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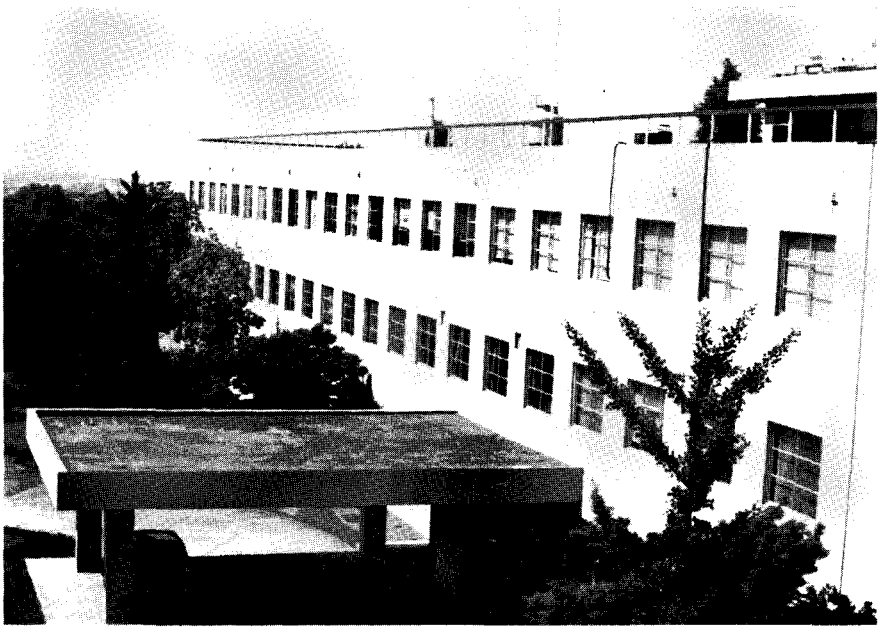
National Institute of Genetics

No. 26, 1975



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GENERAL STATEMENT

Dr. Daigoro Moriwaki retired from the Director's post at the end of February, 1975. He was elected in April, 1969, as the successor of Dr. H. Kihara and dovoted all his energy to the development of the institute ever science. Especially he made strenuous efforts to characterize, in the current of modern genetics, the divisional organization of the institute with staff members who had taken root in the classical genetics. His idea was to develop the whole fields of genetics in good harmony. He completed setting up of the 10th research department, Molecular Genetics, and then planned to establish the Genetic Stock Center. He had also paid close attention to the improvement and perfection of research facilities and equipments.

On March 1st I was appointed, assuming the heavy responsibility, the 4th director of the institute of its 25 year's tradition established under the directorship of Dr. K. Oguma, Dr. H. Kihara and Dr. D. Moriwaki. I am firmly determined to exert myself to the utmost for further development of the institute and sincerely solicit for the advice and cooperation of all persons concerned.

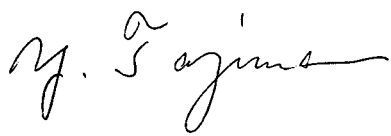
"The institute aims at fundamental researches on the principle of genetics as well as on its practical application." Needless to say, we should take up essential research subjects among so many problems which may arise from genetic studies, so that we can contribute to the creation of new concepts. Furthermore, there are several problems which human being is confronted with—on food, population, environment, energy, etc. Researches to meet such social needs of the times may be no less important than the former. Innumerable research subjects are in front of us, but, our power to tackle with them is only limited. Accordingly, we must carefully select to investigate essential problems of primary importance. We should not go on in the same old rut.

The institute opens a door to the public one day every year during Science and Technology Week in the middle of April. Visitors have been increasing year after year. This year we had more than 7,000 visitors, which recorded the highest figure since the foundation of the institute. This indicates the deep interest of the people in genetic researches. Another event open to the public annually is the public lecture, which was

held on November 1st at the lecture hall of the Science Museum in Tokyo. Speakers were Dr. Y. Hirota, Head of the Department of Microbial Genetics and Dr. Tomoko Ohta, Chief Researcher, Department of Population Genetics. They talked on "Cell Division" and "Molecular Evolution," both of which gave a deep impression on the minds of the audience.

Until now several summer seminars had been held at irregular intervals. This summer it was organized during July 7–9th by the staff members of the Department of Population Genetics, with the cooperation of several scientists from outside the institute, entitled "Population Genetics and Molecular Evolution." The participants came up to over 100 from all over Japan and the auditorium was full of enthusiastic atmosphere of learning.

Looking back the year from a budgetary aspect, i) the Genetic Stock Center, a new establishment, which started last year with the Plant section, was granted for adding the Animal section. Budgetary requirement for setting up Microbial section has already been approved for the fiscal year 1976. Accordingly, it will not take long before the Genetic Stock Center will function as we planned originally. ii) In addition to radiation laboratory and radioisotope laboratory budget for setting up low level radioisotope laboratories was also approved on the expenses for remodelling the old annex building. It will offer a great convenience for molecular and microbial genetics groups. iii) The purchasing plan of the neighboring area, where a junior high school once occupied, will be completed with the next fiscal year budget. Now we are in a position to draw up our future plan of building construction with enough space. Herewith I wish to express my deep gratitude to the authorities concerned for their assistance given by them for the materialization of the necessary budget.



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WADA, Bungo, Manager, Emeritus Professor of University of Tokyo
TAZIMA, Yataro, Manager, Director of the National Institute of Genetics
OSHIMA, Chozo, Manager, Head of the Physiological Genetics Department

PROJECTS OF RESEARCH FOR 1975

Department of Morphological Genetics

Genetic studies of the silkworm (TAZIMA and ONIMARU)
Radiation and chemical mutagenesis in the silkworm (TAZIMA and ONIMARU)
Cytogenetics in the silkworm (MURAKAMI and IMAI)
Studies on recombination in the silkworm (MURAKAMI)
Genetic studies on insect cells in tissue culture (KURODA and MINATO)
Developmental genetic studies on carcinogenesis in tissue culture (KURODA)
Genetics of somatic mammalian cells in culture (KURODA and MINATO)

Department of Cytogenetics

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

Department of Physiological Genetics

Behavior genetics of *Drosophila* (OSHIMA and WATANABE)
Analysis of delecterious and inversion chromosomes in natural populations of *Drosophila melanogaster* (WATANABE)
Studies on fitness of *Drosophila* under controlled environment (OSHIMA and WATANABE)
Ecological genetic studies on the differentiation of *Chrysanthemum* species (NAGAMI)
Genetic studies on the effects of adverse environments on *Drosophile* flies (OSHIMA and WATANABE)

Department of Biochemical Genetics

Studies on transformation in higher organisms (NAWA and YAMADA)
Genetical and biochemical studies of pteridine metabolisms in insects (NAWA)
Analysis of gene action on cell differentiation in higher organisms (NAWA and YAMADA)
Biochemical studies on the differentiation of muscle proteins in animals (OGAWA)
Genetical and biochemical studies of human serum proteins (OGAWA)
Genetical and biochemical studies on Japanese middle size dog (OGAWA)
Genetics of isozymes in plants (ENDO)
Effects of exogenous DNA on plant seed formation (ENDO)
Genetic analysis of developmental mechanisms in hydra (SUGIYAMA and FUJISAWA)

Department of Applied Genetics

Quantitative genetic studies in poultry (KAWAHARA and FUJISHIMA)
Genetic studies in wild populations of Japanese quails (KAWAHARA)
Theoretical studies on breeding techniques (IYAMA)
Behavioral genetic studies in mice (FUJISHIMA)
Genetic studies in natural stands of forest tree species (IYAMA)
Simulation studies on artificial selection (IYAMA)
Evolutionary studies in wild and cultivated rice species (OKA and MORISHIMA)
Ecological genetic studies in some grass species (MORISHIMA)
Genic analysis for isozyme variations in rice (ENDO and OKA)
Genetic effects of environmental pollution on plants (IYAMA, Morishima and OKA)

Department of Induced Mutation

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

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

Department of Human Genetics

Genetic studies on common disorders with complex inheritance (MATSUNAGA)
Genetic studies on retinoblastoma (MATSUNAGA)
Clinical cytogenetic studies of congenital abnormalities in man (NAKAGOME and IINUMA)
Molecular cytogenetic studies of human chromosomes (NAKAGOME)
Studies on human chromosome variants (IINUMA, MATSUNAGA and NAKAGOME)

Department of Microbial Genetics

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

Department of Population Genetics

Theoretical studies of population genetics (KIMURA, MARUYAMA and OHTA)
Studies on molecular evolution from the standpoint of population genetics (KIMURA and OHTA)
Mathematical studies on the genetics of structured populations (MARUYAMA)
Linkage disequilibrium in finite populations (OHTA and KIMURA)
Experimental studies on protein polymorphism in *Drosophila* (YAMAZAKI)
Statistical studies on protein polymorphisms in natural populations (MARUYAMA and YAMAZAKI)

Department of Molecular Genetics

Studies on the chemical structure of genome of viruses containing double-stranded RNA (MIURA, SUGIURA, SHIMOTOHNO)

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

Studies on structure and function of messenger RNA (MIURA, SHIMOTOHNO)

Genetical and enzymological studies on *E. coli* RNA polymerase (SUGIURA)

Genetic Stock Center

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

On the sex expression in monoecious plants (FUJII)

Specificity of mutagen tolerance in higher plants (FUJII)

RESEARCHES CARRIED OUT IN 1975

I. MOLECULAR GENETICS

The Modified Structure at the 5′-terminus of mRNA in a Bone Marrow Cell which Produces Hemoglobin Vigorously

Kin-ichiro MIURA, Yasuhiro FURUICHI and Yasusada MIURA

As a novel modified structure was discovered by us at the 5′-terminus of messenger RNAs of cytoplasmic polyhedrosis virus and vaccinia virus as written in the last issue of this annual report (No. 25, 1974 p. 11), presence of the similar structure was surveyed in a cellular mRNA. Since hemoglobin messenger RNA is well known as an instance of a eukaryotic mRNA which contains an information for a single protein, the 5′-terminal structure of this mRNA was studied. In order to detect small amount of a modified structure in a long RNA molecule, radioisotope labeling of RNAs with [^{32}P] and [methyl ^{3}H] methionine was carried out. A good condition was obtained with the primary suspension culture of bone marrow cell of an anemic rabbit. Major part of the mRNA molecules synthesized in this cell is hemoglobin mRNA. Total RNA was extracted from cell and fractionated by an oligouridylic acid-Sepharose column, which can adsorb poly A-containing mRNA molecule. The [methyl ^{3}H] radioactivity was contained in the poly A-containing mRNA fraction. This RNA was analyzed as mentioned in the last annual report (No. 25, 1974, p. 11). In the 5′-terminal part isolated by the treatment of *Penicillium* nuclease P_1, the methyl label was involved as the structure $m^7G^{5'}ppp^{5'}Cm$-N-. 7-methyl guanylic acid blocks the 5′-terminal nucleotide through two pyrophosphate linkages as shown in mRNAs of CP virus and vaccinia virus.

Modified Structure in Messenger RNA of Reovirus

Kunitada SHIMOTOHNO and Kin-ichiro MIURA

A modified structure of mRNA discovered in silkworm cytoplasmic polyhedrosis virus was tested for human reovirus as another instance of

the double-stranded RNA-containing virus. As reovirus contains RNA polymerase in its coat, RNA is synthesized in a test tube, if the trypsin-treated virus particles were added with four kinds of substrate NTP. If [methyl ^{3}H] S-adenosylmethionine (SAM) was added to this system as a methyl donor, the synthesized mRNA contained [^{3}H] methyl groups. In order to identify the modified structure, [β ^{32}P] GTP (which is incorporated to the 5′-terminal nucleotide—see the last annual report No. 25, 1974, p. 14) was also added to the reaction mixture. After all the terminal modified structure of reovirus mRNA was determined as follows: $m^{7}G^{5'}ppp^{5'}Gm$-C------.

Modified Structure in Messenger RNA of Rice Dwarf Virus

Kunitada SHIMOTOHNO and Kin-ichiro MIURA

Rice dwarf virus (RDV) containing double-stranded RNA can synthesize RNA *in vitro* as cytoplasmic polyhedrosis virus and reovirus. When [methyl ^{3}H] S-adenosylmethionine was added to the reaction mixture, [^{3}H] methyl was incorporated to the 5′-terminal part of the RDV mRNA. The terminal structure was identified as follows: $m^{7}G^{5'}ppp^{5'}Am$-Y------.

Structure of the Double-stranded RNA in a Virus of *Penicillium chrysogenum*

Kazumori YAZAKI and Kin-ichiro MIURA

Recently virus particles which contain double-stranded RNA have been found in many kinds of fungi. The RNA extracted from these virus consists of three or four different size segments. In order to get a view for organization of these genome segments and mechanism of gene expression, the terminal structure of these RNA segments in *Penicillium chrysogenum* Virus (Pc V) was investigated.

The 3′-terminus was labeled with [^{3}H] by borohydride reduction. Two kinds of the 3′-terminal structure was identified as follows for every three segment: ---G-U and ---Y-A.

The 5′-terminus was tried to label with [^{32}P] phosphate by polynucleotide kinase. Only one kind of the 5′-terminal nucleotide sequence A-G-Y- was labeled. The efficiency of the labeling reached only about one mole for one RNA duplex. This means that one strand of the duplex can be phosphorylated, but another strand cannot. The latter must carry the blocked

terminal structure as shown at the 5′-terminus of the mRNA strand of cytoplasmic polyhedrosis virus and reovirus.

The nucleotide sequence of the unblocked 5′-terminus, A-G-Y-, is not complementary to both the 3′-terminal nucleotide sequences. To test the possibility that the double-stranded RNA of Pc V may have a single-stranded part near the terminal region of a polynucleotide chain, the 3′-[^{3}H] labeled RNA and the 5′-[^{32}P] labeled RNA were mixed and treated with nuclease S_1 from *Aspergillus,* which selectively cleves single-stranded region in a polynucleotide chain under some conditions. Only [^{32}P] labeled nucleotide was released from the double-stranded RNA. This means that the 3′-termini should be in a double-stranded state, but at least one 5′-terminus, that is, the 5′-terminus of the unblocked chain carrying the sequence A-G-Y-, is in a single-stranded stretch.

Mechanism of Formation of the Modified Structure at the 5′-terminus of mRNA of Silkworm Cytoplasmic Polyhedrosis Virus

Kunitada SHIMOTOHNO and Kin-ichiro MIURA

The modified structure including the 7-methylguanosine blocking through 5′-5′-pyrophosphate linkage and the ribose-methylation at 2′-position of the first nucleotide was found at the 5′-terminus of mRNA of cytoplasmic polyhedrosis (CP) virus (Annual Rep. No. 25 (1974) p. 12; Nature 253 (1975) 374–375). CP virus, which contains double-stranded RNA as a genome and enzymes including RNA polymerase, phosphohydrolase and methylases, is able to synthesize mRNA carrying the 5′-terminal modification *in vitro* in the presence of a methyl-donor S-adenosylmethionine (SAM). The process of the formation of the modified structure at the 5′-terminus of this mRNA was studied.

The results show that methylation at the 5′-terminus of the mRNA occurs at first on the blocking guanylate at 7-position of the base residue, and the methylation at the 2′-position of the ribose moiety of the first adenylate starts only after RNA chain has reached three nucleotide length.

In order to confirm the steps of methylation the confronting nucleotide structure without methyl groups, G$^{5'}$ppp$^{5'}$A and G$^{5'}$ppp$^{5'}$G, were prepared by chemical synthesis and added to the reaction mixture containing CP virus. The result shows that GpppA can be a substrate for the methylase associated

to CP virus, but GpppG can not. This is not conflict with the 5′-terminal structure of CP virus mRNA, m^7GpppAm. The methylation enzyme in CP virus seems to recognize the substrate structure strictly and to methylate after the blocked structure is constructed. In fact, GTP in the reaction mixture was not methylated. The methyl group incorporated into GpppA was found only in 7-position of G, but not in 2′-position of A. Here again it is clear that methylation at the 7-position of the blocking guanosine residue is carried out at first, and the ribose moiety in the A residue is not methylated before a few nucleotides linked to it.

Based on these results, the process of the 5′-terminal modification during the synthesis of CP virus mRNA are written as follows:

$$\text{GTP} + \text{ATP} \longrightarrow \text{GpppA}$$
$$\text{GpppA} + \text{SAM} \longrightarrow m^7\text{GpppA}$$
$$m^7\text{GpppA} + \text{GTP} \longrightarrow m^7\text{GpppA-G}$$
(with or without SAM)
$$m^7\text{GpppA-G} + \text{UTP} + \text{CTP} + \text{SAM} \longrightarrow m^7\text{GpppAm-G-U-C------}$$

This work was published in FEBS Letters 64: 204–208 (1976).

A New Acid Phosphatase from *Escherichia coli* Cells

Masahiro SUGIURA

There are many phosphatase activities in *Escherichia coli* cells. Some of them have been purified and characterized. During the course of polynucleotide kinase purification from bacteriophage T4-infected *E. coli* cells, we found a new type of acid phosphatase activity. This enzyme was not bacteriophage T4-coded one because the activity was detected in uninfected *E. coli* cell extract.

