NATIONAL INSTITUTE OF GENETICS

JAPAN

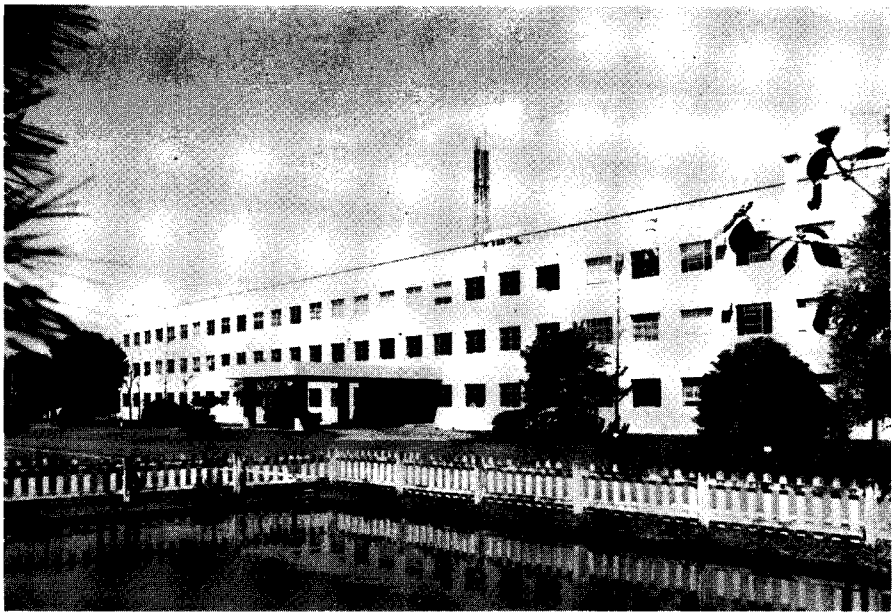
ANNUAL REPORT

No. 22 **1971**

Published by
THE NATIONAL INSTITUTE OF GENETICS
Misima, Sizuoka-ken, Japan
1972

Annual Report
of the
National Institute of Genetics

No. 22, 1971



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GENERAL STATEMENT

To our deep regret, Dr. Kan Oguma, the founder and the first director of our institute, died at the age of eighty-six years on September 10, 1971 at Odawara. Indeed Dr. Oguma was the creator of the new institute of genetics. The start was made on the occasion of the Twelfth Annual Meeting of the Genetics Society of Japan in October, 1939. In 1949 he took office as its first director. Devoted to his duties, in this short time of six years, he has placed the institute on a stable basis, assembling researchers of ability.

The new library was completed at the end of March of this year as scheduled. The first main floor is now occupied by an office room and a reading room. Rooms on the second and third floors are for conferences or seminars. The rear half of the building is constructed upon the design of four-storied bookrooms. On the wall of the third floor Dr. Kihara's portrait is immortalized in bronze, greeting the visitor with his famous saying, "The History of the Earth is recorded in the Layer of its Crust; The History of all Organisms is inscribed in the Chromosomes. Hitoshi Kihara, 1946."

Dr. Mitsuo Tsujita, Head of the Department of Biochemical Genetics, retired in March, according to the age limit. Devoting himself to his studies in this institute for twenty-one years since 1950 he has produced many valuable achievements and contributed his share to the institute prosperity.

This year three members of the staff of the institute traveled abroad as follows:

Dr. K. I. Sakai, March 1–21, and April 12—June 26, Formosa, for the purpose of genetic investigations of forest trees in Formosa on one hand, and for giving courses on the other hand at Chung-Hsing University.

Dr. Y. Tazima, June 13–30. U.S.A., to attend the 21st Session of UNSCEAR (the United Nations Scientific Committee on the Effect of Atomic Radiation) (New York).

Dr. E. Matsunaga, June 24—July 16, Switzerland, West Germany and Austria, to attend the Experts Committee of the WHO (Geneva): Sept. 4–26, France, West Germany and U.S.A., to attned the 4th International Congress of Human Genetics (Paris).

