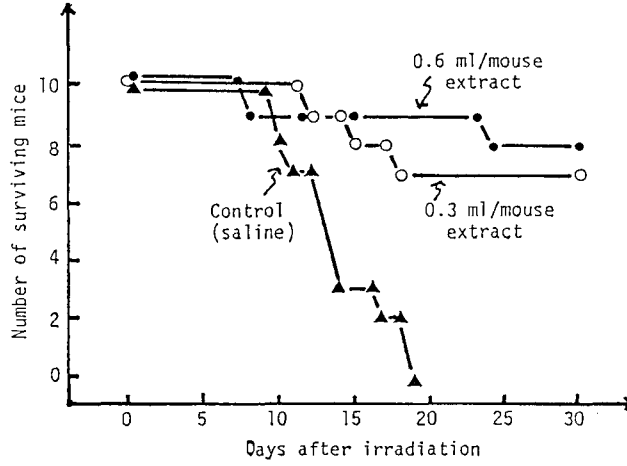

Fig. 1. Changes of survival in mice after irradiation with ^{137}Cs γ-rays.

at 22°C with nutritional chow (Oriental Yeast Co. Ltd.) and tap water *ad libitum*.

It was repeatedly found that more than 70% of irradiated mice with the placenta injection survived at least for 30 days. All the mice with saline injection usually died between 10th and 20th days after irradiation. Since promotion of DNA-repair by certain antimutagens was often found in irradiated bacteria, the hypothesis that a similar effect might be involved in the critical target tissue(s) is being examined to explain the present observation.

Effects of DBMA on the Gamma-ray Mutagenesis in Soybean

Taro FUJII

9,10-dimethyl-1,2-benz(a)anthracene (DMBA) is a well-known tumor initiator in mouse-skin carcinogenesis. Mutagenic activity of DMBA was investigated with a soybean test system. Dry seeds were treated at the concentrations of between 50 μg/ml to 2 mg/ml for 24 hrs in 4 experiments, and the mutation frequency was measured as the number of mutant spots on two simple leaves of heterozygous plants. No difference in the average number of spots per leaf was observed in the groups treated with water or different concentrations of DMBA. DMBA has no mutagenic activity in the present test system.

To see the effect of DMBA on the gamma-ray mutagenesis, combination treatment of drug and gamma-ray exposures was conducted. This experiment was divided into 2 parts; in Exp. A, seeds were treated first with gamma-rays and then with DMBA, in Exp. B, seeds were treated first with DMBA and then with gamma-rays. In Exp. A, the mutagenicity of gamma-rays was not affected by post-treatment of seeds with DMBA. The spotting frequencies of 2.26 and 2.45 per leaf obtained when irradiated seeds were post-treated with DMBA at 1 and 2 mg/ml, respectively, were similar to those obtained when irradiated seeds were post-treated with water (2.66 spots per leaf). On the other hand, interesting results were observed in Exp. B. Spotting frequency of 9.86 were obtained when water pre-soaked seeds were irradiated with 150 R. However, frequency of spots per leaf was much lower in DMBA pre-treatment lots than that of water pre-treatment lot. Although severe killing effect was observed at 2 mg/ml pre-treatment plot, the spotting frequencies of 5.69 and 3.83, respectively, were

observed when 1 and 2 mg/ml DMBA pre-treated weeds were irradiated with 150 R of gamma-rays. Namely, decrease in mutation frequency was noticed with DMBA pre-treatments, while most of chemical mutagens enhance the damage or the mutation frequency induced by radiations.

Absence of Mutagenic Activity of BP in the Soybean Mutation Test

Taro FUJII and Tadashi INOUE

Mutagenic effect of benzo(a)pyrene (BP) in higher plants was studied using the soybean test system. Concentrations of 5 to 400 μg/ml of BP were applied in the first experiment. There were no differences in the average number of spots per leaf in treatment with water or 4 different concentrations of BP treatments. However, some abnormal gorwth or deformed leaf development, without increase in germination ability, was observed at highest concentration treatment. To examine the BP action on presoaking seeds, combined treatments of water presoaking and BP were conducted (2nd experiment). Seeds presoaked for 2 to 6 hrs in water were immersed in 50 to 200 μg/ml of BP for 24 hrs. No increase of spotting frequency was noticed.

Activation by liver S9 mixture is necessary for BP to be mutagenic in bacterial mutation assay system. We therefore applied BP to dry seeds together with rat liver S9-mixture. Severe toxic effects of the S9-mixture were observed in this experiment. In spite of such a physiological damage by the rat liver S9-mixture, the number of mutant spots did not differ throughout all treatments. The results indicated that rat S9 is useless for the soybean test system. It has been reported that microsomes from Jerusalem artichoke tubes are capable of activating BP *in vitro*. We therefore examined whether the extract of Jerusalem artichoke is effective in our soybean mutation test. In these experiments, dry or presoaked seeds were treated with BP in the presence of plant S9-mixture, or seeds presoaked in plant S9-mixture were treated with BP in water. The average number of spots in each experiment was similar to those of respective control plot. Mutation induction by a mixture of BP with plant S9-mixture could not be detected either in treatments for dry or water-presoaked seeds, or BP to seeds presoaked with the S9-mixture.

BP is known to have strong carcinogenic and mutagenic activity after

metabolic activation with cytochrome P450 and NADPH. However, the present experiments indicated no mutagenic activity of BP irrespective of the various combination treatments, Some hidden action of BP may not be excluded because a concentration of 400 μg/ml to dry seeds induced some physiological damage, and when BP was mixed with rat liver S9-mixture, a decrease in germination ability was paralled to an increase in BP concentrations. We are currently investigating this observation in more detail to see if mutagenic activity is present in the chemical because BP is one of the environmentally significant substances having potential hazardous effect on living organisms, and contamination in food stuffs has been noticed. At present, our experiments demonstrated negative results.

VIII. POPULATION GENETICS (THEORETICAL)

Diffusion Model of Intergroup Selection, with Special Reference to Evolution of an Altruistic Character

Motoo KIMURA

Let us assume a diploid species consisting of an infinite number of competing demes, each having N_e reproducing members and in which mating is at random. Then consider a locus at which a pair of alleles A and A' are segregating, where A' is the "altruistic allele," which has selective disadvantage s' relative to A with respect to individual selection, but which is beneficial for a deme in competition with other demes; namely, a deme having A' with frequency x has the advantage $c(x-\bar{x})$ relative to the average deme, where c is a positive constant and $\bar{x}$ is the average of x over the species.

Let $\phi=\phi(x;t)$ be the distribution function of x among demes in the species at time t. Then, we have $\partial\phi/\partial t=L(\phi)+c(x-\bar{x})\phi$, where L is the Kolmogorov forward differential operator commonly used in population genetics [i.e., $L=(1/2)(\partial^2/\partial x^2)V_{\delta x}-(\partial/\partial x)M_{\delta x}$], and $M_{\delta x}$ and $V_{\delta x}$ stand for the mean and variance of the change in x per generation within demes. As to migration, assume Wright's island model and denote by m the migration rate per deme per generation. By investigating the steady state, in which mutation, migration, random drift, and intra- and interdeme selection balance each other, it is shown that the index $D=c/m-4N_es'$ serves as a good indicator for predicting which of the two forces (i.e., group selection or individual selection) prevails; if $D>0$, the altruistic allele predominates, but if $D<0$, it becomes rare and cannot be established in the species. For details, see Proc. Natl. Acad. Sci. USA **80**, 6317–6321.