The acid phosphatase was purified from *E. coli* strain A19 (RNase I^-) by streptomycin sulphate fractionation followed by successive column chromatographies on DEAE-cellulose, phosphocellulose and Sephadex G-100. The purified enzyme had an optimal pH of 4.1 and was activated by magnesium and cobalt ions. The enzyme catalyzed the hydrolysis of nucleoside 5′-diphosphates and nucleoside 5′-triphosphates to nucleoside 5′-monophosphates, and nucleoside 3′,5′-diphosphates to a mixture of nucleoside 3′-monophosphates and nucleoside 5′-monophosphates. Nucleoside 3′-monophosphates and nucleoside 5′-monophosphates were not hydrolyzed

but nucleoside 2′-phosphates were hydrolyzed at slower rate. 5′- and 3′-Terminal phosphates of RNA were removed by this enzyme. The phosphatase hydrolyzed *p*-nitrophenylphosphate but not bis-*p*-nitrophenylphosphate. This enzyme was differ from any of the *E. coli* phosphatases previously reported and we tentatively called it acid polynucleotide phosphatase. This enzyme may be useful for the chemical analysis of nucleic acids and their components because of its unique specificity.

Induction of RNA Ligase Activity in Bacteriophage T4-Infected *Escherichia coli* Cells

Masahiro SUGIURA and Kazuko SEGAWA

RNA ligase has been isolated from bacteriophage T4-infected *E. coli* cells. This enzyme catalyzes inter- and intra-molecular joining reactions of ribopolynucleotides. Deoxyribooligonucleotides were also joined by this enzyme action. Therefore, we investigated the relationship between DNA ligase and RNA ligase. *E. coli* strain A19 (RNase I^-, Su^-) cells were infected with phage T4 amber mutant of gene 30 (DNA ligase), *am* H39, *am* C72 or *am* E605 and the RNA ligase activity was assayed. All mutant phages induced the RNA joining activity comparable to wild-type phage. This indicated that the induction of RNA ligase activity was nothing to do with the structural gene of T4 DNA ligase. A T4 mutation called SP62, which overproduced some of early enzymes in combination with DNA-negative mutation, did not cause any overproduction of RNA ligase. A double amber mutant N82 X E1140 induced the highest activity of RNA ligase among T4 DNA-negative mutants tested.

II. MICROBIAL GENETICS

An *Escherichia coli* Mutant Defective in Murein Lipoprotein Synthesis

Yukinobu NISHIMURA, Hideho SUZUKI, Sei-ichi YASUDA, Hiroko IKETANI and Yukinori HIROTA

The mutant lacking murein lipoprotein in the outer membrane has not been available so far. Such a mutant is expected to contribute very much toward the elucidation of function of the lipoprotein. During the genetic manipulation of an F prime, we found a mutation defective in the production of the lipoprotein on the F prime. The gene necessary for the lipoprotein production was mapped at 36.5 min between *uidA* and *aroD* on the *E. coli* linkage map.

It has been established that *lpm*$^{+}$ and its closely linked markers are mapped in the order of *-man-uidA-lpm-aroD-pps-*. An F prime carrying the genetic loci of *man* and *aroD* was constructed by the cross between HfrB7 and JE5507 (*recA1, man, aroD*), and introduced into the *rec*$^{+}$ strain having *lpm*, in order to obtain *lpm*-carrying F prime. Among transductional derivatives of this strain, we found two clones in which the lysozyme digests of their murein showed no electrophoretic band characteristic to the bound form of the lipoprotein.

One of the clones designated as JE5508 isolated by transduction with P1 was examined further with respect to the lipoprotein. The electrophoretic analysis of the outer membrane proteins solubilized with hot-SDS showed no band of the lipoprotein detectable by staining with Coomasie blue. Thus the free form of the lipoprotein as well as the bound form was absent in this strain. This was further confirmed by labelling the cellular proteins with radioactive arginine. The cells of JE5508 were labelled with ^{3}H- or ^{14}C-arginine, mixed with those of the parental strain labelled with the counter species of the radioisotope, respectively, and disrupted to prepare the membrane fractions. The electrophoretic analyses showed that the radioactivity peaks found at the position of the bound or unbound lipoprotein in the parental strain were missing in the mutant. The serological assay on an Ouchterlony agar plate showed that materials cross-reacting with anti-lipoprotein serum was absent in the samples prepared from the mutant cells

by sonic-disintegration and subsequent solubilization with hot-SDS. The messenber activity of RNA was examined if it could stimulate the synthesis of lipoprotein, by the cell-free system for protein synthesis followed by the serological purification of the product and the electrophoretic analysis. The RNA extracted from the mutant did not stimulate the synthesis of the lipoprotein, whereas that from the parental strain directed the lipoprotein synthesis *in vitro* at the level amounting to 1.9% of the general protein synthesis. These results indicate that the cells of JE5508 lacked the lipoprotein because of their inability to synthesize active mRNA for the lipoprotein.

The mutation leading to the loss of lipoprotein appeared to have occurred in the process of production of the F prime, since the merodiploid isolated at first by the cross of HfrB7 and the *rec*$^-$ strain was shown to carry this mutation on the F prime by the transduction experiments. This mutation will be referred to as *lpo*.

The colour of the colony of lipoprotein-less strains was found to become darker after prolonged incubation. This character was shown to be absolutely parallel with phenotypes identified by electrophoresis, and aided the genetic analyses of the *lpo* mutation.

Our experimental results demonstrated that the map position of the *lpo* was at 36.5 min on the Tayler's map (Bachmann B. J. *et al.*, 1976, Bacteriol. Rev., **40**: 116), as determined by transduction with P1. The merodiploids carrying *lpm*$^+$/F'*lpo* and *lpm*$^-$/F'*lpo* exhibited phenotypes of the wild and the modification, respectively. The *lpo* mutation could be a delection mutation which is extending over at least two loci, *lpm* and a hypothetical structural gene coding for polypeptide of the lipoprotein, *lpp*.

The physiological nature of this mutant was examined with isogenic strains obtained by P1 transduction. The *lpo* mutant grew normally over the wide range of growth conditions and remained susceptible to the following bacteriophages; λ, ϕ80, P1, P2, T1, T2, T3, T4, T5, T6, T7, f1, f2 and MS2. The mutant revealed hypersensitivity to EDTA, acriflavin and ethidium bromide. The mutant leaked periplasmic enzymes; ribonuclease 1, alkaline phosphatase, cyclic phosphodiesterase and penicillinase, more than the wild type strain. The effect of EDTA was conspicuous. The addition of EDTA to cell suspension of the *lpo* mutant brought about rapid cell lysis, but this was rescued by the simultaneous addition of magnesium. The passive transport of β-galactosides was not changed by the *lpo* mutation, as observed by the measurement of the crypticity of β-galactosidase activity

of bacterial cells (Cohen, G. N. & Monod, J., 1957, Bacteriol. Rev., **21**: 169).

From these physiological characteristics of the mutant, it was suggested that the lipoprotein participates in maintaining the stability of the outer membrane structure by anchoring the murein layer of the cell, but does not in the vital processes of growth and division.

† *lpm*: a gene for the altered lipoprotein (SUZUKI et al., 1976 J. Bacteriol. **127**: 1494).

III. BIOCHEMICAL GENETICS AND IMMUNOGENETICS

A Simplified Method for Reading Hemagglutinations on a Flat Bottom Microtitiration Plate in the Mouse H-2 Assay

Kazuo MORIWAKI, Tadashi AOTSUKA and Toshihiko SHIROISHI

The original polyvinylpyrrolidone (PVP) method of Stimpfling (Transpl. Bull. 27: 109, 1961) has been successfully modified to the more dependable form by Kaliss (Transpl. 15: 251, 1973). As his method is still laborious in the larger scale assay, a simplified method for reading the hemagglutinations microscopically on a microtitration plate with flat bottom wells has been developed in our laboratory. The recipe for working solutions followed Kaliss. Fresh blood for target RBC was obtained from the orbital sinus using a microhematocrit tube. We usually pour the full tube of blood (75 μl) into 10 ml Alsever solution immediately after bleeding, mix gently and wash three times with PBS by centrifugation at 2,000 rpm for 3 min. Final volume was adjusted to make 0.75% RBC suspension.

Titrations are performed in the Microtiter Plate for tissue culture (Cooke Laboratory Products) with 96 flat bottom wells. One trop (25 μl) of 1.2% (only for 1st row) or 0.6% PVP BSA was dispensed to each wells, then the antisera were serially diluted by 25 μl loops. Finally 25 μl RBC suspension was dispensed to each wells and shaked for about 10 seconds by a Voltex mixer. After incubation at 37°C for one hour and at 5°C overnight, each plate was shaked again for 10 seconds just before reading. Microscopic observation of the hemagglutinations was conducted by the inverted type microscope with X10 objective lens. Though the plate method seems to be slightly less sensitive than the original Kaliss' method, its operational simplicity could enough compensate the small defect.

H-2 Antigenic Specificities in the Japanese Wild Mouse, *Mus musculus molossinus*

Kazuo MORIWAKI, Toshihiko SHIROISHI, Tadashi AOTSUKA, Mitsuru MINEZAWA[1] and Kyoji KONDO[1]

The Japanese wild mouse, *Mus musculus molossinus* has likely derived

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from an aboriginal mouse, *Mus musculus manchu,* which geographical distribution was quite distant from the other aboriginal mice, *Mus musculus wagneri* and *Mus musculus spicilegus* (Schwarz and Schwarz, J. Mammal.

Table 1. Occurrence of twelve H-2 specificities in the Japanese wild mice, *Mus musculus molossinus*

Sample No	Place of collection	Sex	H-2 antigenic specificities											
			Public									Private		
			1	3	5	8	11	13	25	28	35	2	4	23
1	Teine	M	−	−	−	−	−	−	−	−	−	−	−	−
2	Teine	F	−	−	−	−	−	−	−	−	−	−	−	−
3	Teine	F	−	+	+	−	−	−	−	−	−	−	−	−
4	Sunagawa	M	−	−	−	−	−	−	−	−	−	−	−	−
5	Sunagawa	F	−	+	+	−	−	−	−	−	−	−	−	−
1	Sunagawa	F	−	+	+	−	−	−	−	−	−	−	−	−
6	Sunagawa	F	−	+	+	−	−	−	−	−	−	−	−	−
7	Johoji	M	−	+	+	−	−	−	−	−	−	−	−	−
8	Natori	M	−	+	+	−	−	−	−	−	−	−	−	−
9	Niigata	M	−	+	+	−	−	−	−	−	−	−	−	−
10	Kanazawa	F	−	+	+	−	−	−	−	−	−	−	−	−
11	Kanazawa	F		−	+	−	−	−	−	−	−	−	−	−
12	Utsunomiya	M	−	−	+	+	−	−	−	+	−	−	−	−
13	Hattabata	M		+	+	−	+	+	−	−	−	−	−	−
14	Haranoya	F		−	−	−	−	−	−	−	−	−	−	−
15	Anjyo	M		−	−	−	−	−	−	−	−	−	−	−
16	Anjyo	M		+	+	+	−	−	−	−	−	−	−	−
17	Mizuho	M		−	−	−	−	−	−	−	−	−	−	−
18	Ohtsu	M		−	−	−	−	−	−	−	−	−	−	−
19	Ohtsu	M		−	−	−	−	−	−	−	−	−	−	−
20	Kochi	M		+	+	−	−	−	−	−	−	−	−	−
21	Kochi	F		+	+	−	−	+	+	−	+	−	+	+
22	Matsuyama	M	+	+	−	−	−	−	−	+	−	−	−	−
23	Matsuyama	F		−	−	−	−	−	−	−	−	−	−	−
24	Sasaguri	F	−	+	+	−	−	−	−	−	−	−	−	−
25	Sasaguri	M	−	+	+	−	−	−	−	−	−	−	−	−
26	Sasaguri	F	−	−	−	−	−	−	−	−	−	−	−	−
27	Sasaguri	F	−	−	−	−	−	−	−	−	−	−	−	−
28	Hakozaki	F	−	+	−	−	−	−	−	−	−	−	−	−
29	Okinoerabu Is.	M	−	+	−	−	−	−	−	−	−	−	−	−
30	Yonakuni Is.	M	+	+	+	−	−	−	−	−	−	−	−	−
Average freq.			.11	.57	.53	.07	.03	.07	.03	.07	.03	.00	.03	.03

54: 59, 1945). These two subspecies are possible origins of the European wild mice, *Mus musculus musculus* and *Mus musculus domesticus,* in which many H-2 specificities, especially public ones being fairly common in the various inbred strains of mouse, have been detected with the higher frequency. The present investigation aims to survey serologically those H-2 antigenic specificities in the Japanese wild mice, *Mus musculus molossinus,* from view point of H-2 gene evolution in the mouse species.

Assay of H-2 antigenic specificities was carried out using a modified Kaliss method (Moriwaki *et al.* Experientia, in press) which allowed us to directly observe PVP-hemagglutinations of the microtitration plate with flat·bottom by the inverted microscope. Twelve alloantisera employed in this study were given by the Transplantation Immunology Branch, NIAID, NIH. Twelve numericals listed in Table 1 represent antigenic specificities against which each of the alloantisera mainly directed. In many cases the hemagglutination titers for each H-2 specificities in *M. m. molossinus* were relatively lower than those in the standard mice.

Table 1 summarizes the present data on the H-2 specificities of *M. m. molossinus.* For the comparison between *M. m. molossinus* and *M. m. musculus* or *M. m. domesticus,* we adopted nine public specificities which seem to be considerably common in the European and American wild mice, (Klein, Science 168: 1362, 1970. Mickova & Ivanyi, Immunogenetics of the H-2 system: 20, 1971) and three private specificities as well. Only two public specificities, H-2·3 and H-2·5, were apparently common in the Japanese wild mice and the others were very rare.

Considering numerous differences in the biochemical characteristics between the Japanese and the European feral mice (Minezawa *et al.,* This Annual Report), the frequency differences in their H-2 antigenic specificities may reflect a certain remote distance between them. In the other words, H-2·3 and H-2·5 public specificities likely are rather older evolutionarily than the other. They might have their origin in a common ancester of the three subspecies, *M. m. molossinus, musculus* and *domesticus.*

Studies on Protein Polymorphism of Japanese Wild Mouse, *Mus musculus molossinus*

Mitsuru MINEZAWA[1], Kazuo MORIWAKI and Kyoji KONDO[1]

The protein polymorphism of *Mus musculus molossinus* in Japan was surveyed on Hbb, Es-1, Es-5, Id-1, Es-2, Es-6, Amy-1, Amy-2, Amy-3, Mod-1 and Trf loci by gel electrophoresis, and Gus locus by spectrophotometry. Table 1 demonstrates the localities of collection, number of mice observed in each locality and phenotypic distributions of Hbb, Es-1, Es-5 and Id-1 loci.

Hbb locus (Hemoglobin): P, PC, D and PS types were observed, but Hbb^s-gene was rare and Hbb^d and Hbb^p were common alleles in *molossinus*. Es-1 locus (Serum esterase): A, AB and B types were observed and A type was considered to be popular among them. Es-5 locus (Serum esterase): A, B and C types were found and the C type showed slower migrating band, and was not yet observed in laboratory strains of mice. A type was common one. Id-1 (Kidney isocitrate dehydrogenase): 7 phenotypes, namely A, AB, B, AC, BC, C and BD types, were observed. The C type or C band was a slower migrating band than A band, and the D band was migrated faster than B band. Both two types, C and D bands, were not seen in laboratory mice. The B type is considered to be common type of *molossinus*.

Results of the electrophoretic survey for other proteins, which are not shown in Table 1, were as follows. Es-2 locus (Kidney esterase): A, B, BC and C types were found. The C type was common in *molossinus* (49/60 observed). Es-6 locus (Kidney esterase): A, B, AB and C types were observed, and the C type was slower migrating band than B band. The type A was common type. Amy-1 locus (Salivary amylase): AB and B types were observed and B type was common one. Amy-2 locus (Pancreatic amylase): A and B types were observed and B type was popular. Amy-3 locus (Pancreatic amylase): All the mice showed A type (49 mice). Mod-1 locus (Liver supernatant malic enzyme): A and AB types were found, and A type was thought to be common type of *molossinus*. Trf locus (Serum transferrin) and Gus locus (Liver β-glucronidase): At these loci, all of the mice observed are monomorphic, namely, B type at Trf and A type at Gus respectively.

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Table 1. Phenotypic distributions of proteins, Hbb, Es-1, Es-5 and Id-1 in Japanese *Mus musculs molossinus*

	No. Mice	Hbb					Es-1				Es-5				Id-1							
		P	PD	D	PS	n.t.	A	AB	B	n.t.	A	B	C	n.t.	A	AB	B	AC	BC	C	BD	n.t.
Okinawa	4			4			3			1	2	1		1					1			3
Kyushu																						
Hakozaki	26	6	10	7	3		7	8	11		17	1	7	1	1		6		3			16
Sasaguri	11		1	10			5	4	2		3		3	5		2	1	2	5	1		
Miyazaki	1	1							1		1						1					
Tsushima	11					11	5	6						11		1	1	5		4		
Shikoku																						
Kochi	8	8					5	3			8						1					8
Matsuyama	3	2				1	2		1		3						1					2
Honshu																						
Mine	6	1	3	1	1		2		4		6						1					5
Anjyo	2	2					2				2						2					
Hattabata	5	5					5				5											5
Haranoya	5	2	2			1			5		4		1				1					4
Kanazawa	5	3	1	1			5				5											5
Niigata	8		4	4			8				8											8
Johoji	4	4					4				4											4
Hokkaido																						
Nemuro	14	1		13			14							14			13				1	
Teine	4			4			3		1		2		1	1			1					3
Total	117	35	21	44	4	13	70	21	25	1	70	2	12	33	1	3	28	7	9	5	1	63

n.t.: not tested.