STAFF

Director

MORIWAKI, Daigoro, D. Sc., Emeritus Professor of Tokyo Metropolitan University

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

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FUKUI, Teijiro, Chief of the Finance Section

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KUWADA, Yoshinari, D. Sc., Member of Japan Academy, Emeritus Professor of Kyoto University

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OSHIMA, Chozo, Managing Director, Head of the Physiological Genetics Department
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MATSUNAGA, Ei, Manager, Head of the Human Genetics Department
SINOTO, Yosito, Manager, Professor of International Christian University
WADA, Bungo, Manager, Emeritus Professor of University of Tokyo

PROJECTS OF RESEARCH FOR 1971

Department of Morphological Genetics

Genetic studies of the silkworm (TAZIMA and ONIMARU)
Repair processes in radiation mutagenesis (TAZIMA and ONIMARU)
Studies on radiosensitivity in the silkworm (TAZIMA and MURAKAMI)
Chemical mutagenesis in the silkworm (TAZIMA and ONIMARU)
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Developmental genetic studies on carcinogenesis in tissue culture (KURODA)
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Department of Cytogenetics

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Chromosome studies in rodents (YOSIDA, KATO, TSUCHIYA and SAGAI)
Chromosome alteration and development of tumors (YOSIDA)
Cytogenetical study on monosomic and trisomic cultured mammalian cells (KATO)
Cytogenetical and immunological studies on mouse plasma cell tumor (MORIWAKI, K. and SADAIE, T.)
Biochemical studies on serum transferrin variations in rodents (MORIWAKI, K., SADAIE, and TSUCHIYA)
Experimental breeding and genetics of mice, rats and other wild rodents (YOSIDA, MORIWAKI, K., TSUCHIYA, SAKAKIBARA and TAKAHASHI)
Cytogenetical study of ants (IMAI)

Department of Physiological Genetics

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Mechanisms of persistence of some lethal genes (OSHIMA and WATANABE)
Physiological and population genetics of sterility genes in natural populations of *Drosophila melanogaster* (OSHIMA and WATANABE)
Analysis of fitness in a fluctuating environment (OSHIMA, WATANABE, INOUE and CHOO)

Analysis of inversion chromosome in natural populations of *Drosophila melanogaster* (OSHIMA and WATANABE)
Cytoplasmic inheritance in *Drosophila* (OISHI)
Nucleus substitution in wheat and related species (KIHARA, SAKAMOTO OHTSUKA and YOSHINO)
Cytogenetic studies in the tribe Triticeae (SAKAMOTO)
Studies on ecotypic differentiation in Japanese *Agropyron* (SAKAMOTO)

Department of Biochemical Genetics

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Genetical and biochemical studies of pteridine metabolisms in insects (NAWA and TSUJITA)
Studies on a gene for retarded moulting (*rm*) in the silkworm (TSUJITA)
Studies on the pteridine granule formation in larval hypodermal cells of the silkworm (TSUJITA and SAKURAI)
Analysis of gene action on cell differentiation in higher organisms (TSUJITA and NAWA)
Biochemical studies on the differentiation of muscle proteins in animals (OGAWA)
Genetical and biochemical studies of human serum proteins (OGAWA and ODAKI)
Genetical and biochemical studies on Japanese middle size dog (OGAWA)
Genetical and biochemical studies of the membrane protein of pteridine granules in the silkworm (SAKURAI and TSUJITA)
Genetics of isozymes in plants (ENDO and PAI)
Effects of exogenous DNA on plant seed formation (ENDO)

Department of Applied Genetics

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Genetic studies in wild populations of Japanese quails (KAWAHARA)
Theoretical studies on breeding techniques (SAKAI, IYAMA and FUJISHIMA)
Studies on competition in plants (SAKAI and IYAMA)
Genetic studies in natural stands of forest tree species (SAKAI, IWAGAMI and PARK)

Simulation studies on artificial selection (IYAMA)
Evolutionary studies in wild and cultivated rice species (OKA and MORISHIMA)
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Genetic analysis of isozymes in rice plants (PAI and ENDO)

Department of Induced Mutation

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Biochemical factors involved in cellular repair of genetic damage (NOGUTI, SADAIE and KADA)
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Studies on structure of human immunoglobulins (SHINODA)
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Genetics of cellular regulatory mechanisms (SUZUKI and ISHIDSU)
Genetics of bacterial flagella (IINO, ENOMOTO and SUZUKI)