Rare Variant Alleles in the Light of the Neutral Theory

Motoo KIMURA

Based on the neutral theory of molecular evolution and polymorphism, and particularly assuming "the model of infinite alleles," a method is proposed which enables us to estimate the fraction of selectively neutral alleles

(denoted by P_{neut}) among newly arisen mutations. It makes use of data on the distribution of rare variant alleles in large samples together with information on the average heterozygosity. The formula proposed is $P_{neut}=[\bar{H}_e/(1-\bar{H}_e)][\log_e(2\bar{n}q)/\bar{n}_a(x<q)]$, where $\bar{n}_a(x<q)$ is the average number of rare alleles per locus whose frequency, x, is less than q; $\bar{n}$ is the average sample size used to count rare alleles; $\bar{H}_e$ is the average heterozygosity per locus; and q is a small preassigned number such as $q=0.01$. The method was applied to observations on enzyme and other protein loci in plaice, humans (European and Amerindian), Japanese monkeys, and fruit flies. Estimates obtained for them range from 0.064 to 0.21 with the mean and standard error $P_{neut}=0.14\pm0.06$. It was pointed out that these estimates are consistent with the corresponding estimate $P_{neut}(\mathrm{Hb})=0.14$ obtained independently based on the neutral theory and using data on the evolutionary rate of nucleotide substitutions in globin pseudogenes together with those in the normal globins. For details, see Molecular Biology & Evolution **1**, 84–93.

Selective Constraint in Protein Polymorphism: Study of the Effectively Neutral Mutation Model by Using an Improved Pseudosampling Method

Motoo KIMURA and Naoyuki TAKAHATA

To investigate the pattern of allelic distribution in enzyme polymorphism, with special reference to the relationship between the mean ($\bar{H}$) and the variance (V_H) of heterozygosity, we used the model of effectively neutral mutations involving multiple alleles in which selective disadvantage of mutant alleles follows a Γ distribution. A simulation method was developed that enables us to study efficiently the process of random drift in a multiallelic genetic system and that saves a great deal of computer time. It is an improved version of the pseudosampling-variable (PSV) method [Kimura, M. (1980) Proc. Natl. Acad. Sic. USA **77**, 522–526] previously used to simulate random drift in a diallelic system. This method will be useful for simulating many models of population genetics that involve behavior of multiple alleles in a finite population. By using this method, it was shown that, as compared with the model of strictly neutral mutations, the present model gives the reduction of both $\bar{H}$ and V_H and an excess of rare variant alleles. The results were discussed in the light of recent observations on

protein polymorphism with special reference to the functional constraint of proteins involved. For details, see Proc. Natl. Acad. Sci. USA **80**, 1048–1052.

Theoretical Study on the Accumulation of Selfish DNA

Tomoko OHTA

The accumulation of selfish DNA in eukaryotic genomes was studied from the standpoint of population genetics. Selfish DNA is assumed to replicate itself within a haploid set. For the selectively neutral case, the fate of a single self-replicating DNA segment (unit) within a population was investigated by the method of the probability generating function, and by Monte Carlo simulation, with special reference to the probability of survival and average number of units per haploid set. For the selectively deleterious case at the organismal level, the equilibrium between new occurrence and selective elimination was studied, and the average and variance of the number of units per haploid set in the population was examined by Monte Carlo simulation. It is shown that the process of self-replication (duplication-deletion) plays an essential role for the maintenance and elimination of selfish DNA. For details, see Genet. Res., Camb. **41**, 1–15.

On the Evolution of Multigene Families

Tomoko OHTA

Multigene families are classified into three groups: small families as exemplified by hemoglobin genes of mammals; middlesize multigene families, by genes of mammalian histocompatibility antigens; and large multigene families, by variable region genes of immunoglobulins. Facts and theories on these evolving multigene families are reviewed, with special reference to the population genetics of their concerted evolution. It is shown that multigene families are evolving under continued occurrence of unequal (but homologous) crossing-over and gene conversion, and that mechanisms for maintaining genetic variability are totally different from the conventional models of population genetics. Thus, in view of widespread occurrence of multigene families in genomes of higher organisms, the evolutionary theory based mainly on change of gene frequency at each locus would appear to need considerable revision. For details, see Theor. Pop. Biol.

23, 216–240.

Time until Fixation of a Mutant Belonging to a Multigene Family

Tomoko OHTA

Time until fixation of a mutant that occurs in one copy of a multigene family was investigated from the standpoint of population genetics. Because of the complexity of the double process of random drift (on the chromosome and in the population), an approximate method based on the rate of steady decay of genetic variability is applied. The simple model of gene conversion with constant gene family size is also used. The expectation based on the approximate method is shown to be valid by extensive Monte Carlo simulations, and the results are useful for understanding the mechanisms of turnover of multigene families, when comparison is available between closely related species. For details, see Genet. Res., Camb. **41**, 47–55.

Population Genetics of Multigene Families that are Dispersed into Two or More Chromosomes

Tomoko OHTA and Gabriel A. DOVER

The evolution of multigene families whose members are dispersed into two or more nonhomologous chromosomes is studied from the standpoint of population genetics. By using a simple model of gene conversion, equilibrium and transient properties of the probability of identity of genes belonging to the family are investigated. Also, the time until fixation of a mutant belonging to a subdivided multigene family is studied by an approximation method. It is shown that dispersion of the family into two or more chromosomes has a relatively minor effect on the extent of identity between genes, unless the conversion rate between genes on nonhomologous chromosomes is extremely low, or unless the number of nonhomologous chromosomes on which gene members are dispersed is large. For details, see Proc. Natl. Acad. Sci. USA **80**, 4079–4083.

Gene Identity and Genetic Differentiation of Populations in the Finite Island Model

Naoyuki TAKAHATA

A formula for the variance of gene identity (homozygosity) was derived for the case of neutral mutations using diffusion approximations for the changes of gene frequencies in a subdivided population. It is shown that when gene flow is extremely small, the variance of gene identity for the entire population at equilibrium is smaller than that of the panmictic population with the same mean gene identity. On the other hand, although a large amount of gene flow makes a subdivided population equivalent to a panmictic population, there is an intermediate range of gene flow in which population subdivision can increase the variance. This increase results from the increased variance between colonies. In such a case, each colony has a predominant allele, but the predominant type may differ from colony to colony. The formula for obtaining the variance allows us to study such statistics as the coefficient of gene differentiation and the correlation of heterozygosity. Computer simulations were conducted to study the distribution of gene identity as well as to check the validity of the analytical formulas. Effects of selection were also studied by simulations. For details, see Genetics **104**, 497–512,

Evolutionary Dynamics of Extranuclear Genes

Naoyuki TAKAHATA and M. SLATKIN

We studied the evolutionary dynamics of extranuclear genes taking into account simple kinds of selection, two modes of inheritance and the multiplicity of genomes within a cell. Particular attention was paid to the accumulation of advantageous or deleterious mutations in an extranuclear genome. Within-generation drift due to multiplicity of genome and non-Mendelian segregation promotes the fixation of advantageous mutations and prevents deleterious mutations from accumulating. We show also that the extent of paternal contribution makes little difference in the rate, but, in contrast, the configuration of the genome and the mode of transmission both make a large difference. These results are compatible with what is known about extranuclear genomes. For details, see Genet. Res., Camb. **42**, 257–265.

Linkage Disequilibrium of Extranuclear Genes under Neutral Mutations and Random Genetic Drift

Naoyuki TAKAHATA

To evaluate the genetic constitution of extranuclear or non-Mendelian genes, several formulas for the variance of linkage disequilibrium (non-random association of linked genes) were derived under the neutral mutation hypothesis. Two kinds of the variances are mainly considered. One is the variance for the entire population and the other is the variance within a single germ cell. At equilibrium, both variances differ from that of a Mendelian population with the same parameters. In particular, when the cytoplasmic contribution from a male gamete β is small, as is found in most organisms, the effect of recombination between extranuclear genes is weak because of low within-cell heterogeneity. Then, the total variance for the entire population is large. Other important quantities determining the variances are the number of extranuclear genomes per cell, n, and the number of cell divisions in a germ cell line per generation, λ. Unless $\beta=0$, even a small contribution from a male gamete becomes significant depending on n and λ. For details, see Theoretical Population Biology **24**, 1–21.