Based on the results of survey of protein polymorphism, several clear differences between *molossinus* and laboratory mice were able to be observed. For examples, P type of Hbb locus is a rare variant in laboratory mice, but common in *molossinus,* and C type of Id-1 and also C type of Es-5 are not observed in laboratory mice. Besides the difference between *molossinus* and laboratory mice noted above, some differences among localities were seen in the ratio of protein types.

IV. DEVELOPMENTAL AND SOMATIC CELL GENETICS

Estimation of Genetic Load and Isolation of Mutants of Hydra from Sexual Crosses

Tsutomu SUGIYAMA and Toshitaka FUJISAWA

Sexual crosses were made between field strains of *Hydra magnipapillata* collected from various localities in Japan, and viability and other characters of the progenies were examined.

The results showed that the chance for the fertilized eggs to hatch and grow to adults was generally very low due to genetic and non-genetic factors. Statistical analysis showed that field strains of *H. magnipapillata* contained an average of between 3.6 and 4.1 lethal equivalent units of recessive deleterious genes per gamate (between 7.2 and 8.2 per animal).

The results also showed that among the surviving F_1 progenies, there were many types of developmentally defective strains such as mini strains, maxi strains, multi-head strains, nematocyte deficient strains, regeneration deficient strains and male sterile strains. The defective traits of these strains were stably transmitted to their progenies produced by asexual budding. These strains, therefore, were all propagated asexually and maintained as clonal lines to be used later for further characterizations.

Nematocyte Deficient Strains of Hydra

Toshitaka FUJISAWA and Tsutomu SUGIYAMA

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

The nematocytes deficient strains of *H. magnipapillata* were isolated by crossing male and female strains collected from the same ponds and examining their F_1 and F_2 progenies for the food capturing ability and the nematocyte compositions. Two strains selected from the six nematocyte deficient strains were subjected to detailed analyses.

Nem-3, which virtually lacked type B nematocytes in its tentacles, was found to contain a greatly reduced level of the interstitial cells within its

body. Since interstitial cells are known to be the precursors of the nematocytes, it is likely that the lack of the interstitial cells is responsible for the nematocyte deficiency of this strain.

Nem-4, which was deficient in type A nematocyte in its tentacles, contained a normal level of the interstitial cells. Several lines of evidence indicated that the primary defect of this strain is probably in the migration of the matured type A nematocyte from the body column to the tentacles.

Regeneration Deficient Strains of Hydra

Tsutomu SUGIYAMA and Toshitaka FUJISAWA

Three strains of *Hydra mgnipapillata* selected from the seven regeneration deficient strains isolated by sexual crosses were subjected to detailed examination.

The number of the tentacles regenerated per animal was used as a measure of the capacity for regeneration. Wild type strains generally had about 6 tentacles per animal and, after amputation of the head and the foot, they regenerated practically the same number of tentacles within 5 to 6 days. In contrast, one of the regeneration deficient strains (reg-1) regenerated only about 40% and the other two strains (reg-16 and reg-19) regenerated only about 10% of the original tentacles during the same period of time.

It was however found that the efficiency with which these strains regenerated depended greatly upon how the animals were disected. When reg-16 and reg-19 were further disected into smaller pieces after head and foot amputation, their ability to regenerate increased markedly, and they produced up to 50% of their original tentacles.

Tissues taken from the distal and proximal ends of regenerating animals were transplanted to intact host animals to analyse their ability to regenerate into head ("head determination"). It was found that head determination took place within 24 hours after the head and foot amputation of the wild type animals. This determination, however, was abnormal with reg-16 and reg-19. It was suggested that the regeneration deficiency of these strains was due to this abnormal head determination, and that this in turn was probably caused by defective polarity gradient(s) in these strains.

A Tissue-Specific Defect in Developmentally Lethal Cells from *fused* Embryos in *Drosophila melanogaster* in Culture

Yukiaki KURODA

The *fused* (*fu*) strain is known to be an X-linked (1–59.5) recessive lethal mutant. Homozygous or hemizygous eggs for the *fu* gene die during embryogenesis when they have the cytoplasm given by homozygous mothers. In the present experiment cells from these lethal embryos were cultured to study when and what types of cells were affected by the factors produced at a specific locus (17D or 17E) of the *fu* gene on the X chromosome.

When the effective lethal phase of 605 embryos obtained from matings of *fu*/*fu* females with *fu*/Y males was examined, it was found that 0.5% died after blastoderm formation, 5.0% died after gastrulation, 8.6% died after head and trunk segmentation, 4.0% died after formation of a sac-like midgut, 0.7% died after chitinization, 76.0% died after entrance of air in trachea, 5.3% died after active movement and none became larvae.

When cells from *fu* embryos after gastrulation were cultured, spindle-shaped muscle cells, flat polygonal epithelial cells, fibroblastic cells, and nerve cells were maintained in a functionally active state for longer than the effective lethal phase of the embryos. Some muscle cells contracted at regular intervals and others produced the syncytial complexes. Epithelial cells grew slowly and a brown chitinous pigmentation was observed in the cytoplasm. After several days of cultivation baloon-like cellular spheres which are suspected to be imaginal disc materials were formed.

In contrast to rather normal natures and functions of these cell types, nerve cells from *fu* embryos were found to have some defects in extension and branching of nerve fibers. The droplet formation on nerve fibers which was found in cultures of wild-type embryonic cells and seemed to show some secretions, was also not found in cultures of *fu* embryonic nerve cells.

Reverse Mutations of 8-Azaguanine Resistance to Sensitivity in Human Lesch Nyhan Diploid Cells in Culture

Yukiaki KURODA

Human diploid cells derived from the elbow skin of an 11 year Lesch Nyhan boy were treated with ethyl methanesulfonate (EMS) at various

concentrations for 2 hours, incubated in normal medium for mutation expression time of 48 hours, and selected in HAT medium to induce reverse mutations from 8-azaguanine (8AG) resistance to sensitivity.

To examine the effect of EMS on cell survival, the D_0 values of cells treated EMS were calculated from the concentration-survival curves. The D_0 values for 2 hour- and 14 day-treatments with EMS were 5.8×10^{-2} M and 4.8×10^{-4} M, respectively. The D_0 value for 14 day-treatment with 8AG was 14 μg/ml, which were about 20 times more resistant than that of normal human diploid cells.

When Lesch Nyhan cells were treated with 10^{-3} M of EMS for 2 hours, the induced mutation frequency of 8AG sensitive cells (surviving in HAT medium) was 30.0×10^{-5}. When the cells were treated with higher concentrations of EMS, the induced mutation frequencies increased gradually. Cells treated with 10^{-2} M EMS gave the induced mutation frequency of 54.9×10^{-5}. This reverse mutation frequency of Lesch Nyhan cells by EMS was about two times higher than the forward mutation frequency in normal human diploid cells treated with the same concentration of EMS. This indicates that the induction of reverse mutations may be more sensitive than that of forward mutations in human diploid cells, as far as a sex-linked recessive 8AG resistance (at the HGPRT locus) is concerned.

The Effect of Cell Density on Induction of 6-Thioguanine Resistant Mutations in Chinese Hamster Cells in Culture

Yukiaki KURODA and Tohru SHIBUYA

Chinese hamster lung cells, Don-6, were treated with ethyl methanesulfonate (EMS) for 16 hours, incubated for 48 hours in normal medium, and selected in medium containing 6-thioguanine (6TG). In this procedure for detecting the induced 6TG resistant mutations, it was found that the replating of dissociated cells in new petri dishes after mutation expression time in normal medium enhanced the induced mutation frequency of cells.

The number of replated cells in each petri dish also markedly affected the induced mutation frequency: 4.4 replated cells per mm^2 of surface area of petri dishes produced the highest induced mutation frequency among various cell densities employed. The effects of replating and small cell density in enhancing the induced mutation frequency may be accounted by the reduc-

tion in cell-to-cell contact (metabolic cooperation) in monolayer culture. The concentrations of 6TG used for selecting 6TG resistant cells did not significantly affect the induced mutation frequency.

Two 6TG resistant cell clones, ET-1031 and ET-0011, were isolated from the original 6TG sensitive cells after treatment with the mutagen. Their D_0 values for 7 day-treatment with 6TG were 18.3 μg/ml and more, indicating that they were highly resistant to 6TG compared with the original cells. These 6TG resistant cells showed a cross-resistance to 8-azaguanine (D_0 =40.2 μg/ml).

Changes in Cell-to-Cell Cohesive Activity during Cell Cycle of HeLa S3 Cells in Rotation Culture

Yukiaki KURODA

The cell-to-cell contact is known to have a close relationship to the proliferation and differentiation of animal cells in development. In the present experiment changes in cell-to-cell cohesive activity during cell cycle was examined using synchronized cell population of HeLa S3 cells.

HeLa S3 cells were treated with 2.5 mM thymidine for 24 hours, cultured in normal medium for 10 hours, and treated with 0.5 μg/ml colchicine for 2 hours. Thus mitotic cells which showed a synchronization of 96.5% were collected, inoculated in 60-mm petri dishes in replicate, and incubated in a CO_2 incubator for various times. Cells in petri dishes were harvested every 3 hours, and 3×10^5 cells in 3 ml medium were transferred into 25 ml Erlenmeyer flasks which were rotated at 70 rpm at 38°C for 1 hour. The number of single cells remaining in cell suspensions was counted and the cell-to-cell cohesive activity of cells at each phase of the cell cycle was compared.

The highest cell-to-cell cohesive activity was found at the M phase (0 hour after cell collection) of cells. The cohesive activity of cells decreased gradually during the G_1 phase. At the early period of the S phase (12 hours after cell collection) the cohesive activity increased again and reached the second highest value. The cohesive activity decreased gradually at the late period of the S phase (18 hours after cell collection). During the G_2 phase the cohesive activity showed a gradual increase and reached the highest value of the M phase. The cyclic changes in the cohesive activity of cells may have some correlations with the DNA and protein syntheses of cells during cell cycle.

Protective Effect of Vitamin E on Bisulfite-Caused Inhibition of Aggregation of Embryonic Quail Liver Cells in Culture

Yukiaki KURODA

Vitamin E is known to have a specific role in the redox reaction and antioxidant effects on various metabolic processes in higher animals. In the previous experiment it was found that vitamin E counteracted the effect of bisulfite in reducing colony formation of cultured human cells. In the present work the protective effect of vitamin E on inhibition by bisulfite of aggregation of dissociated embryonic quail liver cells was examined in rotation-mediated cell culture.

Liver cells from 7-day quail embryos were dissociated by the standard procedure. Samples of 3 ml of cell suspension, containing 10^6 cells, were rotated at 70 rpm at 38°C for 24 or 48 hours. Then the aggregates were harvested and their mean diameters were determined.

Bisulfite had a concentration-dependent inhibitory effect on the aggregation of cells, and in the presence of 10^{-2} M bisulfite, the mean diameters of aggregates formed after 24 and 48 hours were less than half those of aggregates in control cultures. Addition of 10^{-7} M vitamin E significantly reduced the inhibitory effect of bisulfite on cell aggregation. A concentration of 10^{-5} M vitamin E had the most protective effect on aggregation of embryonic liver cells. Further investigations on the mechanism of inhibition by bisulfite and protection by vitamin E of aggregation of embryonic liver cells are in progress.

Effective Lethal Phase of the *Curly* Mutant in *Drosophila melanogaster*

Yukiaki KURODA and Kiyoshi MINATO

A number of studies on the phenotypic expression of genes during development of higher animals have been carried out by determining the specific tissue and time of the gene action. The lethal genes in *D. melanogaster* provide a useful tool for analysing the tissue- and time-specificity of the gene action in development. In the present experiment the effective lethal phase of the *Curly* (*Cy*) mutant (which involves inversions at 22D1-2 and 33F5–34A1) on the second chromosome was examined.

F_1 eggs obtained from matings of *Cy*/+ females with *Cy*/+ males were allowed to develop on a moistened filter paper, and hatched larvae were grown on the standard cornmeal-sugar-agar medium. The numbers of eggs collected, hatched larvae, pupae and adult flies were determined. Among 5063 eggs collected, 11.2% died during embryonic development, 25.9% died during larval period, 4.1% died during pupal period, and 58.7% reached the adult flies. In adult flies the ratio of *Cy*/+ : +/+ was about 2 : 1 (1853 : 962). These results indicate that *Cy*/*Cy* individuals may have been about one fourth of total eggs collected and most of them may have died during larval period of development. Investigations on the tissue-specificity of the action of the *Cy* gene is also in progress.

V. CYTOGENETICS

Population Survey of B-chromosomes in Black Rats

Tosihide H. YOSIDA

Black rats (*Rattus rattus*) have been classified into 3 types according to chromosome numbers; Asian type with $2n=42$, Oceanian type with $2n=38$ and Ceylon type with $2n=40$. B- or supernumerary chromosomes in Asian type black rats have been reported by some investigators (Gropp, A. *et al.* 1970, Z. f. Söugetier. 35: 363; Ray-Chaudhuri, S. P. and Pathak, S. 1970, MCN, 11: 135; Yosida, T. H. and Sagai, T. 1975, Chromosoma 50: 283). Distribution of the B-chromosomes and their types in the black rats were investigated in the animals collected from several localities in the world. Among 271 Japanese black rat (*Rattus rattus tanezumi*), 190 were collected from five different localities of Japan (Misima, Nagasaki, Hokkaido, Niigata and Tokyo), and the remaining 81 were obtained from our breeding colony of the animals originated from Japanese black rats. Almost all black rats, except one which came from Hokkaido, showed diploid chromosome numbers ($2n=42$). This one rat showed 43 chromosomes including one small submetacentric B-chromosome. Nine *R. rattus flavipectus* collected from Hong Kong, 19 *R. rattus mindanensis* from Philippines, 14 *R. rattus* from northern India and 20 *R. rattus* from northern Pakistan showed all exact diploid number ($2n=42$). On the other hand, among 20 *R. rattus diardii* from Malaysia, 11 rats showed 43 chromosomes with one B-chromosome and 3 rats had 44 chromosomes with two B-chromosomes. These B-chromosomes are all small metacentrics and they were stained heavily by C-band technique.

In the Oceanian type black rats ($2n=38$) two from Iran, 4 from Australia and 10 from the United States showed normal diploid chromosome numbers (38). However, among 41 black rats from southern India (20 from direct observation from collected rats, and 21 from our breeding colony originated from the southern Indian rats), 8 rats had 39 chromosomes with one small metacentric B and 2 rats showed 40 chromosomes by having 2 small metacentric B. In Sri Lanka, 13 Ceylon type black rats (*R. rattus kandianus*) were collected. Among them one small metacentric B-chromosome was found in two rats.

Morphology of the B-chromosomes in these black rats was exactly the same by the small metacentric type except only one found in Hokkaido, Japan which showed the small submetacentric type. From these findings it is likely that the small metacentric B-chromosome is common in the black rats, and it may have developed somewhere in Asia and propagated there, from which they were distributed widely to the other localities irrespective of the karyotype evolution from $2n=42$ to 40 and 38. One small submetacentric B-chromosome found in Hokkaido, northern Japan, must have been developed independently from the above B.

Further Studies on the Frequencies of Chromosome Polymorphism in Pairs No. 1, 9 and 13 of the Black Rats in Japan

Tosihide H. YOSIDA

Pair No. 1 chromosome as well as Nos. 9 and 13 in the black rat (*Rattus rattus*) were polymorphic consisting of A/A, A/S and S/S complexes with regard to acrocentric (A) and subtelocentric (S) centromeres. A population survey of the No. 1 chromosome polymorphism in 453 black rats collected in various localities of Japan has been reported (Yosida *et al.* 1971, chromosoma 33: 30), but a numerical survey of No. 9 and 13 chromosomes has not yet been carried out, except for a small samples (16 rats) in Japan (Yosida 1971, this report 21: 55). A statistical survey of A/A, A/S and S/S types in the three chromosome pairs (Nos. 1, 9 and 13) has been carried out in 185 black rats in Japan. Among them 95 black rats came from four localities, Sapporo (22 rats), Niigata (15), Misima (35) and Nagasaki (23), which are located in northern, northwestern, southeastern and southern districts of Japan. Remaining 90 animals were derived from our breeding colony originated from a few rats collected in Misima.

Frequencies (%) of three chromosome types (A/A, A/S and S/S) of the pair No. 1 chromosome in total 95 black rats coming from the field were 75, 24 and 1, respectively, those in the pair No. 9 were 91, 5 and 4, respectively, but those in the pair No. 13 were 49, 36 and 18, respectively. The pair No. 1 of all rats from Sapporo and Niigata carried the monomorphic A/A type, while that in specimens from Misima showed A/A (54%), A/S (43%) and S/S (3%) polymorphism, and that from Nagasaki by A/A (74%), A/S (21%). With regard to pair No. 9, all 22 black rats from Sapporo were of the A/A type, while in Niigata, Misima and Nagasaki populations the rats

with A/S or S/S pair were observed together with a high frequency of the A/A type. Frequencies of the pair No. 13 were markedly different from those of pairs No. 1 and 9, because in all four localities rats with A/A, A/S and S/S types were usually found. About one half of the rats were A/S and S/S types.

Frequencies of chromosome polymorphism in pairs No. 1, 9 and 13 in 90 Japanese black rats bred in the laboratory were shown that rats with A/S and S/S types in all three chromosome pairs were observed at higher frequencies than those in the natural population of Japan, although mode of the occurrence of three chromosome pairs was somewhat different in each chromosome pair. Based on the above findings it is likely that the chromosome inversion of the pair No. 13 has occurred in earlier period than those of the other No. 1 and 9 pairs, and thus the rats with the inverted pair No. 13 chromosome have distributed widely in Japan.

Frequencies of Chromosome Polymorphism in Pairs No. 1, 9 and 13 in the Black Rats Distributed in the World

Tosihide H. YOSIDA

Frequencies of three chromosome types (A/A, A/S and S/S) in the three chromosome pairs (Nos. 1, 9 and 13) in the black rats (*Rattus rattus*) collected from 9 different localities in Southeast, Southwest Asia and Oceania were investigated. Total 141 rats examined were as follows; 7 from Hong Kong, 19 from Philippines, 6 from Kualalumpur, Malaysia, 8 from northern India, 8 from southern India, and 22 from northern Pakistan showed the Asian type idiogram ($2n=42$); 14 from Davis, United State, 13 from Brisbane, Australia, 25 from southern India, 3 from southern Pakistan and 3 from Iran showed Oceania type ($2n=38$) and the remaining 13 from Kandy, Sri Lanka, showed Ceylon type idiogram ($2n=40$). The frequencies of chromosome polymorphism of the three chromosome pairs in the Hong Kong population was similar to those of the Japanese one, but in the Philippine rats most of the pairs No. 1 and 9 were subtelocentrics, and pair No. 13 was polymorphic, most of the animals showing an acrocentric pair. The Malayan population was similar to the Philippines, but pair No. 1 in the former was more polymorphic than the latter. In the northern Indian population, the chromosome polymorphism was rather few, pairs No. 1 and 9 being subtelocentrics and the pair No. 13 acrocentrics. On the northern

Pakistan population pairs No. 1 and 9 were also usually subtelocentrics, but pair No. 13 was polymorphic showing high frequency of subtelocentric chromosomes. The Asian type rats of Mysor population in southern India was slightly different from those of northern Indian and Pakistan ones, because pairs No. 9 and 13 were acrocentrics.

Pair Nos. 1 and 9 in Oceanian and Ceylon type black rats were always the subtelocentric homologous pair, but no polymorphism was found in these chromosomes. Pair No. 13, however, was polymorphic in regard to acrocentric and submetacentrics. This was similar to the rats in northern India and Pakistan. Frequencies of three chromosome types in pair No. 13 were similar to all rats distributed in southern India and Pakistan, Iran and Sri Lanka. They were remarkable by showing high frequency of the acrocentric pairs. On the other hand, those of the pair No. 13 in the black rats collected from Davis, U.S.A. and Brisbane, Australia, were quite different from those of the Asian population by showing a high frequency of the subtelocentric pair. Based on the above findings it is likely that the pericentric inversion of pair No. 13 occurred in earlier period than that the other pairs No. 1 and 9, and therefore it was involved widely in the black rats distributed in the world.

Karyotype and Chromosome Polymorphism in Indian gerbil *Tatera indica*

Tosihide H. YOSIDA and Yuriko OCHIAI

A few Indian gerbils, *Tatera indica*, were collected from Mysor, India, in 1972 by us, and then they were bred in this laboratory. Diploid chromosome number of this animals was 68 (33 autosome pairs and X, Y), among which 50 acrocentrics (autosome pairs No. 1 to 25), 16 subtelo- or submetacentrics (autosome pairs No. 26 to 33) and a large metacentric X and a small acrocentric Y being included. The smallest biarmed autosome pair No. 33 was remarkable by showing chromosome polymorphism with three chromosome types. Type A is a considerably large submetacentric, type B a smaller submetacentric and type C the smallest subtelocentric. These types seems to be developed by deficiency of the chromosome arm of the smallest pair No. 33. Identification of the A and B types is really difficult because they are the same submetacentrics. The type A, however, is almost the same size as the pair No. 32, but the B was much smaller than that pair. Type C

is remarkable because of its smaller size than the above two and by having a tiny short arm. This will be developed by breakage of the short arm of the type B chromosome. Seven animals sampled at random from our breeding colony showed the following combinations; AB (1 rat), AC (2), BB (3) and BC (1). Six types were expected theoretically, but four types were observed in the present materials. The other two types (AA and CC) could be observed by sampling of more number of animals.

Mechanisms for Sister Chromatid Exchanges

Hatao KATO

By taking advantage of the fact that visible light (VL) induces strand breaks only in bromodeoxyuridine (BrdU)-substituted DNA, and that those breaks eventually lead to the formation of sister chromatid exchanges (SCEs), the response of SCEs to VL was studied carefully in Chinese hamster chromosomes in which, out of four DNA strands, BrdU-substitution and occurred either in one or three strands. The frequency of VL-induced SCEs did not differ greatly between these two types of chromosomes. This apparently contradicts to the expectation that the SCE frequency might be higher in trifilarly-substituted chromosomes than in the unifilarly-substituted ones previded that each break has an equal opportunity to evoke an exchange event, and seems to suggest that breaks induced in the post-replicational regions of the trifilarly-substituted chromosomes do not contribute to the induction of SCEs. On the other hand, when cells were submitted to caffeine treatment after VL illumination, a drastic increase in the SCE frequency was detected in the trifilarly-substituted chromosomes while a significant decrease in the unifilarly-substituted ones. It is very likely that breaks induced in the post-replicational regions of DNA in the trifilarly-substituted chromosomes now participate in the exchange event, possibly due to interference of repair processes by caffeine treatment.

Based on these results, a working hypothesis is developed that the SCE can arise by at least two different mechanisms, one operating at replicating points probably utilizing the machinery of DNA replication, and the other acting only in the post-replicational DNA portion, probably in a similar fashion as assumed in a general model of crossing over in the eukaryote. The majority of SCEs induced by VL would arise through the first mechanism irrespective of whether chromosomes are unifilarly- or trifilarly-substituted

with BrdU. The second type of mechanism might operate only under situation where two broken ends localized in two sister DNA duplexes can interact. Such a situation would be provided if caffeine disturbs repair of breaks while permitting the displacement of single strands at the break points.

The presence of these dual mechanisms may probably account for the discrepancy encountered in the explanations of the induction of SCEs by various exogenous agents as well as spontaneous SCEs.

B10BR Mouse with a Partially Deleted Y-chromosome

Kazuo MORIWAKI and Katsumi SAKAKIBARA

A B10BR mouse with a partially deleted Y-chromosome has been found in the progenies of a non-pedigreed litter mate pair which was obtained from the Jackson Laboratory, U.S.A. in 1968. Actually the karyotype was observed for the first time at the 3rd generation in our institute by using bone marrow cell preparation, revealing about half size of Y-chromosome in the 40 diploid chromosomes. G-banding pattern analysis of this mouse failed to show any translocation of the Y-chromosome fragment to the other chromosome. Observation of the spermatogonia has also shown no tetravalent meiotic chromosomes due to Y-autosome translocation.

This subline with a small Y-chromosome was designated B10BR-Y^{del} and has been successfully propagated until now through sister-brother mating. The litter size of them is almost the same as the normal B10BR line, though the sex ratio apparently deviated to the female. Recently the Y-chromosome was able to be introduced to the three congenic strains of mouse for establishing B10-Y^{del}, B10A-Y^{del} and B10D2-Y^{del}, respectively.

Furthermore, microscopic survey of the spermatozoa of B10BR-Y^{del} mouse demonstrated significantly higher occurrence of mal-formation. Whether the partial deletion of Y-chromosome could cause the malformation of the spermatozoa directly in the Y-carrying cells or indirectly through the male having the partially deleted Y remains unsolved.

VI. MUTATION AND MUTAGENESIS IN ANIMALS

Mutagenic Effectiveness of an Internal β Emitter, Tritium. (2) Measurement of Mutagenic Effectiveness on Germ Cells of *Bombyx*

Yataro TAZIMA, Kimiharu ONIMARU and Yosoji FUKASE

We reported in the last issue of this report (Ann. Rept. Natl. Inst. Genet. No. 25) that mutagenic effect of tritium was able to be demonstrated by somatic mutation expressed in F_1 larvae of *Bombyx* by means of intraperitoneal administration of the isotope to mother moths. The method, however, hardly permitted the estimation of induced mutation frequency. Hence, this experiment was undertaken with use of a germinal mutation system. ^{3}H-TdR in aqueous saline was injected into the body cavity of wild type females of a strain C108 at a mid-pupal stage (100 μCi/pupa). The average radioactivity transmitted to a deposited egg was 5.2×10^{-9} Ci. Injected and non-injected (control) females were crossed to *pe ok*$+^{re}$ males. There were 164 days from injection to the hatching of F_1 eggs. Calculated exposure dose was 3.17 rad per day and 519.5 rad in total.

F_1 larvae exhibited tiny *ok* spots at high frequency. Those females were crossed to *pe*$+^{ok}$ *re* males which made possible the assessment of germinal mutation frequency at *re* locus. Cluster-like appearance of *re* mutants was marked. The mutation frequencies were 4.9×10^{-5} and 4.1×10^{-3} in the control and treated group respectively, giving induced frequency of 7.96×10^{-6}/rad. By assuming Poisson distribution mutation frequency per cell generation was calculated to be 1.21×10^{-6}/rad.

On an Effective Stage of Chemical Mutagens as Detected by the Silkworm Oöcyte System

Yataro TAZIMA and Kimiharu ONIMARU

In the silkworm oöcyte system (Tazima, 1973), chemicals to be tested for mutagenicity are injected at mid pupal stage, 5–6 days before emergence. It was conjectured that injected chemical was incorporated maximum into the oöcytes after the injection at this stage and reacted with genetic materials around meiotic metaphase which takes place just after oviposition. Our finding, that AF-2 was mutagenically effective even when it was injected five

days before supposedly sensitive stage, i.e. around meiotic metaphase, raised a question, because the compound had been known to be easily metabolized in bacterial cells or mammalian liver tissues. Immediate mutation response of genetic materials to injected chemicals has been elucidated by an experiment using EMS, a biological half life of which was known about 48 hours at 25°C. However, the obtained response patterns were not necessarily consistent.

Experiment was, therefore, continued using DES, a biological half life of which was known to be few hours. The chemical was injected into female pupa at various developmental stages every 24 hours at the dose of 50 μg/pupa. The mutation response curve was clearly mono-modal representing a peak at mid pupal stage, six days before emergence. Further experiment with EMS, in which data were collected at daily basis regarding time interval between injection and emergence instead of on average duration basis, also exhibited clear cut mono-modal curves. It was concluded that genetic materials in silkworm oöcytes at mid pupal stage are in a state that can respond to chemical mutagens. The system may, therefore, be applicable also to the detection of chemical mutagens of short biological half life.

Nature of Chemically Induced Mutations Obtained by Oöcyte System in the Silkworm

Yataro TAZIMA, Kimiharu ONIMARU and Akio OHNUMA

The purpose of this experiment was to know whether there was qualitative difference in the recovered mutation at specific loci by oöcyte system between those induced by chemical mutagens and by ionizing radiations. The experiments were performed by treating oöcytes of the silkworm with chemical mutagens, EMS and DES. Mutations obtained at speficic loci were examined of their spectrum with regard to lethality expressed in homozygote, so as to be compared with those induced by ionizing radiations.

Wild type females of strain C108 were treated with those chemical mutagens at mid pupal stage and mated to non-irradiated males of *pe re*, after emergence, to pick up mutations to *pe* (or *re*) in F_1. Of those mutants only females were used to cross with $+re$ (or $pe+$) males, so as to separate the mutation-bearing chromosome from its homologue (*pe re*). All those crosses showed segregation of $1+:1$ *re* (or $1+:1$ *pe*) in the next generation. After discarding the red (or pink) eggs, only mutation-bearing heterozygotes

were isolated, and they were mated *inter se*.

The recovered phenotype (*pe* or *re*) appeared again in all mutant strains with a frequency of approximately 25%. Since the marker chromosome used for the detection of the mutation were already been discarded and there are no crossingover in the female heterozygotes in the silkworm, the segregated phenotype could be regarded as a homozygote for a newly arisen mutation.

Lethality was examined for all new mutations. For mutations which were obtained first as mosaics, lethality test was performed only in female lines. They were then crossed to *pe re* males and BF_1 females of mutant phenotype were subjected to the further analysis by the same procedure as mentioned above. The results are given in the Table, together with those obtained for X- or γ-ray irradiation in our previous experiments (Y. Tazima, Japan. J. Genet. **36**, Suppl. 50, 1961).

Table Results of lethality test for induced mutations in homozygotes

Mutagen	Homozygote				
	Viable	Lethal	Semi-L	Trans.	Total
EMS, DES	15	16	1	(2)	32
X-, γ-rays	5	31	—		36

When compared to radiation-induced mutations, where lethal is overwhelming, it may be noted that the proportion of lethal is lower in chemically-induced mutants. This indicates that mutational lesions produced by chemical mutagens, as EMS and DES, are less drastic than those produced by X- or γ-rays. Anyway, it should be noted that the proportion of lethal is still fairly high after chemical treatment and even chromosome aberrations of two hit events as translocation were induced after chemical mutagen treatment.

Estimation of Incorporated Amount of Furylfuramide into Egg-plasm after Injection into Silkworm Pupa

Yumi SHIMADA, Akio MURAKAMI and Yataro TAZIMA

In the silkworm oöcyte method, developed for mutagenicity testing, the most effective stage of injection of mutagenic compound was determined to be 5–6 days before emergence. Screening of various mutagens has been

performed by utilizing this method. It seems to be essential for this method to know exactly the amount of incorporated mutagenic compound into the ovum for the evaluation of the mutagenic effectiveness. Using furylfuramide which is labelled with radioactive ^{14}C this work has been carried out. Wild type female pupae of a strain C108 were injected, 5 days before emergence, into the body cavity with 0.025 ml each of 0.5% CMC saline suspension of ^{14}C-AF-2. Injected doses of the compound were 10 μg and 20 μg per pupa. Treated females were mated to non-treated males. After oviposition, measurement of radioactivity was performed on urine, deposited eggs, ovarioles with some remaining ova, body fluid, and the remnant body. All those samples were well grinded and shaked with 2 ml distiled water and 0.1 ml of each sample was dissolved in a Bray scintillator for counting the radioactivity by scintillation counter. The results are given in the Table.

Table Proportion of radioactivity incorporated into deposited eggs, remnant body, etc. after injection of ^{14}C-AF-2, five days before emergence

	Remnant body	Body fluid	Genital organ*	Deposited egg**	Urine	Total
Female	6–7	2–3	2–3	6–7	82–83	100
Male	8.2	2.9	0.0	—	88.9	100

* Including undeposited ova in ovariol for the female and testes and other genital organs before ejaculation for the male.

** On an average deposited eggs per batch were 467 in number and 280 mg in weight.

Radioactivity per egg grain (weighing 0.6 μg) was calculated 0.02% of total amount of injected radioactivity of the chemical. With an assumption that chemical structure of the injected AF-2 is kept unchanged, it can be estimated that the amount of AF-2 incorporated into deposited egg is 2×10^{-3} μg (3.3 ppm) per egg grain, when 10 μg of AF-2 had been injected. The amount is very close to that had been legally permitted for addition to meat sausages and soybean curd, maximum concentration being 5 ppm.

Differential Stage Sensitivity to the Induction of Mutations of Post-Meiotic Male Germ-Cells by Mitomycin C in the Silkworm

Akio MURAKAMI and Tetsuro MUROTA

Mitomycin C (MC) is highly mutagenic for inducing egg-color specific

locus mutations in the sperm of adult moths (Inagaki and Oster, 1969), but not for dominant lethal mutations in the sperm at the mid-pupal stage or five days before emergence (Murota and Murakami, 1975). In mice, accumulated evidences indicated that, whatever the basic explanation might be, some chemicals having the ability to produce dominant lethals also produce so called point mutations and *vice versa*. If such the statement is applicable to the silkworm, the frequency of specific locus mutations in the sperm at the mid-stage pupae by treatment with MC would not markedly increase as that of dominant lethals, while the frequency of MC-induced dominant lethals in the sperm at the late stage of pupae (including adult moths) would increase as that of specific locus mutations.