Population Genetics of Extranuclear Genomes under the Neutral Mutation Hypothesis

Naoyuki TAKAHATA

Population genetics of extranuclear genomes is further developed under the neutral-mutation random-drift hypothesis, and the characteristic evolutionary aspects are summarized. Several formulae derived here are concerned with the variances of genetic variability (gene identity) at a single extranuclear locus and the evolutionary distance between two isolated populations which is estimated from a comparison of homologous linked nucleotide sites. Two types of variance are considered; one is the variance in the entire population (V_Q) and the other is the variance within a single germ cell (V_H). When compared with a Mendelian genetic system in a panmictic population, an extranuclear genetic system has the following equilibrium properties: (1) the mean genetic variability is low despite the high multiplicity of the genome in a cell if the proportion of the cytoplasmic

contribution from the male's gamete is small, (2) the effect of recombination is small and a large amount of variance of linkage disequilibrium tends to be maintained, (3) the overall relationship between the mean and variance of genetic variability does not much differ but $V_Q(V_H)$ is expected to be small if the paternal contribution is small, and (4) the evolutionary distance estimated depends on the extent of intrapopulational variation in a common ancestor population which in turn depends on within-cell variation. I argue that there is an analogy between the model of extranuclear genomes in a finite population and that of nuclear genes in a subdivided population. The analogy helps our understanding of some properties in an extranuclear genetic system. For details, see Genet. Res., Camb. **42**, 235–255.

A Quantitative Genetic Model of Reciprocal Altruism: A Condition for Kin or Group Selection to Prevail

Kenichi AOKI

A condition is derived for reciprocal altruism to evolve by kin or group selection. It is assumed that many additively acting genes of small effect and the environment determine the probability that an individual is a reciprocal altruist, as opposed to being unconditionally selfish. The particular form of reciprocal altruism considered is TIT FOR TAT, a strategy that involves being altruistic on the first encounter with another individual and doing whatever the other did on the previous encounter in subsequent encounters with the same individual. Encounters are restricted to individuals of the same generation belonging to the same kin or breeding group, but first encounters occur at random within that group. The number of individuals with which an individual interacts is assumed to be the same within any kin or breeding group. There are $1+\bar{i}$ expected encounters between two interacting individuals. On any encounter, it is assumed that an individual who behaves altruistically suffers a cost in personal fitness proportional to c while improving his partner's fitness by the same proportion of b. Then, the condition for kin or group selection to prevail is

$$r>\{(c/b)-[1-(c/b)]\bar{i}\bar{C}\}/\{1+[1-(c/b)]\bar{i}[\bar{C}+(T_b/V_b)]\}$$

if group size is sufficiently large and the group mean and the within-group genotypic variance of the trait value (i.e., the probability of being a TIT-FOR-TAT strategist) are uncorrelated. Here, $\bar{C}$, V_b, and T_b are the popula-

tion mean, between-group variance, and between-group third central moment of the trait value and r is the correlation between the additive genotypic values of interacting kin or of individuals within the same breeding group. The right-hand side of the above inequality is monotone decreasing in $\bar{C}$ if we hold T_b/V_b constant, and kin and group selection become superfluous beyond a certain threshold value of $\bar{C}$. The effect of finite group size is also considered in a kin-selection model. For details, see Proc. Natl. Acad. Sci. USA **80**, 4065–4068.

Stochastic Theory of Population Genetics

Takeo MARUYAMA

Stochastic models of population genetics are studied with special reference to the biological interest. Mathematical methods are described for treating some simple models and their modifications aimed at the problems of the molecular evolution. Unified theory for treating different quantities is extensively developed and applied to some typical problems of current interest in genetics. Mathematical methods for treating geographically structured populations are biven. Approximation formulae and their accuracy are discussed. Some criteria are given for a structured population to behave almost like a panmictic population of the same total size. Some quantities are shown to be independent of the geographical structure and their dynamics are described. Bulletin of Mathematical Blology **45**: 512–554.

Analyses of the Age of Genes and the First Arrival Times in a Finite Population

Takeo MARUYAMA

The age of a mutant gene is studied using the infinite allele model in which every mutant is new and selectively neutral. Based on a time reversal theory of Markov processes, we develop a method of mathematical analysis that is considerably simpler for calculating the various statistics of the age than previous methods. Formulas for the mean and variance and for the distribution of age are presented together with some examples of relevance to cases in natural populations.—Theoretical studies of the first arrival time of an allele to a specified frequency, given an initially monomorphic condi-

tion of the locus, are presented. It is shown that, beginning with an allele that has frequency $p=1$ or an allele with frequency $p=1/2N$, there is an initial lag phase in which there is virtually no chance of an allele with a specified intermediate frequency appearing in the population. The distribution of the first arrival time is also presented. The distribution shows several characteristics that are not immediately obvious from a consideration of only the mean and variance of first arrival time. Especially noteworthy is the existence of a very long tail to the distribution. We have also studied the distribution of the age of an allele in the population. Again, the distribution of this measure is shown to be more informative for several questions than are the mean and variance alone. Genetics **105**: 1041–1059.

Models of Evolution of Reproductive Isolation

Takeo MARUYAMA

Mathematical models are presented for the evolution of postmating and premating reproductive isolation. In the case of postmating isolation it is assumed that hybrid sterility or inviability is caused by incompatibility of alleles at one or two loci, and evolution of reproductive isolation occurs by random fixation of different incompatibility alleles in different populations. Mutations are assumed to occur following either the stepwise mulation model or the infinite-allele model. Computer simulations by using Itô's stochastic differential equations have shown thRt in the model used the reproductive isolation mechanism evolves faster in small populations than in large populations when the mutation rate remains the same. In populations of a given size il evolves faster when the number of loci involved is large than when this is small. In general, however, evolution of isolation mechanisms is a very slow process, and it would take thousands to millions of generations if the mutation rate is of the order of 10^{-5} per generation. Since gene substitution occurs as a stochastic process, the time required for the establishment of reproductive isolation has a large variance. Although the average time of evolution of isolation mechanisms is very long, substitution of incompatibility genes in a population occurs rather quickly once it starts. The intrapopulational fertility or viability is always very high. In the model of premating isolation it is assumed that mating preference or compatibility is determined by male- and femaic-limited characters, each of which is controlled by a single locus with multiple alleles, and

mating occurs only when the male and female characters are compatible with each other. Computer simulations have shown that the dynamics of premating isolation mechanism is very similar to that of postmating isolation mechanism, and the mean and variance of the time required for establishment of premating isolation are very large. Theoretical predictions obtained from the present study about the speed of evolution of reproductive isolation are consistent with empirical data available from vertebrete organisms. Genetics **103**: 557–579.

An Approach to Population and Evolutionary Genetic Theory for Genes in Mitochondria and Chloroplasts, and Some Results

Takeo MARUYAMA

We developed population genetic theory for organelle genes, using an infinite alleles model appropriate for molecular genetic data, and considering the effects of mutation and random drift on the frequencies of selectively neutral alleles. The effects of maternal inheritance and vegetative segregation of organelle genes are dealt with by defining new effective gene numbers, and substituting these for $2N_e$ in classical theory of nuclear genes for diploid organisms. We define three different effective gene numbers. The most general is N_i, defined as a function of population size, number of organelle genomes per cell, and proportions of genes contributed by male and female gametes to the zygote. In many organisms, vegetative segregation of organelle genomes and intracellular random drift of organelle gene frequencies combine to produce a predominance of homoplasmic cells within individuals in the population. Then, the effective number of organelle genes is N_{eo}, a simple function of the numbers of males and females and of the maternal and paternal contributions to the zygote. Finally, when the paternal contribution is very small, N_{eo} is closely approximated by the number of females, N_f. Then if the sex ratio is 1, the mean time to fixation or loss of new mutations is approximately two times longer for nuclear genes than for organelle genes, and gene diversity is approximately four times greater. The difference between nuclear and organelle genes disappears or is reversed in animals in which males have large harems. The differences between nuclear and organelle gene behavior caused by maternal inheritance and vegetative segregation are generally small and may be

overshadowed by differences in mutation rates to neutral alleles. For monoecious organisms, the effective number of organelle genes is approximately equal to the total population size N. We also show that a population can be effectively subdivided for organelle genes at migration rates which result in panmixis for nuclear genes, especially if males migrate more than females. Genetics **103**: 513–527.

A Mathematical Model of Codon Substitution

Takashi GOJOBORI

A mathematical model for codon substitution is considered, taking into account unequal mutation rates among different mucleotides and selective constraint against amino acid changes. This model is constructed by using a 61×61 transition probability martix for the 61 nonterminating condons. Under this model, a computer simulation is conducted to study the numbers of silent (synonymous) and amino acid-altering (nonsynonymous) mucleotide substitutions when the underlying mutation rates among the four kinds of nucleotides are not equal. It is assumed that the substitution rates are constant over evolutionary time and the codon frequencies are at equilibrium. Thus, the numbers of synonymous and nonsynonymous substitutions both increase linearly with evolutionary time. It is shown that, when the mutation rates are not equal, the estimate of synonymous substitutions obtained by F. PERLER *et al*'s (Cell 1980) "Percent Corrected Divergence" method increases nonlinearly, although the true number of synonymous substitutions increases linearly. It is, therefore, possible that the "saturation" of synonymous substitutions observed by PERLER *et al.* is due to the inefficiency of their method in detecting all synonymous substitutions. For details, see Genetics **105**: 1011–1027.

IX. HUMAN GENETICS AND HUMAN CYTOGENETICS

Modern Medical Practice Versus Environmental Mutagens: Their Possible Dysgenic Impact

Ei MATSUNAGA

We do not know how many mutations are being produced in human populations by exposure to environmental mutagens. If these mutagens caused a persistent rise in mutation rates, then ultimately there would be a proportional increase in the frequency of a variety of genetic diseases, including those that are difficult to treat and that require life-long care of affected individuals. In contrast, modern medical practices are relaxing selection pressure selectively with respect to disease, leading to a gradual increase in the frequency of certain genetic and partly genetic diseases that can be effectively treated. The pattern of this increase would differ from condition to condition, depending upon the mode of inheritance and the extent to which selection is relaxed; except for some special cases, the anticipated increase would generally be slow. Additional economic burdens on future society and families imposed by relaxed selection would mainly involve expenditures for relatively inexpensive treatments, and not those for expensive life-long care. Moreover, individuals treated successfully can be expected to contribute productively to society. With education and counseling for those who survive serious dominant and X-linked disorders, and with the development of accurate, inexpensive prenatal diagnosis, the presumed dysgenic effects of relaxed selection could be balanced. For details, see Mutation Res. **114**: 449–457, 1983.

Retinoblastoma and ABO Blood Groups

Ei MATSUNAGA and Kensei MINODA[1)]

Data for the ABO blood groups were obtained from records of 193 children with bilateral retinoblastoma and 346 with sporadic unilateral cases. They were ascertained by a nationwide survey from 1975 to 1981, supported by the Children's Cancer Association of Japan. As controls, data

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by Fujita *et al.* (Jpn. J. Hum. Genet. **23**: 63, 1978) were utilized. The blood group distribution in either group of the patients was essentially the same as the control. Furthermore, comparison was made of the patients with early onset, who could be regarded as the most susceptible, with the general population. The blood group distribution in those patients was also close to the control. Thus, we conclude that the ABO blood groups are not involved in the inherited host susceptibility to the development of retinoblastoma. For details, see Hum. Genet. **63**: 87, 1983.

Origin and Structure of a Minute Chromosome in Patients with Congenital Malformations

Yasuo NAKAGOME and Yutaka NAKAHORI

It is not unusual to find a minute chromosome of unknown origin during the course of a cytogenetic survey on patients with mental retardation and/or congenital malformations. Because of its small size, the origin and structure of it is usually not identifiable by the standard banding techniques i.e., G, C or Q. Even the high resolution banding is often useless in the identification of it.

In the present study, a total of 12 such patients were studied by some of the new banding techniques in addition to the standard techniques. The distamycin-DAPI (4'-6-diamidino-2-phenylindole) dual staining technique (Schweizer *et al.* 1978) followed by the destaining and DAPI staining proved itself to be the most useful of all, so far tested.

Table 1. Cases with a Minute Chromosome

47, XX, +i (15p)	2584, 2587*
47, XY, +del (15) (q21.2q26.3)	2667
47, XX, +psu dic (15) (q12 or 13)	2608, 2493
46, XX, −14, −15, +t(14q15q), +psu dic (15) (q12 or 13)	2618
45, X/46, X, +psu dic (13) (q13)/47, X, +psu dic (13), +psu dic (13)/47, X, del (Y) (q11), +psu dic (13)	2646
46, X, i (Yp)	2590
46, X, del (Y) (q11)	2611
46, X, minute ring	2498
47, XY, +psu dic (14) or (21)	2630
45, X/46, X, +min	2300

* mother of 2584.

In 6 cases, the minute chromosome was present as an extra chromosome (47, XX or XY plus a minute), in the 3 it replaced one of the normal complement, two cases showed mosaicism and a case had both Robertsonian translocation and, to our surprise, an extra psu dic chromosome. In 9 cases, the origin and structure of the minute chromosome were determined (Table 1). In the two cases of i(15p) and two cases involving Y, phenotypic abnormality of the propositus presumably had notheing to do with the minute chromosome. The former, in fact, included phenotypically normal mother of the propositus. In other 5 cases, each minute chromosome involved euchromatic segment of an autosome and thus would have been responsible for phenotypic abnormalities.

Cloning and Characterization of a Highly Y-specific 3.5 Kb Repeated Fragment

Yutaka NAKAHORI and Yasuo NAKAGOME

We attempted to obtain a highly Y-specific DNA probe, presumablly consisting of repeated sequences rather than a unique sequence. The former is expected to be a very sensitive probe in the *in situ* hybridization-study in addition to the standard Southern blot analysis.

The male specific repeated sequence was first reported by Cooke as a 3.4 Kb *Hae*III fragment. Though there have been described multiple families of repeated sequences on the human Y chromosome, this represents the largest fraction of all and constitutes about 40% of the total Y chromosome DNA.

It was presumed that the fragment has a 3.4 Kb interval for both *Hae*III sites and *Eco*RI sites. *Eco*RI-digested human male DNA was electrophoresed on polyacrylamide gel and a segment corresponding to the 3.4 Kb band (male-band) was cut out. DNA fragments were eluted from it and cloned in the *Eco*RI site of the plasmid pBR325. Clones were selected by the colony hybridization technique using the nick translated male-band DNA as a probe. Three independent clones proved to be highly male specific in the Southern-blot analysis each being 3.5 Kb in length and gave an identical restriction pattern. They were mainly constructed of the 5 bp repeating unit (TTCCA) and its related sequences.

Mitochondrial DNA Polymorphism in Japanese

Satoshi HORAI and Ei MATSUNAGA

The mitochondrial genome of human is a small (16.5 Kb), naked, circular, supercoiled, maternally inherited DNA. Since Potter *et al.* (1975 Proc. Natl. Acad Sci. USA **72**: 4496) showed that a restriction enzyme can detect variability in mammalian mitochondrial DNA (mtDNA) sequences, restriction enzyme analysis has become a powerful tool for detecting genetic variation in humans (Brown and Goodman, 1979 Extrachromosonal DNA: 485; Brown, 1980 Proc. Natl. Acad. Sci. USA 77: 3605). In the present study an attempt was made to obtain enough amount of purified mtDNA from human placenta in order to examine mtDNA polymorphism with a large number of restriction enzymes without using Southern blotting analysis and end labeling method. A total of 120 placentas were obtained from Japanese and closed circular mtDNA were prepared. Recoveries of closed circular form of mtDNA ranged from 200 to 200 μg per individual placenta. Preliminary studies with 8 restriction enzymes that recognize six base pairs showed that 5 enzymes (*Eco*RI, *Hind*III, *Pst*I, *Hinc*II and *Xho*I) were polymorphic, while 3 enzymes (*Bam*HI, *Kpn*I and *Xba*I) were monomorphic. Particularly, in the digestion with *Hinc*II, seven distinct cleavage patterns were observed.