1–50 μg per individual of MC which had been dissolved in saline was injected into the abdomen of wild type (*C108* strain) silkworm males at different developmental stages, mature larvae, mid- and late-stage pupae. In the testes of mature larvae, there are a predominant number of late spermatids and sperm in addition to pre- and meiotic germ-cells. The most advanced germ-cells throughout the whole pupal stage (10–12 days at 25°C) seems to be at the stage of sperm. The treated males were then mated with non-treated marker females having double recessive egg-color genes, *pe* and *re*. The incidence of the dominant lethal mutations was detected by the reduction in hatchability of fertilized eggs as compared with control eggs produced from non-treated parents and that of the recessive visible mutations was detected by the egg-color specific locus method.

The results of dominant lethal tests were indicated that LD_{50} values of sperm in mature larvae and late pupae were ca. 30 μg and 15 μg of MC per individual, respectively. However, an LD_{50} value of the sperm in mid-stage pupae could not be estimated in doses lower than 50 μg since over this dose female moths mated with male moths which are derived from the treated pupae did not deposit a sufficient number of fertilized as well as unfertilized eggs. Thus, the sperm in late-stage pupae was the most sensitive to MC when judged by the incidence of dominant lethals, and followed by the germ-cells in mature larvae and mid-stage pupae. The results of the specific locus mutation tests were indicated that the sensitivity to the induction of mutations was quite similar to that to dominant lethals, suggesting that the frequency of MC-induced specific locus mutations in post-meiotic germ-cells is in parallel with that of dominant lethals. This may support the view that chemicals having the ability to induce dominant lethal mutations

also induce the specific locus mutations and *vice versa,* suggesting that the nature of the specific locus mutations is partly similar to that of the dominant lethal mutations which may be due to chromosome aberrations.

For the mechanism of the differential stage sensitivity to the induction of those mutational incidences, three possible interpretations may be given: the first is a differential susceptibility to the germinal selection of the damaged DNA in the different post-meiotic germ-cell stages prior to fertilization (or penetration of sperm into ova) and the second is a differential repairability of the damaged DNA molecules. Lastly, a combination of those above-mentioned possibilities may be the cause of the mitomycin C stage sensitivity.

Comparison of Recombinogenic Effects of Radiomimetic Alkylating Agents on Oogenic Cells in the Silkworm

Akio MURAKAMI

Recombinogenic effects of several radiomimetic alkylating agents having a difference in functional arm numbers on silkworm larval prophase I oocytes were compared in order to throw light on the mechanisms underlying those of ionizing-radiations. This cell stage is showing the highest incidence of X-ray induced recombinational events. One monofunctional alkylating agent, ethyl methane sulphonate (EMS) and two polyfunctional ones, mitomycin C (MC) and trimethylmelamine (TEM), were used. For estimating recombination frequencies the egg-color method (Murakami, this Report 23: 66–67 (1973)) was employed. Mature female larvae (prophase I oocytes) were treated with these alkylating agents.

The results indicated that no significant increase of recombinational events in prophase I oocytes treated with EMS was observed. On the other hand, when the oocytes were treated with MC and TEM, the frequencies of recombinations were clearly higher than those of controls as observed in X-rayed oocytes. The present finding and others tend to suggest that X-ray induced recombinational events may rise primarily through the double strand breakage of DNA within the vicinity of the complementary strand similar to the breakage event being derived from cross-links between the both DNA strands in oocytes treated with polyfunctional alkylating agents.

Mutagenesis of Furylfuramide in Mature Sperm of the Silkworm

Akio MURAKAMI

Furylfuramide (FF) is proved to be mutagenic in pupal oocytes of the silkworm (Tazima and Onimaru, 1973) as well as in many of microbes and mammalian cultured cells. However, it has been reported that FF is non-mutagenic in pupal spermatozoa of this insect (MURAKAMI and FUJIOKA. MUTATION Res. 31, 266–267, 1975). These findings and others indicated that in the silkworm genetic effect of FF is clearly dependent on the cell stage (or type) and mature sperm seems to be resistant to the chemical in contrast to oocytes.

Current evidence indicates that although FF itself is a hydrophobic and/or non-mutagenic compound, it becomes mutagenic (and hydrophilic) compound(s) after metabolic activation by rat liver homogenates. If this is the case, FF may be not activated in the male pupae before reaches it the spermatozoa. To check this possibility mutagenic effects of FF on the spermatozoa was investigated by means of the metabolic activation of the compound with rat liver homogenates. Mutagenicity was determined by the egg-colour specific locus method. Livers from 10 week old male Wistar rats were used in this experiment. The incubation mixture with containing required cofactors and a supernatant from the rat liver homogenate were incubated in a shaking incubator at 37°C for 15–30 min. For the experimental group FF was added at concentration of 7.5 μg per pupa. After incubation 0.025 ml/pupa of the reaction mixtures with or without FF was immediately injected into wild-type (*Aojuku*) male silkworm mid-pupae, which were then mated to marker females having *pe* and *re* gene loci.

The killing effect of FF on pupae was increased with doses of the compound and an estimated LD_{50} was around 5–15 μg/pupa depending on body weight. While the treated FF did not show any significant killing effects within the doses tested ranging from 0.5 to 50 μg per pupa, indicating that FF might be converted into non-toxic intermediates. The frequency of the egg-color specific locus mutation in males after treatment with the complete incubation mixture containing FF slightly but significantly increased as compared with the control. Next, 5 or 10 μg/pupa of FF labeled with ^{14}C at carbon-3 of the acrylamide moiety was injected into the mid pupal male. After emergence the radioactivity remained in testis, blood and the remnant part of the moth as well as in meconium was measured. The result

so far obtained indicating that a very slight radioactivity (0.2%) was detected in tests. A few percents of the activity was remained in blood as well as in the remnant part of the moth. Above 90% of the radioactivity was recovered in meconium, suggesting that almost treated FF was excreated.

Thus, it may conclude that the lack of mutagenic activity of FF in the spermatozoa of the silkworm is absence of the incorporation of the compound probably due to lack of an activating enzyme(s) for FF in the male pupal body or in the testis.

Spontaneous Diabetes in Djungalian Hamster, *Phodopus sungorus*

Tosihide H. YOSIDA

Djungalian or striped hairy footed hamsters, *Phodopus sungorus,* were obtained by courtesy of Dr. H. E. Pogosiantz in 1967 and from one pair of them a colony of the animals was established in this laboratory. They were maintained by inbreeding and/or outbreeding. Among 35 animals (18 femels and 17 males) sampled at random from the colony, the spontaneous diabetes was found at a high frequency; about one third (12 animals) among them showed the heavy diabetes. The disease was found in both males and females and also in adults any youngs. The diabete was found by reaction of the urine to the test paper (test-paper) prepared by Shionogi Pharmaceutical Co. LTD., Japan. The urine reaction of the disease animals showed all serious sympton as ++ or +++ to the test paper. The behaviour of the diabetes animals seems to be normal, and they are fertile. The genetical study of the disease is in progress now.

Dominant Black Coat Color in the Black Rat

Tosihide H. YOSIDA

Over five hundred black rats, *Rattus rattus,* were collected in several localities of Japan. Their coat color was agouti except one black color. The rat with the black coat was bred in the laboratory and then crosted to the rats with the wild agouti rats. Then the rats were bred for several generations. The black coat color in the Norway rat and the mouse is always recessive character which is subjected by a non-agouti gene (a). From the segregation ratio of the offspring obtained by crossing between

the wild and the black color, it was found that the black coat color in the black rat was completely dominant as seen in Table 1.

Table 1. Segregation of coat color in the black rats

Parents			Offspring		
			No. of litters examined	No. of rats examined	Segregation
					wild : black
wild (+/+)*	×	black (BL/+)	8	31	18 : 13 (+/+) (BL/+)
black (BL/+)	×	black (BL/+)	7	36	15 : 21 (+/+) (BL/+) (BL/BL)
black (BL/BL) (BL/+) (BL/BL)	×	black (BL/BL) (BL/BL) (BL/+)	12	50	0 : 50 (BL/BL) (BL/+)
wild (+/+)	×	wild (+/+)	50	206	206 : 0 (+/+)

* Probable conbination of genes. BL: dominant black gene.

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

Comparative Studies on DNA Damage Induced by Gamma-ray and ^{3}H-decay

Tsuneo KADA, Tadashi INOUE and Yoshito SADAIE

In order to evaluate damage induced in DNA by ionizing radiations and decay of radioisotopes incorporated in the cell, comparative studies have been carried out on inactivation of transforming DNA, induction and inactivation of spores of *Bacillus subtilis,* as well as on production of DNA single-strand breaks of colicin E1 plasmids by irradiation with gamma-ray and treatment with ^{3}H-glycerine.

In the transforming study, inactivation of an arginine marker was measured using cells of the strain H17 (*arg, try*) as recipient. For mutation induction, spores were prepared on Schaeffer medium by cultivating the strain with a supprressible *his* mutation and histidine reversions were studied. Circular DNA was extracted from *E. coli* harvoring colicin E1 plasmids and neutral sucrose gradient sedimentation analyses were made on irradiated DNA molecules. Irradiation or treatment with ^{3}H-glycerine was done in appropriate buffer at 0°C.

Based on the theoretical estimation that the absorbed energy of β-ray from ^{3}H (83 μCi/ml/hr) corresponds to 1 R of gamma-ray, we concluded that relative effectiveness of ^{3}H-decay is very similar to that of gamma-irradiation with regard to inactivation of the arginine marker of transforming DNA and to production of DNA single-strand breaks, indicating that the ^{3}H-decay effects are solely explained by β-irradiation.

On the other hand, at identical absorbed doses, ^{3}H-decay was more than 100 times more efficient in inactivating and in inducing mutation in spores. These results were presented at the 1976 annual meeting of Japanese Society of Radiation Research.

Enzymatic Repair Mechanisms of DNA Damage Induced by Ionizing Radiation: Identification of a "Cleaning Enzyme"

Tadashi INOUE and Tsuuneo KADA

Studies have been developed to elucidate enzymatic mechanisms of repair of DNA damages induced by ionizing radiation. Previous studies (Biochi. Biophys. Acta *395* 284, 1975) revealed the existence of endonucleolytic m exonucleolytic activities specific for gamma-irradiated DNA in extracts of *Bacillus subtilis* cells, both promoting the priming activity of irradiated DNA for type I DNA polymerase. The detailed properties of the endonuclease were described earlier (Biochim. Biophys. Acta *395* 294, 1975).

Further studies were made on the enzyme which had exonucleolytic activity on gamma-irradiated DNA. The enzyme was purified more than 340 fold by means of salt fractionation and chromatographies on DEAE- and phospho-cellulose. The purified enzyme preferentially degraded irradiated DNA exonucleolytically releasing mono-phosphorous substances and produced effective priming sites for DNA polymerase. Hypothesis is under examination in which this enzyme is a promissing candidate for the exonucleolytic "cleaning" of damaged nucleotides which was produced with gamma-irradiation.

Enhanced Mutagenesis, Repair Deficiency, Mutator Activity, and Thermal Prophage Inducibility in *DNA-8132* Strains of *Bacillus subtilis*

Yoshito SADAIE, Kikuko NARUI and Tsuneo KADA

The integrity of the bacterial chromosome is maintained by a number of cellular components. Isolation of *dna* mutants is an attempt to clarify the role of each component whose synthesis is controlled by respective *dna* loci. We have found pleiotropic effects of a *ts* mutation *dna-8132* at permissive temperature which was isolated by Hara and Yoshikawa and mapped in the region of chromosome replication origin of *Bacillus subtilis* (Nature New Biol. *244*, 200). This mutation arrested DNA synthesis (within 30 min) at a restricted temperature without any change in the *purA/metB* ratio and was classified to be neither of the initiation nor the continuation type. We have found that strains carrying this mutation exhibited a lower

capacity for repairing radiation or chemical damage at a permissive temperature. Introduction of *polA59* mutation (Gass *et al.* J. Bacteriol. *108*, 364) further enhanced the repair deficiency, indicating that the *polA59* or the *dna-8132* blocks independent and complementary pathways. The *dna-8132* mutation alone did not reduced cellular capacity of reactivation of UV irradiated phages, whereas the *polA59* mutation did. However, the effect of *polA59* was enhanced in the presence of *dna-8132*. Therefore, the wild-type *dna-8132* gene product appears to be involved in phage DNA repair in the absence of polymerase I activity. Spores of *dna-8132* strains were resistant to UV while *polA59* spores showed enhanced sensitivity to UV irradiation. The mutations *dna-8132* and *polA59* did not affect the resistancy of spores to gamma-rays. Spontaneous reversion mutations of a suppressible nonsense *his* mutation were observed at a higher frequency in the *dna-8132* strains and the frequency was much enhanced in the simultaneous presence of *polA59* mutation. These results suggest that the *dna-8132* gene product may be involved directly in the chromosome replication and repair. SPO2 lysogens carrying this mutation produced mature phages upon a temperature shift from 30 to 48°C. Phage production at a nonpermissive temperature suggests that there are few defects in the precursors of DNA synthesis in the mutant. Base change type mutations (from His^- to His^+) were induced by chemicals and UV irradiation. Mutation was abnormally enhanced in the *dna-8132 polA59* double mutant strain. Thus it is suggested that the *dna-8132* gene product may be involved in a error prone repair and mutations observed may have originated from errors in the base selection. Though the *dna-8132* mutation was found to be linked to *rec-43* (J. Bacteriol. *125*, 489), no drastic reduction in recombination ability was observed in the strains with *dna-8132*. Some of the results were reported earlier (J. Bacteriol. *126*, 1037 and Ann. Rep. Natl. Inst. Genet. *25*, 51).

Detection of Environmental Mutagens and by the Rec-assay and Reversion-assay Procedures

Tsuneo KADA

We have been carrying out screening of chemical mutagens in our environment and detected upto now more than 30 new mutagens, by means of the rec-assay and reversion-assay procedures, including phloxine, AF2, DAPA, reaction products between $NaNO_2$ and sorbic acid, etc. (for review see T.

KADA, Scientific Publication No. 12 of International Agency for Research on Cancer, p. 105 1975 and Y. Shirasu *et al.* Proceeding of the 1976 Cold Spring Harbor Symposium, in press). The rec-assay screening was further carried out in this laboratory by Dr. N. Kanematsu of Gifu Dental College on about 80 metal compounds and some 25 of them were found to be clearly positive. They include compounds of chromium, arsenic, mercury, cadmium, etc. on which reversion-assays were made. Except those compounds whose mutagenicities are already known, some compounds of rhodium ($Rhcl_3$) and Vanadium ($VOCl_2$) were found to induce tryptophane reversions in *Escherichia coli* WP2 *try*. We have recently carried out the rec-assay screening on about one hundred food samples, homogenates prepared from vegetables, fruits, species and other natural food. Many species including garlic black pepper, cinnamon, paprika, nutmeg, clove, oregano, mustard, and curry gave positive results.

Waxy Starch Mutations in Rice

Etsuo AMANO

Induction of waxy starch mutations in rice plant, *Oryza sativa* var. Norin No. 8 has been continued and five new mutants were obtained in 1975. Following mutagen treatment was effective. Unhulled seeds which had been presoaked in water for 24 hours were soaked in 0.05 M ethyl methanesulfonate (EMS) aequous solution for five hours. After the mutagen treatment, seeds were rinsed and incubated in water for 20 hours. A 27°C shaking incubator was used for these treatments. Seeds were sown in soil in germinating flats and grown in them until harvest. A few panicles were obtained from each treated seed. Harvested and dried panicles hulled between two wooden plates with rough surfaces. Visually selected waxy starch mutant grains were confirmed by iodine staining. Each panicle was considered as a unit of mutation and all grains of the mutant segregating panicles were kept for further examinations.

When Norin No. 8 was treated, only a few endosperm mutants other than waxy starch could be detected visually. In the present experiments only four opaque endosperm of different degrees and a case of short and round grains were found. Compared to those five possible endosperm mutations, 15 waxy starch mutants were detected, including a very leaky case (74wx3) confirmation of which is under way. Of these 15 waxy mutants, seven were

leaky type of some degrees, or in other words mutations fixed somewehere between non-waxy starch and fully waxy starch. Ten mutants were planted in 1975, as M_2 or M_3 (one mutant) generations. In most of the mutants, some unfavorable growing characters and/or low fertilities were accompanied. However, segregation of these characters in homo- or heterozygotes of the waxy mutants suggested that most of these unfavorable mutations accompained might locate in different chromosomes or very weakly linked. These results were very alike to those of maize experiments and may be helpful in practical mutation breeding, provided frequent and various degrees of leaky mutations are taken into consideration.

It was noticed during examinations of hulled grains of heterozygotes for segregation scoring that in many waxy mutants some of the non-waxy grains could be classified as intermediate phenotype. In 1975, some of these intermediate grains were planted separately and all of them turned out to be heterozygous for the waxy gene. In this case, about half of the remaining non'waxy grains were also heterozygotes although the number of plants examined was not so many. Further examination of grains of waxy heterozygotes revealed that most of the waxy starch mutants obtained in the present experiments seemed to have gene dosage effect in endosperm. The expressions of heterozygotes differ from grain to grain and distributed rather continuously between waxy and non'waxy. However, in some cases, possible four classes of the genotype in triploid endosperm could be separated though with difficulty. Quantitative measurement of the phenotype and confirmation of these four classes are in progress. These intermediate phenotypes differ in their genetics and expression from those of the leaky waxy mutants. The latter ones showed uniform waxy characters within the mutant and could be established as homozygous mutant lines. Intermediate phenotypes of heterozygotes were genetically unstable and always segregated in the following generation into waxy, intermediate- and non-waxy endosperm classes.