Differential Enzyme Activities in Human Esterase D Phenotypes

Satoshi HORAI and Ei MATSUNAGA

Recently, studies of families with hereditary retinoblastoma demonstrated close linkage of the gene for this tumor with the ESD locus (Sparkes *et al.* 1983 Science 208: 971). Benedict *et al.* (1983 Science **219**: 973) reported a patient with retinoblastoma who had 50% ESD activity in all normal cells but no deletion of 13q14 at 550 band level, indicating that the smallest constitutional chromosomal deletion within 13q14 is associated with susceptibility to retinoblastoma. This suggests that examination of ESD activity is essential for the detection of a minute constitutional deletion or inactivation of DNA segment involving the retinoblastoma locus. For this purpose, however, it is necessary to know about the range of quantitative variation in the enzyme activity among normal individuals. In the present study, the ESD activity and phenotype were determined in blood from 200

unrelated individuals. The distribution of different ESD phenotype is as follows; ESD-1 (39%), ESD2-1 (47%) and ESD-2 (14%). The gene frequencies of *ESD**1 and *ESD**2 are estimated at 0.625 and 0.375 respectively, which are not different from those of other Japanese populations in earlier reports. The mean enzyme activities of three phenotypes were estimated at 276.7 (ESD-1), 216.6 (ESD2-1) and 171.5 (ESD-2) expressed as 10^{-7} M methylumbelliferone produced/h/gHb, respectively. The activities associated with a single *ESD**1 gene are estimated to be 60% higher than *ESD**2.

Establishment of a Human Genomic Library with MboI-Digested DNA

Yasuo NAKAGOME, Yutaka NAKAHORI and Jun KUSUDA

It is attempted to establish a human genomic library from which unique DNA probes for RFLP (restriction fragment length polymorphism) studies can be obtained. Lambda phage Charon 30 was chosen as it can take a passenger DNA fragment up to 19.10 kb long into its *Bam*HI sites. DNA was isolated from leukocytes of the senior author and partially digested with a restriction enzyme *Mbo*I. Its recognition site is ↓GATC and a fragment generated with it can be inserted into a *Bam*HI site with G↓GATCC sequence. After the insertion, *Bam*HI sites may not be preserved, however, the passenger can be cut out with *Hpa*I together with attached small pieces of lambda DNA on both ends (470 bp from the left arm and 770 bp from the right arm).

So far, about 300,000 recombinant phages have been obtained. Preparation of additional 700,000 clones are in the progress.

X. BEHAVIORAL GENETICS

The Behavioral Changes in Mice Continuously Reared under Noise Conditions

Tohru FUJISHIMA

Mice continuously reared under noise conditions have been examined for the behavioral changes in comparison with those reared under normal, or non-noise conditions. The mice of the two groups were originally derived from the same full sib families. It has been found previously that the mice having been successively reared under noise conditions have come to show a chronic and non-recovering emotionality. It was also suggested in the previous experiment that noise might have an influence on the mice at least during the prenatal period. To examine the validity of this suggestion, a portion of the pregnant mice of the non-noise group was moved to the noise conditions and returned to the non-noise conditions at the first day of birth. The result showed that the adult mice having experienced noise conditions during their prenatal period expressed a high emotionality. These experiments revealed that noise would have an influence upon the mice during the prenatal period and arouse their non-recovering emotionality.

XI. ECOLOGICAL GENETICS

Observations on Wild and Cultivated Rices in Thailand for Ecological-Genetic Study

Hiroko MORISHIMA, Yoshio SANO, Yo-Ichiro SATO
and Yoshiya SHIMAMOTO*

We stayed in Thailand from November 21 to December 24, 1983, travelling in the North, North-East, South and Central Plain. The objective of the present tour was firstly to study population dynamics of wild rice, *Oryza perennis*, and secondly to collect information and seed materials of Thai native rice cultivars for understanding their phylogenetic status. For these purposes, we made general survey of 131 wild and 92 cultivated rice sites, and then permanent observation-sites were established for demographic study. In each site, various items of habitat conditions (biotic and abiotic) and characteristics of rice plants were recorded.

Wild rice: Both perennial and annual types were widely distributed all over the country. Their habitats, however, precisely segregated according to water regimes conditioned by macro- and micro-topography. Introgression between wild and cultivated rices was prevailing particularly in the direct-seeded fields. Hybrid derivatives were frequently observed in various habitats such as in and at the fringe of deep-water rice fields, marshy waste land, roadside ditch, forming hybrid swarms or uniform weedy populations depending on their breeding system.

Demographic study: Seven permanent study-sites were chosen in the suburb of Bangkok. Those sites were inhabited by perennial, annual and intermediate or weedy type of wild rice, and had contrasting environmental conditions. In the permanent quadrats established in respective site, detailed records were taken in addition to general observation. Demography and population flux will be monitored in these sites.

Cultivated rice: Rice varieties grown in Thailand show a wide variation in adaptability to water condition (deepwater-lowland-upland) as well as in photoperiodic response. Further, it is known that Japonica type varieties exist in the northern hilly areas. We found in the present trip that upland

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varieties grown in the South where upland fields are located less than 200–300 m above sea level contained a high proportion of Japonica-like varieties. This suggests that existence of Japonica type in tropics is not necessarily due to its adaptability to highland as often argued, but due to adaptability to upland condition.

Seed samples of 363 wild and 145 cultivated strains were collected for future genetic study.

Invasion of *Drosophila simulans* into Inland Area and the Effect of Winter Temperature on its Frequency

Takao K. WATANABE and Yutaka INOUE

A nine year survey of *D. simulans* population around the area of Mt. Fuji revealed that the species gradually invaded inland from the Pacific coast area since 1975. The frequencies of 12 autumn populations are presented by the percent of *D. simulans* among *D. melanogaster* and *D.*

Table 1. Percents of D. simulans among D. melanogaster and D. simulans in autumn populations around Mt. Fuji and average temperatures of winter and summer in Mishima

Bite	Year								
	1975	1976	1977	1978	1979	1980	1981	1982	1983
Mishima	95	95	95	99	94	92	93	85	96
Susono	76	44	19	57	35	43	46	72	77
Gotenba	50	50	2	25	55	56	15	21	75
Fujiyoshida	0	0	0	17	56	50	0	14	33
Otsuki	0	0.5	0.4	2	41	70	1	19	26
Katsunuma	0	0	0	0.1	0.5	7	0	0.5	0
Kofu	0	0	3	2	18	0	1	4	0.6
Nirasaki	0	0	0	0	1	0	0.2	3	0.5
Kajikazawa	0	0	1	2	12	8	1	9	3
Minobu	0	0	1	7	35	62	7	17	13
Tomizawa	39	0	4	33	52	83	11	51	22
Fujinomiya	99	99	92	97	75	78	97	82	90
Mean	29.9	24.0	18.4	28.4	38.0	45.8	22.7	31.6	36.3
Winter*	5.6	6.0	5.1	6.1	7.2	6.6	4.9	5.6	6.3
Summer*	24.3	23.5	23.9	25.7	24.7	23.8	24.2	23.1	23.9

* Average temperatures (°C) of winter (Dec.-Feb.) and summer (June.-Aug) in Mishima.

simulans (Table 1). *D. simulans* was not found in the inland area in 1975. But it was found in every collection site in 1979. Thereafter the species seemed to be colonized there with some temporal fluctuations of its size. However, a remarkable reduction of the *D. simulans* frequency was observed in 1981 in every collection site. In order to correlate this phenomenon with some environmental factors, we compared the summer and winter temperatures preceding collection of flies with the frequency of *D. simulans*. The summer temperature did not correlate with the mean frequency of *D. simulans* but the winter temperature did significantly correlate with ti. Cold winters decreased the size of *D. simulans* population relatively.

Biochemical Phylogeny of the *Drosophila montium* Subgroup

Seido OHNISHI and Takao K. WATANABE

Phylogenetic relationships among twenty nine species in the *D. montium* subgroup were investigated by two electrophoretic techniques (2DE and SGE). Based on data of genetic distances estimated by protein differences detected by 2DE, a dendrogram was constructed (Fig. 1). In addition, species specific allozymes (diagnostic alleles) were surveyed by SGE in order to find a method to distinguish species easily. It was found that six enzymes (*Aldox*, *G6pdh*, *Men*, *6Pgdh*, *Idh* and *Est-6*) are helpful for systematics of this subgroup. For instance, if we run two enzymes of *Aldox* and *G6pdh* in a starch gel, we can distinguish 26 of 29 species. And if we add two enzymes (*Men* and *Est-6*) we can classify all 29 species by the four enzyme systems.

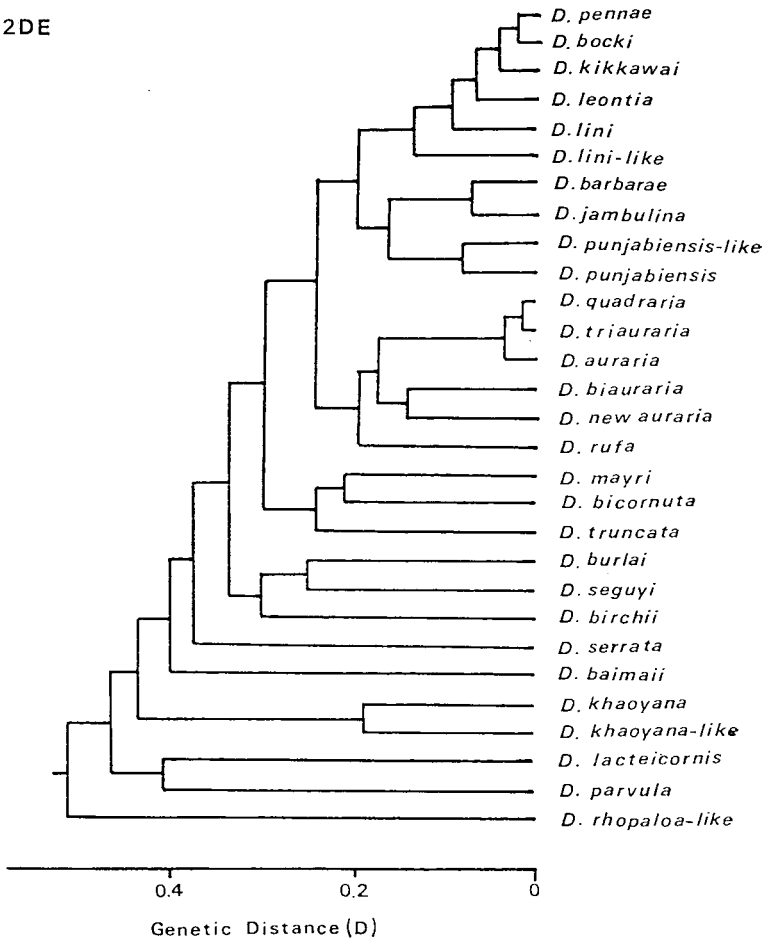

Fig. 1. A dendrogram of 29 species of the *D. montium* subgroup.

XII. APPLIED GENETICS

Correlated Selection Responses in Characters and Allozymes of Rice

Hiroko MORISHIMA

Three hybrid populations of rice, one between wild-annual and wild-perennial type (Cross I) and the other two between cultivated and wild type (Cross II, III), were propagated in bulk. In F_3, the seeds were collected by two different methods; one from naturally shed seeds on the ground and the other from the seeds remaining on the panicles. The same procedure was repeated in each group until F_6. This resulted in selection for early (shed-seed group) and late (non-shed seed group) flowering groups in Cross I, while selection for shedding and non-shedding groups resulted in Cross II and III. In F_3 and F_6, various metric characters, coloration of plant organs and allelic frequencies at two isozyme loci, *Pox-1* and *Acp-1*, were examined.

In F_3, we failed to find out significant correlations between characters and allozymes, except for a loose association of flowering time with *Pox-1* in Cross I. In F_6, some correlated responses to selection and associations between characters and allozymes were detected as follows. In Cross I (peren.$\times$ann.), the early flowering group had shorter awns, greater values of seed productivity components, higher reproductive allocation, lower regenerating ability, shorter anthers than the late flowering group. This pattern of character association is consistent with that found between the perennial and annual types of wild rice except for few traits. In Crosses II and III (cult.$\times$wild), shedding group consistently showed longer awns and more pronounced seed dormancy than the non-shedding group. Further, plants with white grain and those with straw-color hull increased during F_3 to F_6 in non-shedding group (characteristics of cultivated type), but tended to decrease in shedding group.

The parents of each cross carried different alleles at *Pox-1* and *Acp-1*. In Crosses I and III in which 2A and 4A alleles at *Pox-1* segregated, the frequency of 2A generally increased during F_3 to F_6 except in the late-selected group in Cross I. The plants homozygous for 2A/2A and 4A/4A

were compared in each cross regarding various character measurements. Generally, 2A/2A plants had greater values of seed productivity components, higher reproductive allocation, shorter anthers, less pronounced seed dormancy, and in Cross I they flowered earlier than 4A/4A plants. This is consistent with the trend of character association found between the wild perennial and annual types or between the wild and cultivated types. At the *Acp-1* locus, however, no such consistent trend was detected.

From these observations, the following points can be suggested: (1) There could be a key factor governing seed productivity which is located on a chromosome segment marked by *Pox-1*. (2) One of major genes controlling flowering time differeing between the wild perennial and annual types is linked also with *Pox-1*. (3) Seed shedding is partly correlated with awn length and seed dormancy, developmentally or by linkage, but seems to be independent of seed productivity factor. A gene or genes controlling seed shedding is linked with *Rc*/*rc* locus for grain color as well as with one of the three complementary genes for hull color.

Non-random association found among many adaptive traits and gene loci can be attributed to various factors. The present experiment suggests existence of two independent adaptive gene blocks, noe loading linked genes for flowering time and seed productivity, and the other loading linked genes for seed shedding, and grain and hull color. Selection and fixation of the combination of these two blocks could have brought about effective and constant seed productivity which meets the basic requirement for the cultivated type.

The Genetic Basis of Hybrid Chlorosis Found in a Cross between Two Japanese Rice Cultivars

Yo-Ichiro SATO and Seiji MATSUURA*

We incidentally found a case of hybrid chlorosis in the F_2 population of a cross between two Japanese native rice cultivars, J-147 and J-321. Its first symptom was a change in color of the third or fourth leaf-blade to yellowish. The chlorotic plants died within 30 days after germination, hence no progeny seed was obtainable from them.

The F_2 population segregated into 849 normal and 63 chlorotic plants,

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giving a good fitness to the 15: 1 ratio. F_3 strains showing 1: 0, 3: 1 and 15: 1 ratios were 72, 35 and 37, respectively. The F_3 ratio fitted 7: 4: 4, which was expected by assuming two independent recessive genes. The data thus indicated that there was a set of duplicate genes independent of each other whose double-recessive combination causes chlorosis. They were symboled *ch-1-a* and *ch-1-b*, tentatively.