Germination and Early Growth of Some *Podostemaceae* Seeds

Etsuo AMANO

Seeds of *Mourera fluviatilis* and two other South American species of *Podostemaceae* could successfully germinate in dilute nutrient solution. The seeds were about 1/4 mm or smaller in length and had a layer of cells

which imbibed water easily, swelled and seemed to secrete some kinds of gluing materials. Thus they could be attached to a slide glass with a drop of distilled water. Once dried, the seeds sticked on the glass all through the surface disinfection procedure and incubation in the culture solution which was basically 1/10 dilution of inorganic recipe of Murashige and Skoog (1962).

Newly obtained *Podostemaceae* seeds from Sri Lanka were tested with the same culture methods. All five groups, or species (unidentified) had capsules and seeds as small as the smaller species in *Apinagia,* a South American genus. Sticking property of wet seeds was the same as those of the South American species. Dry slide glasses on which seeds were attached were surface disinfected by immersing into mixture of one part of ethanol and one part of 3% H_2O_2 for five minutes. Glasses were then rinsed twice in distilled water. After three days of incubation in culture solution in Petri dish kept at 25°C and under continuous illumination, germination started. Basic patterns of the germination were the same for all the species tested. Young plant emerged from seed coat with its root end first. However, there was no conspicuous stem or root on the young plant but only root hairs appeared on the outer end of the part where two cotyledons met. The young plant sticked to the glass by these root hairs after emergence from seed coat. Sri Lanka species were different in shape of cotyledons and size of young plant from the South American species. *M. fulviatilis* and two *Apinagia* species had needle or cylindrical cotyledons which elongated after germination. Fully opened cotyledons reached to 2 to 5 mm from tip to tip. One species from Sri Lanka had cylindrical cotyledons but four other species opened rather flat cotyledons. Seedlings of all five Sri Lanka species were smaller than 1 mm. In all the species tested, leaves were produced from basal part of the cotyledons, crosswise and alternately, and grew in direction of either cotyledon. Disk shaped part between cotyledons grew longer into elliptical form and then the growing point rose in small angle. After this early flat and centipede like forms, they start expressing their adult characteristics.

Fresh *Podostemaceae* seeds showed good germination under the present culture condition, but for further growth they require ample volume of the culture solution and good aeration. Under these favorable condition *M. fulviatilis* grew up to 3 cm in diameter and produced characteristic spinous processes and papillae on their leaves. A species of *Apinagia* (*A. longifolia*)

reached to about 5 cm long with more than seven nodes. In still culture, *M. fulviatilis* seedlings could survive in the aseptic culture solution in deep Petri dishes for two years though growth was very slow. It had not been successful yet to grow the plants in open culture.

This study has been made in cooperation with the Kihara Institute for Biological Research, and seed materials were collected and kindly supplied by the expedition parties of the Kihara Institute.

Sex Expression in Monoecious-like Variants in Cucumber

Taro FUJII

Sex expression in the F_1 plants obtained from the crosses between original gynoecious strain (MSU) with male flowers of monoecious-like plant (MSU-713-5-M) has already been reported (Ann. Rep. 25, 56–57). Make flower development in the F_2 generation was examined successively using seeds obtained by selfed monoecious-like F_1 segregants. The data are diagrammatically given as follows;

	MSU-713-5 × MSU 713-5-M									
F_1 fruit No.	1					5				
No. of plants	Geno.		Mono.			Geno.		Mono.		
(segregation)	17		6			23		18		
F_2 fruit No.		1–8			5–1			5–7		5–32
(male flowers in F_2 plant)		(one male flower on a node)			(49 male flowers over 10 nodes)			(37 male flowers over 6 nodes)		(60 male flowers over 13 nodes)
No. of plants	Geno.		Mono.		Geno.	Mono.		Geno.	Mono.	Geno.	Mono.
(segregation)		5	10		3	10		8	18	7	5

Segregation of monoecious plants was observed in all the F_2 pupulations; even a plant with only one male flower (F_2 No. 1–8) produced a large number of monoecious plants. Although more than half of plants in each strain had shown genoecious habit in the F_1 generation, the number of monoecious types exceeded genoecious types in the F_2. This suggests that the tendency to monoecism is promoted by selfing.

The experiment was repeated again in the second experiment. The same

original strain (MSU-713-5) was exposed to 30 and 50 kR of gammarays. One of 34 M_1 plants in 30 kR lot developed one male flower; of 28 plants in 50 kR lot, 3 plants, designated as MSU-50A, 50B and 50C, developed male flowers similarly as in the first experiment. Selfed seeds were obtained from these plants. Monoecious segregants in the F_1 generation were 19, 21 and 23 in 50A, 50B and 50C, respectively among each 34 plants.

As mentioned above, monoecious-like plants were frequently observed in the irradiated (M_1) generation. If they were mutants, either induced or spontaneous, they would behaved as recessives. Moreover, their incidence was much higher than the usual mutation frequency. Though the mechanisms involved are still not clear, the results of the present experiment suggest that the monoecious-like variants might be due to a retain change in heredity. The change from genoecism to monoecism might be attributed to a radiation-induced change in the gene(s) controlling growth hormone metabolism.

Effects of Gamma-rays and Neutrons on the Seedling and Callus Growth in Rice Seeds

A. A. BARADJANEGARA, Taro FUJII and Etsuo AMANO

Seeds of rice c.v. Norin-8 and two radiation induced dwarf mutants MGS-46 and -95 were used to investigate the effects of gamma- and 14 MeV neutron-radiations in different culture systems. The following three different culture systems were adopted in the present study. (1) Irradiated seeds were sown in soil. (2) The surface of irradiated seeds was sterilized and the seeds were sown onto agar slant media in test tubes. Two types of media, modified-White's (M-W) and modified-Eriksson's (M-E) were used. (3) To examine effects of gamma-irradiation on the callus formation and growth, the third culture system was used consisting of the M-W and M-E media with 20 mg/liter and 2 mg/liter 2,4-D supplimentation. Seedling growth of irradiated seeds in soil, and in two types of synthetic media, as well as the callus growth on 2,4-D supplemented media were measured as an index of radiation damage. The seedling height in soil culture indicate considerable decreases in growth with increasing of gamma-ray doses in the parent Norin-8, and similarly in two mutant strains. However, two mutant strains were relatively more sensitive to the neutron treatment compared to the Norin-8. The seedling growth on media was indicated that the seedling height in the control lots of MGS-46 and -95 on the M-E medium were

almost twice of those found on the M-W medium, while the control lots of Norin-8 showed a slight difference between two types of media. In the irradiated lots, Norin-8 showed striking decrease in growth especially on the M-W medium, while growth of two mutant strains had repressed slightly on the same medium. The callus formation on the M-W varied with gamma-ray doses. In the unirradiated controls as well as in the lowest dose lots, 100% callus induction was seen in all strains examined. The callus formation ability was slightly reduced with 18 krad treatment and drastically depressed at 27 krad exposure. On the other hand, 100% callus growth formation took place on the M-E medium in every lot. The callus growth was clearly retarded after radiation treatment, and the reducing rates along with the dose increment were almost the same as those observed in soil culture. From these experiment, the M-E medium seem to be appropriate for both the seedling and callus growth of rice.

VIII. POPULATION GENETICS (THEORETICAL)

Mathematical Contributions to Population Genetics

Motoo KIMURA

Mathematical theories have played a vital role in the development of population genetics. In this paper, recent progress in the field of mathematical population genetics is reviewed, with special reference to the diffusion models treating the stochastic behavior of mutant genes in a finite populations. Also, recent developments of theories treating linkage disequilibrium due to random drift are reviewed. For details see Genetics 79: 91–100 (1975).

Distribution of Allelic Frequencies in a Finite Population under Stepwise Production of Neutral Alleles

Motoo KIMURA and Tomoko OHTA

A formula for the distribution of allelic frequencies in a finite population is derived assuming stepwise production of multiple alleles. Monte Carlo experiments were performed to check the validity of the formula, and excellent agreement was obtained between theoretical distribution and experimental results. The formula should be useful for analyzing genetic variability in natural populations that can be detected by electrophoretic methods. For details, see Proc. Nat. Acad. Sci. 72: 2761–2764.

Theoretical Analysis of Electrophoretically Detectable Polymorphisms: Models of Very Slightly Deleterious Mutations

Tomoko OHTA and Motoo KIMURA

The nature of mutation-selection balance was investigated using models of mutation which assume stepwise production of new alleles in the allele space occupied by one or two type genes and multiple slightly deleterious mutations. The distribution of allelic frequencies and the effective number of alleles at equilibrium were obtained based on deterministic treatments. It was shown that they depend on the ratio between the selection coefficient

and the mutation rate (s/v). When this ratio is large, the frequencies of the deleterious alleles become too low to form polymorphism; however, when the ratio is small, such a mutation-selection balance becomes important as a cause of polymorphism. In addition to analytical treatments, extensive Monte Carlo experiments were performed to investigate the stochastic effect due to small population size. When the effective population size (N_e) is small so that both $N_e v$ and $N_e s$ are small, the allele distribution is indistinguishable from the neutral case, while it approaches the deterministic equilibrium as N_e gets larger. The bearing of the present models on observations regarding protein polymorphisms was discussed. For details, see Amer. Natur. 109: 137–145.

Moments for Sum of an Arbitrary Function of Gene Frequency along a Stochastic Path of Gene Frequency Change

Takeo MARUYAMA and Motoo KIMURA

A diffusion model is developed to compute any moment of the sum of an arbitrary function of the gene frequency along sample paths between any two specified frequencies. This is used to calculate the mean age of a mutant of frequency x, including or excluding the possibility of its having been at value $x=1$ during the process, and the total frequency of heterozygotes involving an allele since its origin. For details, see Proc. Nat. Acad. Sci. 72: 1602–1604.

The Effect of Selected Linked Locus on Heterozygosity of Neutral Alleles (the Hitch-hiking Effect)

Tomoko OHTA and Motoo KIMURA

A diffusion model was developed to investigate the effect of a mutant substitution by natural selection on heterozygosity at a linked neutral locus. Using this theory, we made extensive numerical analyses to compute the expected total heterozygosity (i.e. the sum of the fraction of heterozygotes over all generations until fixation or loss) at the neutral locus. It was shown that the hitch-hiking effect is generally unimportant as a mechanism for reducing heterozygosity. The effect becomes significant only when the recombination fraction between the selected and the neutral marker loci is smaller than the selection coefficient. In order to check the validity of the

mathematical theory, Monte Carlo experiments were performed, and the results were in agreement. It has been suggested that linkage is important only in transient small populations such as at the time of speciation. For details, see Genet. Res. 25: 313–326.

Statistical Analyses of *Drosophila* and Human Protein Polymorphisms

Tomoko OHTA

By using the distribution function of allelic frequencies which was recently derived by Kimura and Ohta (P.N.A.S. 72: 2761) for the model of stepwise production of neutral alleles, the observed protein polymorphisms of *Drosophila* and man are tested for fit to the theory of neutral protein variation. The observed and theoretical distributions of alleles agree quite well except for the excess of rare alleles in the actual distributions. In human polymorphisms, the alleles with frequencies less than 1% are more numerous than expected, whereas in *Drosophila,* those with frequencies less than 10% are more numerous. It is pointed out that these results support the thesis that mutational pressure rather than balancing selection is the main cause for the maintenance of protein polymorphisms. For details, see Proc. Nat. Acad. Sci. 72: 3194–3196.

The Effects of Linkage in Multilocus System—Overdominance

Tsuneyuki YAMAZAKI

Computer simulations were performed with overdominant multiple alleles under multiplicative fitness model among tightly linked loci. A new measure of non-random association between multi-allelic loci is introduced: $\chi^2/N(n-1)$ was introduced as a new measure of linkage disequilibrium which can also be applied to multiple allele model, where N is the sample size, and n is the number of alleles (n is the smaller one when the number of alleles are different between two loci).

Findings of simulations are: (1) With multiple (three or four) alleles, the approach to the equilibrium is slower and the amount of disequilibrium established is weaker. (2) The number of complementary chromosomes is a function of number of alleles and population size. (3) As population size

increases the approach to the equilibrium is slower. (4) There is an optimum selection coefficient to minimize the transient fixation probability of alleles with linkage.

Distribution of Enzyme Polymorphism and Functional Difference in *Drosophila*

Tsuneyuki YAMAZAKI and Takeo MARUYAMA

Published data on enzyme polymorphisms in *Drosophila* species were examined with special reference to molecular evolution. These data were used to test several rival hypotheses regarding the maintenance mechanisms of genetic variation in natural populations. Three different quantities were used for these tests: (1) Number and frequency of alleles, (2) Distribution of heterozygosity, (3) Variance of heterozygosity.

The following conclusions were obtained from the above tests. (1) Analysis of the data collectively indicates that distributions of most of these polymorphisms are consistent with those expected under the selective neutrality of polymorphic alleles. (2) When the enzymes were classified into two groups according to their substrate specificity, namely substrate-specific and substrate-nonspecific ones, it has become apparent that there are more than one mechanism for the maintenance of these polymorphic alleles. It was found that substrate-nonspecific enzymes are maintained by the balance between the mutational production of selectively neutral alleles and random genetic drift, but that polymorphisms of substrate-specific enzymes appear to be maintained by the balance between mutational production of very slightly deleterious alleles and selective elimination. (3) More detailed examination by analyzing each enzyme separately has, generally speaking, confirmed the above conclusion and has shown that the possibility of balancing selection playing a major role for the maintenance of protein polymorphism seems unlikely at any of the 22 enzyme loci examined. Among them the most likely candidates whose rare alleles are found to be selectively disadvantageous are enzymes such as malate dehydrogenase, fumarase, octanol dehydrogenase, tetrazolium oxidase, and glutamate-oxaloacetate transaminase. The distributions of the various quantities at alkaline phosphotase, isocitrate dehydrogenase, and adenylate kinase loci are relatively closer to those expected under balancing selection than those of other enzymes. The consideration of the step-wise model of mutation rather than the infinite allele

model further decreases the possibility of balancing selection.

Distribution of Gene Frequency in a Structured Population

Takeo MARUYAMA

Steady state distribution of gene frequencies were obtained for populations of general geographical structure. Briefly, it consists of a finite number of colonies of arbitrary, finite sizes, and mating takes place in each colony independently and then migration occurs between colonies. We assume that the population structure is fixed so that steady state exists.

Diallelic Case

Assume that there are two alleles A_1 and A_2 at a locus under consideration, and that mutation rate from A_1 to A_2 is u and the reverse rate is v. Let N_i be the size of colony i ($2N_i$ genes) and x_i be the gene frequency of A_1 in colony i. Now let

$$X=\frac{1}{N}\sum_i x_i N_i ,$$

where $N=\sum_i N_i$ the total population size. Therefore X is the mean gene frequency of A_1 allele reference to the whole population. The X is a random variable and the change of this random variable forms a stochastic process.

Then the steady distribution of X is given by

$$\phi(X)=\frac{C}{H(X)}X^{4Nv\cdot X_1(1-X_1)/H(X_1)}(1-X)^{4Nu\cdot X_2(1-X_2)/H(X_2)} , \tag{1}$$

where $0<X_1, X_2<X$, and

$$H(X)=\frac{1}{N}\sum x_i(1-x_i)N_i . \tag{2}$$

Infinite Allele Case of Kimura and Crow (1964)

$$\Phi(X)=\frac{4Nu}{H(X)}(1-X)^{4Nu\cdot X_1(1-X_1)/H(X_1)} , \tag{3}$$

where $0<X_1<X$ and $H(X)$ is given by (2).

IX. EVOLUTIONARY GENETICS

Spectra of Heterozygosities and of Evolutionary Rates among Different Loci

Takeo MARUYAMA and Tsuneyuki YAMAZAKI

Kimura and Ohta (1971) suggest that protein polymorphism and protein evolution should be regarded not as separate phenomena, but as two aspects of a single process. They further argue that most of naturally occurring polymorphisms are transient and due to random frequency drift of selectively neutral mutants in finite populations. Correspondingly, Kimura (1968) and King and Jukes (1969) have argued that most of molecular evolutionary changes detected as differences in amino acid sequence in homologous proteins, are due to fixation of neutral mutants within each species. The purpose of this note is an attempt to examine the hypothesis. Crow and Maruyama (1971) showed that for neutral polymorphisms at a steady state the mutation rate (u) times the population size (N) can be estimated from the local and global genetic variations:

$$4Nu=\frac{1-f_0}{\bar{f}}\,, \tag{1}$$

where f_0 is the probability that two random homologous genes in a local region are identical by descent and $\bar{f}$ is the corresponding probability for two genes randomly chosen from the entire population. Importantly, this relationship is independent of the population structure. On the other hand, under the neutrality hypothesis of molecular evolution, Kimura proved that the evolutionary rate (R) or the rate of amino acid substitution is exactly equal to the mutation rate (v) and is independent of other factors. Both of these quantities are observable and are known to vary greatly among loci.