Sato and Hayashi (1983) reported the presence of a set of complementary lethal genes, *L-2-a* and *L-2-b* causing F_1 weakness, and that the distribution of *L-2-a* was suggestive of the phylogenetic relationship among varietal groups and the mode of dissemination of rice varieties. The examination of distribution of the hybrid chlorosis genes is under way, and it may also be useful for phylogenetic study.

Chromosomal Location of *Pgi-2* Locus in Rice

Reiko SANO and Hiroko MORISHIMA

Forty isozyme loci of 14 enzyme species were so far proposed in wild and cultivated rice (Second 1982), but formal genic analysis and linkage studies were carried out for very limited loci. In the present study, chromosomal mapping of the isozyme loci differing in allelic frequencies between the Indica and Japonica types of rice was attempted. Linkage relationships of six isozyme loci (*Cat-1*, *Acp-1*, *Pox-2*, *Pgi-1*, *Pgi-2*, *Est-2*) and eight marker genes covering 5 chromosomes (*Rc*, *wx*, d_1, *g*, *gl*, *lg*, *la ph*) were examined using the F_2 and B_1F_1 plants derived from several crosses by starch gel electrophoresis. *Pgi-2* was found to be located on chromosome 6 as it was linked with *wx* and *Est-2* with recombination values of 35% and 13%, respectively (*wx—Est-2—Pgi-2*). Linkage between *Acp-1* and *Pox-2* which was already reported was reconfirmed in the present study. Four loci other than *Pgi-2* and *Est-2* were found to be independent of the all marker genes used. Our previous statement that *Pgi-2* and *wx* was independent (Ann. Rep. **31**: 117–118) was not correct.

Differential Regulation of waxy Gene Expression in Rice Endosperm

Yoshio SANO

In order to examine the effects of different alleles on the gene expression

at the waxy locus, the *Wx* gene product wihch controls the synthesis of amylose was isolated from endosperm starch of rice plants and analysed by electrophoretic techniques. The major protein bound to starch granules was absent in most of waxy strains and increased with the number of *Wx* alleles in triploid endosperms, suggesting that the major protein is the *Wx* gene product. In addition to *wx* alleles which result in the absence or drastic reduction of the *Wx* gene product and amylose, differentiation of *Wx* alleles seemed to have occurred among nonwaxy rice strains. At least two *Wx* alleles with different efficiencies in the production of the major protein as well as amylose were detected. This work was published in Theor. Appl. Genet. **68**, 467–374 (1984)

Temperature Effects on the Wx Protein Level and Amylose Content in Rice Endosperm

Yoshio SANO, Masahiko MAEKAWA and Harumi KIKUCHI

It is well known that the *waxy* locus controls the synthesis of amylose as endosperm starch of *wx* homozygotes almost lacks amylose. In maize, the *Wx* gene specifies a starch-bound glycosyltransferase which is responsible for the production of amylose in the endosperm and pollen. On the other hand, amylose content in rice endosperm is considered to be one of the major determinants of eating qualities. Since lower temperature during grain development increases the amylose content in Japanese cultivars, great efforts for decreasing amylose content have been made to improve the grain quality of local rice cultivars in the northern part of Japan. Recently, it was reported in rice that one, two and three doses of *Wx* alleles in the endosperm showed a progressive increase in the level of the gene product called *Wx* protein as well as in amylose content and that the two alleles of Wx^a and Wx^b control not only the quantitative level of *Wx* protein but also amylose content. Thus, amylose content in rice endosperm seems to be partly controlled by the *Wx* protein level. The objective of this study was to investigate whether low amylose content observed in a low-amylose mutant and in plants grown under low temperature is due to the reduced level of *Wx* protein or not. Two rice strains, Shiokari and its low-amylose mutant, were compared with regard to the levels of *Wx* protein and amylose under different temperature regemes (Table 1). The mutant showed the reduced level of *Wx* protein bound to starch granules compared to Shiokari,

Table 1. Amylose content and the relative amount of *Wx* protein bound to starch granules in the two rice strains, Shiokari and SM-1

Location	Strain	Heading data	Amylose content (%)	Relative amount of *Wx* protein	Mean temperature for 20 days after heading (°C)
Paddy field	Shiokari	August 18	25.4	100	21.1
	SM-1	August 21	17.8	52	21.8
Glasshouse	Shiokari	July 31	16.0	43	26.8
	SM-1	July 26	9.5	18	25.5

showing that the gene carried by the mutant regulates the *Wx* protein level as well as amylose content. In addition, lower temperature during grain development increased the amount of *Wx* protein as well as amylose, indicating that the genic expression at the *wx* locus is influenced by temperature. The present study suggests that the *Wx* protein level is controlled by a *trans*-acting regulatory gene as well as temperature and that amylose content decreases as the *Wx* protein level decreases, so far examined.

(J. Hered. in press)

Genetic Variation of Nitrogen Fixing Activity in the Progeny Population of Rice Hybrid

Shin-ya IYAMA and Theo J. L. van HINTUM

The cross between two rice strains of different nitrogen fixing activity was made in order to investigate the hereditary nature of the character in a progeny population. From F_2 to F_5 the population was maintained by self-pollination with single seed decent method. About 100 F_6 lines were derived from the F_5 population in 1982. Ten plants per line were grown individually in a small plastic pot with 180 g of soil and their nitrogen fixing activity was measured by acetylene reduction method with gas-chromatography. Acetylene reducing activity (ARA) was assayed one day after the heading of each plant. ARA of the lines was expressed by C_2H_4 produced (nmol)/hour/g dry root. Wide variation among lines was found, ranging from 99 to 732 nmol, the average being 326 nmol. The variation among lines was statistically highly significant, and some lines exceeded the parental range in both directions.

In 1983, selfed F_7 progeny lines obtained from each of the F_6 lines were grown and they were examined for estimating heritability of the nitrogen

Table 1. Realized heritability of ARA of rice estimated from the selection for high and low ARA in the F_6 and the genetic gain in the F_7 of T65×C5444

Generation	No. of selected lines	Means of High ($\bar{H}$)	Means of Low ($\bar{L}$)	$\bar{H}-\bar{L}$	Heritability
F_6	10	551.6	185.2	368.4 (3.43)+	—
	20	483.3	210.6	272.7 (2.54)	—
F_7	10	1166.8	611.8	555.0***(1.71)	0.50
	20	1076.1	627.6	448.0***(1.38)	0.54

+) Standardized values in the parentheses.
*** Significantly different from zero at the 0.1% level.

fixing activity. As observed in the F_6, wide variation among F_7 lines was found but the activity in 1983 was generally higher compared with that of the previous year. The variation ranged from 253 to 1584 nmol, the average being 867 nmol. Ten or 20 lines each were selected from the F_6 population in both high and low directions, respectively, and means of the selected F_7 progeny groups were compared. *t*-test showed that the difference between the two groups selected to high and low directions was highly significant, indicating that the selection was effective. Heritability value was estimated by the ratio of the realized gain in the F_7 to the selection differential in the F_6. Since the seasonal variation of ARA was evident, those measurements were standardized by the standard deviation of the F_6 and F_7 populations, respectively, before being used for calculation. Realized heritability or repeatability was estimated as around 0.5 (Table 1), indicating that the selection based on the line means from the progeny population is considerably effective. This estimation was in good accord with the correlation coefficient between the F_6 in 1982 and the F_7 in 1983 calculated based on the whole lines used (r=0.47).

It was concluded from the above results that (1) ARA in the rhizosphere of rice plant is heritable and controlled by rice genotype. (2) Genes controlling ARA in the parents segregate among progenies through crossing of two different genotypes and some progeny genotypes may exceed parental performance through recombination. (3) One may improve ARA of rice by ordinary breeding procedure of hybridization and selection.

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ABSTRACTS OF DIARY FOR 1983

January	24	283rd Meeting of Misima Geneticists' Club
	27	284th Meeting of Misima Geneticists' Club
March	9	285th Meeting of Misima Geneticists' Club
	17	201st Biological Symposium
April	1	202nd Biological Symposium
	28	286th Meeting of Misima Geneticists' Club
June	8	203rd Biological Symposium
July	11	204th Biological Symposium
	19	287th Meeting of Misima Geneticists' Club
August	24	205th Biological Symposium
	30	206th Biological Symposium
September	9	207th Biological Symposium
October	1	208th Biological Symposium
	13	209th Biological Symposium
	20	210th Biological Symposium
	25	211th Biological Symposium
November	17	212th Biological Symposium
	28	213th Biological Symposium
	29	288th Meeting of Misima Geneticists' Club
December	5	214th Biological Symposium

FOREIGN VISITORS IN 1983

Jul. 29, '82–Jul. 28, '83		Huang, J.-T., Institute of Sericultural Research, China.
January	26	Jefferson, Roland M., U. S. National Arboretum, U.S.A.
February	7–Mar.31	Cai, Y.-M., Institute of Sericultural Research, China
	12–Mar. 13	Sueoka, N., University of Colorado, U.S.A.
March	20–21	Weil, T. H., Institut de Biologie Moleculaire et Cellulaire, France.
April	1	Manna, G. K., Kalyani University, India
	24–26	Ullmann, A., Institut Pasteur, France
May	22–May 21, '84	Yoo, I.-D., Institute of Agricultural Sciences, Korea
June	8	Oakberg, Eugene F., Oak Ridge National Laboratory, U.S.A.
	8	Shaw, William P., Kettering Research Laboratory, U.S.A.
	8	Nazerali, Aly-Raza, The United Nations University, Tokyo.
	8	Kokke, Robert, The United Nations University, Tokyo.
	8	Soyasa, Chandra H., The United Nations University, Tokyo.
	15	Bengt, O. B., University of Lund, Sweden.
August	6–Dec. 3	Baradjanegara, Abudul A., National Atomic Energy Agency, Indonesia.
	11–Mar. 11, '84	van Hintum. T. J. L., Agricultural University in Wagenigen, Netherlands.
	24–25	Novotny, Jiri, The Massachusetts General Hospital, U.S.A.
September	7–10	Green, Maurice, University of St. Louis, U.S.A.

October	1–31	Crow, James F., University of Wisconsin, U.S.A.
	4–5	Ward, Oscar G., University of Arizona, U.S.A.
	4–5	Wurster-Hill, Doris H., Dartmouth Medical College, U.S.A.
	10–16	David, Charles N., Universität München, Germany.
	14	Ohno, S., City of Hope Research Center, U.S.A.
	18–Nov. 25	Watterson, Geoff, University of Monash, Australia.
	21–22	Hawkes, J. G., University of Birmingham, U.K.
	24–31	Fuerst, Paul A., Ohio State University, U.S.A.
	29	Cherry, Flore F., University of Tulane, U.S.A.
November	9	Lewis, Herman W., National Science Foundation, U.S.A.
	17–18	Walker, Charles W., University of New Hampshire, U.S.A.
	21	Kasturi-Bai, A. R., Karnataka State Sericultural Development Institute, India
	21–Sept. 24, '84	Zhu, N.-K., Institute of Environmental Chemistry, China.
	24	Jalaludn, B. S. S., Univeriti Pertanian Malaysia.
	24	Mohammod, M. O. B., University of Malaya, Malaysia.
	24	Rahim, M. S. A., University of Malaya, Malaysia.
	26	Tahir, M. Srivastava, J. P. Somaroo, B. H. Nachit, M. M. } International Center for Agricultural Research in the Dry Areas, Syria.
	26–29	Nei, M., University of Texas, U.S.A.

	27–29	Goldberg, Alfred L., University of Harvard, U.S.A.
December	5	Nevo, Eviatar, Haifa University, Israel
	15	Arnould, Daniel, Belgium Embassy, Belgium.

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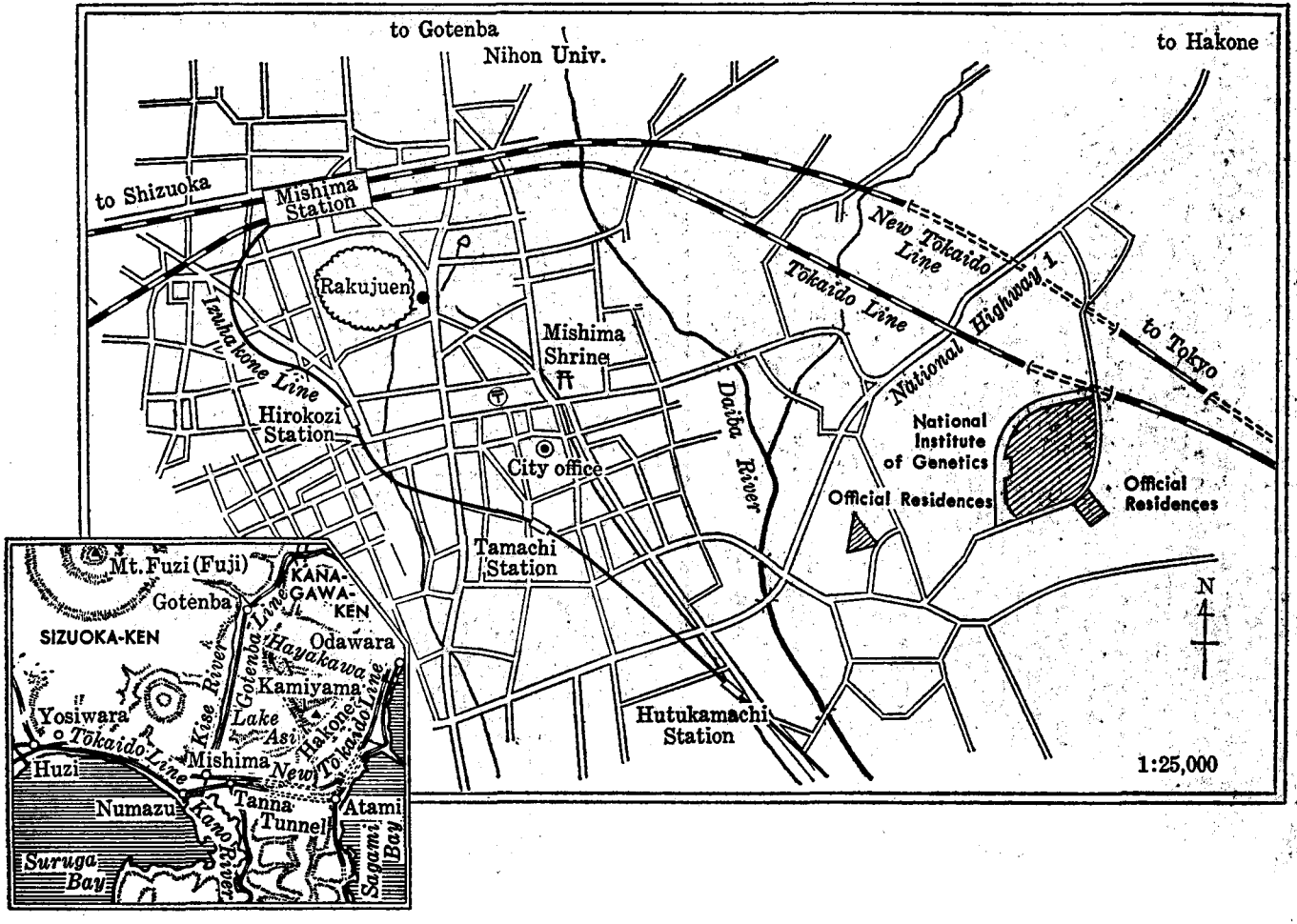
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