Considerable data from which homozygosity (local f_0, and global $\bar{f}$) can be calculated are available in published literature. *Dayhoff's Atlas of Protein Sequence and Structure* (1972) gives the evolutionary rates for a number of proteins. Since both of these quantities are proportional to mutation rate, if they are really two different aspects of a single process, their distributions spectra ought to be similar. The purpose of this note is to obtain the distributions from available data and to examine whether they appear to be

identical or not. This possibility was discussed by Maruyama and Yamazaki (1973) and Harris *et al.* (1974).

It is convenient to compare the distributions on a logarithmic scale. Then if u and v have the same distribution, the distribution patterns of $\log (1-f_0)/\bar{f}$ and $\log R$ should be identical, but one is shifted by a constant ($\log 4N$) from the other. Gene frequency data for some 200 loci were collected from the literature and the value of $(1-f_0)/\bar{f}$ was calculated for each locus. We regard different species of the same enzyme as sample paths of the process and the average value as an estimate of $4Nu$. There are 18 loci for which four or more sample paths are available in all *Drosophila* and only these were used. They are presented Figure 1(a). In Dayhoff's *Atlas* (p. 50), the rate of amino acid substitution is given for each amino acid site. Therefore to make them comparable to polymorphism data which are generated on a protein basis, the value (r) given in the *Atlas* is multiplied by the number (n) of amino acid residues of that protein. On the neutrality hypothesis this value $R(=rn)$ should be the mutation rate per locus per year. Since the rates in Dayhoff's *Atlas* are calculated from mammalian data and mammals have approximately the same length of generation time, the value of R should be proportional to mutation rate per gene per generation which is therefore comparable to the value of u. The distribution of $\log R$ among different proteins is presented in Fig. 1(b).

The variances of the two distributions were calculated. The variance of the estimated $4Nu$ values is 1.51 with 17 degrees of freedom, while that of the evolutionary rates is 2.13 with 16 degrees of freedom. The variance ratio is 1.41. The data are undoubtedly insufficient to draw a definite conclusion. Nevertheless if we take these statistics at face value and apply the variance ratio test, the probability of finding this much difference is more than 20 percent. Therefore the result can be regarded as a supporting evidence for the hypothesis of Kimura and Ohta. Finally it is worth noting that larger variance in the evolutionary rates is mainly due to one very slowly evolving protein, histone. When histone is excluded, the two distributions have almost the same variance and a similar pattern. Furthermore, if the displacement constant 15 is interpreted as $\log_e 4N$, then N is about 10^6, not an unreasonable value for the average effective population number.

The proteins used in constructing these graphs are different, as are the species. The polymorphism graph being based entirely on *Drosophila* data and the evolutionary rates from mammals. Therefore, our comparison of

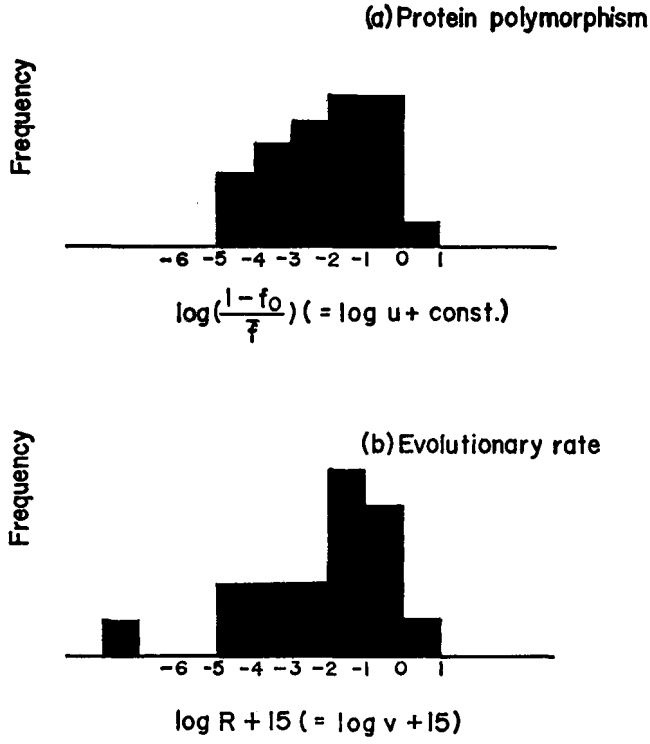

Fig. 1. (a) The distribution of the values of $\log_e$ (average $(1-f_0)/\bar{f}$) which is an estimate of $\log_e u + \log_e 4N$. The f_0, $\bar{f}$, u and N are the average local homozygosity, the global homozygosity, the mutation rate and the population size respectively. (b) The distribution of the values of $\log_e R$ which is an estimate of $\log_e v$. The R is the evolutionary rate and v is the mutation rate.

apples and oranges is meaningful as a test of the neutral hypothesis only if the distribution of mutation rates is comparable for the two groups of proteins in the two groups. It would be better to have the same proteins and the same kinds of animals for comparison.

For this reason we realize that the evidence offered in this note is weak. Furthermore, monomodal distributions skewed to the left are common for logarithmically transformed data, and we have very little statistical power to distinguish differences in shapes. Doubtless there are other hypotheses that could generate graphs of roughly the same shape.

We offer this analysis as weak supporting evidence for a common explanation of both phenomena, but mainly as a new methodology. We hope that in the future data on polymorphism and evolutionary rates for the same proteins will be obtained in sufficient abundance that this kind of comparison can be useful.

As more data become available it should be possible to look for correlations between log R and log $(1-f_0)/\bar{f}$ for individual proteins, and to see if the displacement of one distribution from the other is larger for species with large population numbers than for those with small numbers.

Colonization of *Drosophila simulans* in Japan

Takao K. WATANABE and Masaoki KAWANISHI

In Japan *Drosophila simulans* had been known only in the Bonin Isls. (Ogasawara) since its discovery in 1936 (Komai 1937, DIS 8, 78). Our collection survey work of *Drosophila* from Katsunuma during the past twelve years have failed to obtain *simulans* there. Unexpectedly, since 1972 *D. simulans* has been frequently collected in the mainland of Japan. Although the first landing place of *simulans* has remained unknown, the species has already been distributed in many places of Japan.

In November of 1975 *Drosophila* flies were collected at 29 sites in Yamanashi and eastern Shizuoka Prefectures. A total of 6129 flies was identified as members of the family *Drosophilidae*. *D. immigrans* was most abundant everywhere, and *D. melanogaster* and *D. simulans* followed. The latter two species were nearly the same in the total number, though their relative frequency varied among the sites. *D. simulans* tended to be frequent in the sites nearby the Pacific coast, *e.g.* Mishima (97% *simulans*), whereas *simulans* was not collected in the inland area, *e.g.* Katsunuma (100% *melanogaster*). Yet, the declination of *simulans* frequency toward Katsunuma occurred along several routes from the Pacific coast. For details see Proc. Japan Acad. 52: 191–194 (1976).

X. HUMAN GENETICS

Monitoring Cancer Families

Ei MATSUNAGA

Individuals with certain genotypes or certain chromosomal aberrations are known to be at increased risks of developing specific cancers. The genotypes may be heterozygous for a dominant gene with high penetrance in which multiple tumors (e.g., bilateral retinoblastoma and polyposis of the colon) are developed, or they may be homozygous for a recessive gene such as that for Fanconi's anemia and Bloom's syndrome in which leukemia may develop. For common cancers such as those of the stomach, the breast and the uterus, heredity plays in general a minor role, but studies with a variety of genetic markers, notably those related to enzyme variants and the HLA system, may eventually identify specific genotypes with which cancer formation is closely associated.

These 'cancer genotypes' are usually ascertained by routine diagnostic procedures. If the genotype can be detected by a simple and cheap test with high sensitivity and high specificity, and if some kind of effective treatment is available before they manifest the disease, screening populations or groups at risk can be undertaken as a preventive measure. No such test is, however, at present available for genetic markers closely associated with common cancers. For rare dominantly inherited cancers such as retinoblastoma and polyposis of the colon, population screening by clinical examination is obviously not feasible. However, since relatives of patients with those cancers form a special group at risk, family screening as an extension of genetic counseling would be rewarding; it may allow detection of those at the initial stage of tumor formation and may identify carriers of the gene among apparently healthy individuals.

At present most cases of heritable cancers occur as a result of fresh mutations. Yet, it is clear that, with increasing success of medicine, more and more cases inherited from the survivors will be emerging unless some preventive measures are taken. Therefore, monitoring of cancer families over a long term is of great importance, not only for the families themselves but also for the whole community.

Retinoblastoma in Japan: Follow-up Survey of Sporadic Cases

Ei MATSUNAGA

Previously we presented some genetic and epidemiologic data obtained by a population survey of cases of retinoblastoma which occurred in Hokkaido during the period from 1945 to 1957. Since then we carried out a follow-up survey of these patients, together with a new series of cases, that is, 206 inpatients with retinoblastoma who had been treated in seven university hospitals from 1900 to 1940. While some of the results of preliminary nature had been reported in the meantime, we have recently completed the analysis of the data.

The five-year survival rates for the 206 patients were about 50% for unilateral and 17% for bilateral cases, whereas for 74 patients occurred in Hokkaido from 1945 to 1958 the rates were 60% for unilateral and 32% for bilateral cases. The mean ages at diagnosis of unilateral cases were 34.8 months in the University Hospital series and 31.5 months in the Hokkaido series, while the corresponding figures of bilateral cases were 15.0 months and 17.1 months in the respective series. With respect to the prospect of marriage and fertility of the survivors of unilateral retinoblastoma, there was no essential difference from the general population for the males, but for the females, the chance of marriage seemed to be slightly decreased and, when married, the average number of children was significantly smaller than that of the male survivors, probably because of more eager practice of family planning on the female side. However, among 28 sporadic unilateral cases having children, only one was found to have transmitted the condition to children. Taking into account the distribution of number of children, the fraction of sporadic unilateral cases that can transmit the condition was estimated at 5%. As to sporadic bilateral cases, there were three survivors who produced children, and each had transmitted the disease to their children, suggesting that all sporadic bilateral cases are due to dominant mutations.

The frequency of retinoblastoma in Japan was 4.5×10^{-5} or once in every 22, 326 children who survived the first one year, and about 60% of them were represented by the non-hereditary form. Based on these data, a revised estimate of germinal mutation rate was given by the indirect method as 8×10^{-6} per generation, and the rate of somatic mutation was estimated, according to the hypothesis by Knudson (1971), at 5×10^{-6} per locus in the

course of an individual's development.

Details of the study will be published in the Jap. J. Ophthalmol. 20: 266–282.

Classification with the Size of C-bands in the Human Chromosome Variants and a Case Report of a *de novo* Yq-

Kazuso IINUMA and Ei MATSUNAGA

Heteromorphism of the secondary constrictions in the human chromosomes has been well known, and a new staining method, C-banding, has allowed quantitative evaluation of the size of heterochromatin blocks observed in these regions. After the method described by Sumner, A.T. (Exp. Cell Res. 75, 304, 1972), a total of 52 persons with normal karyotypes have been examined. The common size of the C-band of No. 1 was defined as slightly larger than that of No. 9. The latter had commonly a C-band with the similar size of its short arm. The C-band of the No. 16, in most cases, extended to the half of the long arm. Increases or decreases in the length of these secondary constrictions were expressed by symbols, h+ or h−, respectively. Without these definitions, such various combinations with apparent similarity as h/h−, h+/h or h+/h− could not have been distinguished. The frequencies of 1h+, 1h−, 9h−, 16h+ and 16h− were 0.02, 0.13, 0.14, 0.01 and 0.06, respectively.

A cytogenetic study on a patient with congenital anomalies revealed a deletion on the distal long arm of the Y chromosome. The propositus had severe psychomotor retardation, muscular hypotonicity, high-arched palate, hypertelorism, low-set ears, short upper limbs and hypogenitalism. He had a small Y chromosome, and Q-banding showed a deletion of the fluorescing long arm of the Y. The parents had normal karyotypes and the father's Y had a commonly observed fluorescing portion of the long arm. Results from family studies on the red cell antigens and other genetic markers including haptoglobin, transferrin and isoenzymes showed no inconsistency for paternity.

A part of this study was reported in the 20th annual meeting of the Japan Society of Human Genetics (1975).

Initiation of DNA Replication in Human Chromosomes

Yasuo NAKAGOME

While autoradiography has contributed much to the study of DNA

replication of mammalian chromosomes, the resolving capacity of the technique is rather limited. An alternative method with a higher resolution is now available. The fluorescence of the chromosome stain Hoechst 33258 is quenched by the incorporation of 5-bromodeoxyuridine (BrdUrd) in the DNA. Thus when BrdUrd is given during a part of the S-phase of a cell cycle and the chromosomes at the next mitosis are examined, the segments of chromosome containing BrdUrd appear as less fluorescent bands (Latt, 1973). A modification of this technique involving Giemsa staining has also been used. DNA replication of both random and partially synchronized cultures has been studied using these techniques. The G-positive bands (Paris Conference) of chromosomes appear to continue DNA replication until last part of the S-phase (Grzeschik *et al.* 1974; Epplen *et al.* 1975), while G-negative bands seem to start and finish replication earlier than the former (Kim *et al.* 1976; Epplen *et al.* 1976). On the other hand, Stubblefield (1975) concluded that there was no positive correlation between time of replication and G-band pattern in Chinese hamster chromosomes.

In the present study, DNA replication within the first 10 minutes of the S-phase was studied using synchronized human diploid cells (WI-38). A combination of two techniques, mitotic cell detachment (Terasima & Tolmach, 1963) and 5-fluoro-2′-deoxyuridine (0.1 μg/ml) treatment (Priest *et al.* 1967), was used to obtain good synchronization at the border of the G_1- and the S-phase. Cells entered the S-phase by the addition of small amount of thymidine (1.45 μg/ml). After 10 minutes BrdUrd (50–150 μg/ml) was administered. The "initiation sites" could be distinguished from the rest by more intense fluorescence when stained either with Hoechst 33258 or acridine orange. Alternatively, modified Hoechst-Giemsa technique was employed (Perry & Wolff, 1974).

The former two methods revealed exactly the same results. It appeared that every chromosome in the human genome, including late-replicating X, had segment(s) which initiated DNA replication within the first 10 minutes of the S-phase. The position, the shape and the size of these segments corresponded to those of Q (G)-negative bands suggesting that each of them constitutes a basic unit of initiation of DNA replication. It does not necessarily mean, however, that all of them initiate DNA replication at this time. Q-negative secondary constriction of Nos. 1, 9 and 16 start replication late in the S-phase. They have been shown to contain satellite DNAs (Jones, 1973).

When treated by the Hoechst-Giemsa method, several chromosome arms (1p, 5q, 6p, 11q and 12q) showed inconsistent pattern, i.e., the G instead of the expected R-type bands were observed in most cells. In control cultures that were treated similarly as the initiation-pattern experiment except that BrdUrd was not administered, some cells showed G-bands. Unusual pattern was also observed by Epplen *et al.* (1975) in a few arms including 1p. It may be that some chromosome arms have a tendency to show G-bands when stained with this technique irrespective of incorporation of BrdUrd (for details, see Nakagome: Exp. Cell Res., in press).

XI. BEHAVIORAL GENETICS

Noise Effect on Adult Emergence and Longevity of *Drosophila melanogaster*

Chozo OSHIMA and Won Ho LEE

Four strains, which have homozygous second chromosomes extracted from a natural population of Ishigaki-jima in 1973, were used in this experiment. Adult flies of these two strains emerged with circadian rhythms and of other two strains emerged with arhythmicity under light and dark 12: 12 environment. Many eggs, laid by many female flies for 12 hours, were performed to develope under uncircadian rhythmic white and pink or 2000 cycle pure noise (NQ, NQ 4: 4, 8: 8) 100 phon, constant dark (DD) and temperature (25°C) environments.

A total number of flies, emerged under noise environments was found to be fairly more than that under noiseless environment. Such a stimulus of white and pink noise was assumed to be stronger than pure noise. The noise accelerated remarkably the developmental rate of larvae and pupae, and also the aging of adult flies. Longevity of adult flies was clearly reduced by the noise in addition to constant dark and temperature environments. No significant difference was observed between the responses of four strains for the stimulus of noise environments, but two strains showing circadian rhythms in adult emergence, developed a little more quickly than other two strains showing arhythmic emergence.

Albinism and Learning Performance in Mice

Tohru FUJISHIMA

The relationship between coat color and learning performance, (discriminated avoidance, avoidance and discrimination) was examined in mouse hybrids. The four-way hybrids obtained from crosses among four inbread strains, C3H (agouti), SWM (albino), C57L (leaden) and D103 (albino), were classified according to their coat colors into agouti, cinnamon and albino groups. The results indicated that albino mice, as compared

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with pigmented ones were generally inferior in avoidance learning performance ($P<0.05$). However, when compared with their pigmented sibs from the same litter, the albinos were not inferior.

The inferiority in learning performance of albino mice to the pigmented ones has been reported by several workers. But the data of the present experiment indicated that the inferiority of albinos could differ according to the performances of inbred strains used and the analytical procedures employed.

XII. APPLIED GENETICS

Variations in Copper Tolerance among Strains of Wild and Cultivated Rice Species

Hiroko MORISHIMA and H. I. OKA

Forty strains belonging to *Oryza perennis, O. sativa, O. breviligulata* and *O. glaberrima* were tested for copper tolerance in two plots of gravel culture, in the same manner as were the barnyard grass strains. *O. perennis* showed a wide range of variation in copper tolerance. Some Asian annual types appeared to be highly tolerant, showing better performance in 6 ppm copper plot than in the control. The tolerance of *sativa* strains was comparable to that of susceptible *perennis* strains. *O. breviligulata* and *O. glaberrima* gave a medium degree of tolerance. Measuring copper uptake of the plants showed that *O. perennis* generally had a higher content (40–140 ppm) than *O. sativa* (30–60 ppm), and those with lower content tended to have a higher tolerance. But, copper uptake and tolerance were uncorrelated among the whole strains tested.

Further, 50 F_3 lines from non-tolerant *sativa* (108)×tolerant *perennis* strain (W106, annual) were tested for copper tolerance. The results suggested that a few genes controlling tolerance could be involved and one of them, a recessive gene, was linked with the gene for pericarp coloration, *Rd*. The F_3 lines also showed a wide range in copper uptake, but it was uncorrelated with copper tolerance. Also the copper tolerance and performance in the control plot appeared uncorrelated. Perhaps, there are several genes controlling different physiological responses which take part in copper tolerance.

Variations in Copper Tolerance among Strains of Barnyard Grass

Hiroko MORISHIMA and H. I. OKA

The seeds of *Echinochloa crusgalli* (6X) and *E. oryzicola* (4X) were collected from copper polluted and control rice fields at Ohta (Gumma prefecture), Tsuru (Yamanashi prefecture) and some other places, and also from a slag deposit of Hanaoka copper mine (Akita prefecture). The progeny of a single plant was considered a strain. This year, 69 strains were tested for

copper tolerance using two plots of gravel culture, one (control) with normal culture solution (Matsushima's prescription) while the other containing 6 ppm of copper ($CuSO_4$) in addition. The copper tolerance of the plants was shown by the copper-added: normal ratio in performance (in plant height, tiller number, dry matter weight, root weight, etc., measured at maturity). The data showed that: 1) A population could be highly heterogeneous not only for copper tolerance but also for other characters. 2) The frequency of tolerant plants was higher in polluted than in control fields. 3) When both 6X and 4X plants exist in the same population, 4X plants generally had higher tolerance than 6X plants. 4) Tolerant strains, when tested without copper, generally had a lower performance than non-tolerant ones. This resulted in a negative correlation between copper tolerance and the performance in normal conditions. 5) The plants in the copper-added plot showed at maturity a copper content ranging from 20 to 200 ppm (above-ground part, dried). The copper content was negatively correlated to tolerance, suggesting that the tolerance was at least partly due to a reduced copper uptake.

The Impact of Copper Pollution on the Weed Vegetation in Japanese Rice Fields

Hiroko MORISHIMA and Hiko-Ichi OKA

In several districts in Japan, river water was polluted by the drain of copper mines located on the upper stream resulting in the pollution of rice fields irrigated with the river water. Though the copper content of the soil (to the depth of 20 cm) is not very high (maximum about 450 ppm), a large area of rice farms, for instance, over 6,000 ha in the basin of the Watarase River (north of Tokyo), has become polluted. The grain yield of rice begins to significantly decline at about 150 ppm copper while those of wheat and barley at about 80 ppm. Under the auspice of the Environment Agency, we are working on the genetic effect of soil pollution on the weeds of rice fields. Our work includes observations of *Echinochloa crusgalli* and *Alopecurus aequalis* (with plants from collected seeds) and other weed species (with plants from soil-buried seed stock). The latter part of the work is outlined in this paper.

Soil samples were taken from rice fields in two districts, Ohta, Gumma prefecture, polluted by the Watarase River (Asio mine) and Tsuru, Yama-

nashi prefecture, polluted by the Ohata River (Takara mine). So far, 16 polluted (50 to 310 ppm) and 13 control (less than 30 ppm, from nearest unpolluted rice fields) were examined. The number of plants arising from the soil samples in two months, sorted into species, were recorded, and the plants of several major species were tested for growth rate on normal soil and copper tolerance (as shown by the ratio of growth rates on copper-containing and normal soils). The results are summarized as follows:

1) General—The number of plants of different species were examined assuming that their distributions were lognormal. The rice fields in different localities had a unique composition of weed vegetation (as shown by significant correlations between localities) which markedly differed from that of upland fields. Trends to positive or negative association of species were significant in 9 out of 66 species combinations, when the correlations among 12 major species were examined.

2) Diversity and dominance—The polluted soil samples produced a greater number of weed plants but a smaller number of species than the controls. Evaluating species diversity by the quantity of information ($H=-\Sigma p_i \log_e p_i$) indicated that diversity was significantly reduced by copper pollution. On the other hand, dominance (in terms of $1-H/H_{max}$; $H_{max}=-\log_e \beta$) was found to be promoted by pollution. In a polluted rice fields, the site nearby water inlet has a higher content of copper than water outlet. The above differences between polluted and control fields were similarly recognized when water inlet and outlet of the same fields were compared.

3) Change of vegetation—Polluted rice fields generally gave larger numbers of plants of *Vandellia angustifolia, Lindernia procumbens, Rotala indica* and *Fimbristylis miliacea* and a smaller number of plants of *Mazus japonicus* than unpolluted controls. The same pattern of changes was also found from a comparison of water inlet and outlet of the same fields. Analysis of variance of the data proved the significance of these changes. Significant changes in species association due to pollution were found in 4 species combinations.

4) Copper tolerance—*Cyperus difformis* was highly tolerant while *Fimbristylis miliacea* was most susceptible, and other species (among those given above) appeared to be intermediate. The evolution of tolerant plants in the polluted fields was recognized in three species, *i.e., Vandellia angustifolia, Mazus japonicus* and *Cyperus difformis.* Thus, neither the mean

tolerance for species nor within-species differentiation of tolerant plants did account for the above-mentioned increase and decrease in plant number of different species. Perhaps, a relatively low level of copper pollution as presently observed has its impact on sociological interactions among species.

5) Growth of tolerant plants—When tested on normal soil, the tolerant plants of *Cyperus difformis* and *Vandellia angustifolia* had a lower growth rate than non-tolerant ones. This resulted in a negative correlation between tolerance and performance on normal soil. It seems that the change in metabolism increasing copper tolerance is disadvantageous in normal conditions.

Genetic Loads in Wild and Domestic Japanese Quails

Takatada KAWAHARA

Birds of a wild strain reared for 2 to 4 generations in cages, which showed changes in characters toward the domesticated type as reported elsewhere (Ann. Rep. 1974), were compared with a domestic strain, and their reciprocal F_1 hybrids were investigated. The initial numbers of matings were 191 wild × wild (W), 85 domestic × domestic (D), 83 domestic ♀ × wild ♂ (DW) and 108 wild ♀ × domestic ♂ (WD), and the F_1 birds were subjected to full-sib mating. The amount of genetic load in terms of lethal equivalent was estimated by the regression method (Morton *et al.* 1956 Proc. Natl. Acad. Sci. U.S. 42: 855).

The estimated lower ($2B$) and upper [$2(A+B)$] limits of lethal equivalent per zygote, and B/A ratios as well, were calculated for various traits from the populations with $F=0$ and $F=0.25$ (Table 1). Lethal equivalents in wild strain were higher than those in domestic strain in all the traits examined. The domestic strain may have established themselves by eliminating a part of detrimental genes during their history of domestication. However, the F_1 hybrids had approximately the same lethal equivalent as of the domestic strain. All estimates of the B/A ratio were lower than 14.

Table 1. Estimates of lower and upper limits of lethal equivalent per zygote, and of B/A ratio

Trait	Strain	Lethal equivalent per zygote		B/A
		Lower limit ($2B$)	Upper limit $[2(A+B)]$	
Fertility	W	0.80	1.46	1.23
	D	0.24	0.48	1.03
	DW	0.44	0.86	1.06
	WD	1.12	1.48	3.07
Hatchability	W	5.24	6.32	4.85
	D	3.48	3.82	10.27
	DW	3.60	4.04	8.24
	WD	2.56	3.22	3.91
Viability	W	7.22	7.76	13.49
	D	3.78	4.20	9.16
	DW	4.10	4.54	9.50
	WD	3.32	3.74	7.81
Egg production rate	W	2.18	3.20	2.16
	D	1.24	1.46	2.05
	DW	0.88	1.28	2.19
	WD	1.24	1.58	3.77
Fitness index (Total)	W	15.44	18.74	4.68
	D	8.74	9.96	7.16
	DW	9.02	10.72	5.31
	WD	8.24	10.02	4.63

Viability: up to 20 weeks after hatching; egg production rate: hen-day basis up to 60 days after first egg; fitness index: fertility × hatchability × viability × egg production rate.

Esterase-D Isozymes in Japanese Quail

Takatada KAWAHARA, Seiki WATANABE and Takeshi SHIBATA

The erythrocytes and eleven internal organs from four strains, one wild and three domestic (DN, DS and DM), of Japanese qualis were examined for esterase-D isozymes by the use of starch gel electrophresis.

Three bands were detected in a region. No difference was found among the zymograms from erythrocytes and various organs.

The results of mating experiment indicated that these three bands were controlled by codominant autosomal alleles symbolized $Es\text{-}D^F$ and $Es\text{-}D^S$.

Three domestic strains differed in the frequency of these alleles *e.g.*, the frequency of *Es-D*^{F} was 0.264 in DN, 0.275 in DS and, 0.568 in DM, the differences being statistically significant. The frequency in the wild strain was 0.521.

Variation in Susceptibility of *Echinochloa crus-galli* to a Herbicide

Shinya IYAMA

Echinochloa crus-galli collected from various parts of Japan were investigated regarding its susceptibility to herbicide containing propanil which can kill it but does not damage rice plants. Lines were established on the basis of individual plants collected in the rice fields around Sendai (Northern Japan), Konosu, Ohta and Mishima (Central) and Kochi (Western Japan); twenty to thirty lines from each locality (or population) were tested. Fifty to 100 seedlings per line were grown in a plastic tray, and at the age of two weeks they were sprayed with a given amount of propanil solved in water. The susceptibility was evaluated by percent survival in two weeks after the treatment.

The results showed that 1) all the populations had a wide variation in percent survival (0 to 100%) and 2) they differed in average suceptibility, being high in Konosu, Ohta and Mishima and low in Sendai and Kochi. No line as resistant as the rice plant was found from the present material.

On the Efficiency of Selection for Elite Trees

Shinya IYAMA

The efficiency of a selection procedure for elite trees was investigated by computer simulation. The procedure is a generally used one for selecting elite trees from a standing forest: a candidate tree is compared with the mean of best three (m') taken from 20 to 30 adjacent trees regarding the volume of single tree, and if it exceeds a criterion, say 1.5 times as much as the mean, the candidate is selected. The intensity of selection resulting from this procedure is determined by the relative value of population mean (m) and its standard deviation (s) when a fixed number of adjacent trees are measured. In the case of twenty trees including a candidate, the intensity

of selection is theoretically expected to be 0.32%, 0.06% and 0.01% when $m=2s$, $3s$ and $4s$, respectively. It is suspected that by this procedure, selection is often based on a lower criterion than expected m' resulting in a reduction in effectiveness of selection. Computer simulation revealed that the reduction in the m' was 13.2%, 17.3% and 25.6% when $m=2s$, $3s$ and $4s$, respectively.

Variations in Anaerobic Germinability of Seeds found among Wild Rice Strains

Yoshio SANO

It is well known that the seed of rice can germinate in water by anaerobic respiration. But, how this capacity does differ according to genotypes is not known. The seeds of 40 strains of *Oryza perennis* (wild) stored for 10 to 15 years at 0°–5°C were tested in petri-dishes with moistened filter paper and in flasks filled with water at 25°C, with the seeds of a few strains of cultivated species (*O. sativa* and *O. glaberrima*) taken as the controls. The results showed that: 1) Generally, the seeds of *O. perennis* did not germinate well in water suggesting that they require more oxygen than those of cultivated species. 2) The American form of *O. perennis* showed this trend most strongly, while the African form showed better germination of submerged seeds. The Asian strains, which may be the wild progenitor of cultivated rice, appeared to be intermediate. 3) The capacity of anaerobic germination seemed to be a function of seed dormancy as the germinability differed according to the period of cold storage. Relatively new seeds (less than 10 years in cold storage) showed a low rate of germination in water.

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ABSTRACTS OF DIARY FOR 1975

February	14	226th Meeting of Misima Geneticists' Club
April	12	119th Biological Symposium
	20	120th Biological Symposium
May	16	121st Biological Symposium
	19	227th Meeting of Misima Geneticists' Club
June	16	228th Meeting of Misima Geneticists' Club
	20	229th Meeting of Misima Geneticists' Club
July	14	230th Meeting of Misima Geneticists' Club
November	15	122nd Biological Symposium

FOREIGN VISITORS IN 1975

February	23–28	CROW, J. F., Laboratory of Genetics, University of Wisconsin, U.S.A.
April	20–22	LI, W.-H., Center for Demographic & Population Genetics, University of Texas, U.S.A.
	24–25	KIRK, R. L., Dept. of Human Biology, Australian National University, Australia.
May	19	RANGASWAMI, G., Vice-Chancellor, Tamil Nadu Agricultural University, India.
June	23	STORMONT, C. J., Dept. of Veterinary Microbiology, University of California, U.S.A.
September	12	VILLIERS, J. N., University of Stellenbosch, South Africa.

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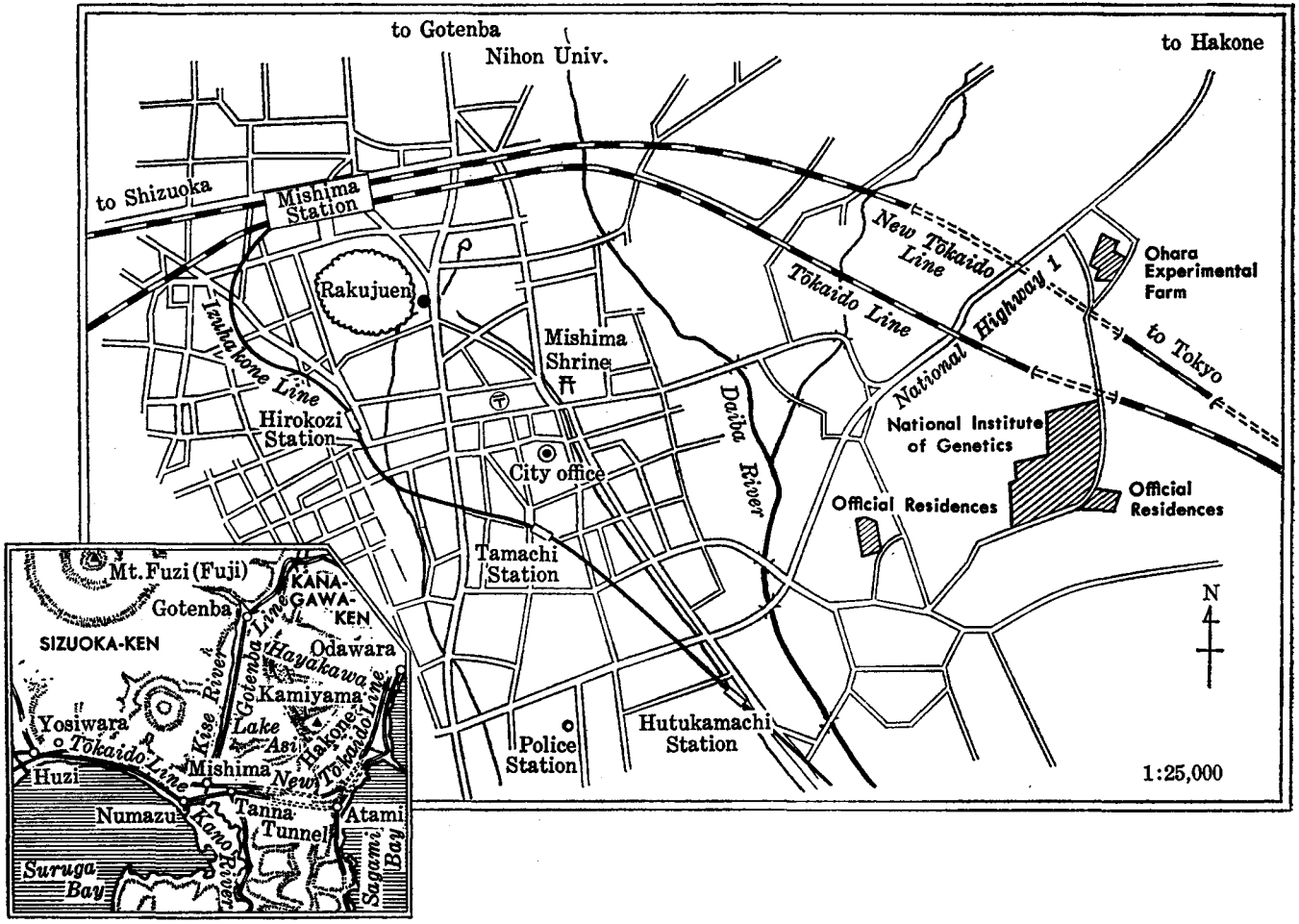
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