Genetics of motility in bacteria (ENOMOTO)
Transduction mechanism of phage P22 (ENOMOTO and ISHIWA)
Genetics of phytopathogenic bacteria (WU and IINO)

Department of Population Genetics

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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)
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Simulation studies on linkage disequilibrium in artificial populations (YAMAZAKI)

Department of Molecular Genetics

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RNA polymerase in a virion containing double-stranded RNA (MIURA, SHIMOTOHNO, FURUICHI and KAGAYA)

RESEARCHES CARRIED OUT IN 1971

I. MOLECULAR GENETICS

Identity of the 3′-terminal sequences in the ten genome segments of silkworm cytoplasmic polyhedrosis virus

Yasuhiro FURUICHI and Kin-ichiro MIURA

Double-stranded RNA preparation isolated from cytoplasmic polyhedrosis virus (CPV) is a mixture of ten different genome segments as reovirus (I. Fujii-Kawata, K. Miura and M. Fuke: J. Mol. Biol. **51** (1970) 247). The 3′-terminal nucleosides of double-stranded CPV-RNA segments consist of 50% cytidine and 50% uridine (Y. Furuichi and K. Miura: J. Mol. Biol. **64** (1972) 619). The distribution of these two kinds of terminal nucleosides among the genome segments was investigated. The 3′-termini of RNA was oxidized and then reduced with ^{3}H-sodium borohydride. The 3′-terminally labelled RNA was separated into three fractions by chromatography on the methylated albumine-Kieselguhr column and into nine fractions by polyacrylamide gel electrophoresis. Each fraction was hydrolyzed with alkaline or ribonuclease T_2, which cleaves every internucleotide bond in RNA. The ^{3}H-labelled nucleoside trialcohols from the 3′-termini of RNA was analyzed by paper or thin layer chromatogrpahy. The results show that every double-stranded RNA segment has an equal amount of cytosine and uracil as its 3′-terminal base.

When the 3′-terminally labelled CPV–RNA was digested with pancreatic ribonuclease A, the result was quite analogous to the alkaline or ribonuclease T_2 digestion, giving equal amounts of C′ and U′ (trialcohol derivatives of cytosine and uracil). This suggests that all the penultimate bases of the 3′-termini are pyrimidine. Ribonuclease T_1, which splits polyribonucleotide at guanylic acid residues specifically, gave CpU′ and CpC′ as 3′-terminally labelled oligonucleotides. This leds to the conclusion that all the penultimate bases of the 3′-termini are cytosine.

From these results, the segmented genome RNA chains in CPV possess the common 3′-terminal structure as shown below:

5′ ——————————pCpU 3′
3′ CpCp ——————————5′

It is possible that the transcriptase of the RNA genome may require a definite and common site for initial binding to the template.

Single-stranded RNA synthesis in vitro by the RNA polymerase associated with cytoplasmic polyhedrosis virus containing double-stranded RNA

Kunitada SHIMOTOHNO and Kin-ichiro MIURA

RNA polymerase associated with cytoplasmic polyhedrosis virus, which contains double-stranded RNA as a genome, synthesizes single-stranded RNA *in vitro*. The optimal conditions for RNA synthesis by this enzyme were investigated.

The RNA product has the same size distribution as the genome segments, showing two fractions on glycerol concentration gradient centrifugation. Each fraction of the synthesized RNA hybridized specifically with the corresponding size fraction of the denatured genome RNA segments.

Only after transcription has been carried out over the whole length of each genome segment does the single-stranded RNA product leave the virus particle. It appears that the transcription of every segment starts at the same time, although the transcribed RNA from the shorter segment group is released from the virion faster than that from the larger group.

II. MICROBIAL GENETICS

A Non-chemotactic Mutant in Salmonella

Tetsuo IINO[1] and Tomoko OGUCHI[2]

Among the mutants of Salmonella strain SJ670 (Asakura, S. *et al.*, J. Mol. Biol. **16**: 302, 1966) which fail to swarm on semisolid nutrient plates (NGA), SJ4006 was found to be fully motile in nutrient broth. The mutant has flagella indistinguishable from the parent strain either in shape or number. It's growth rate and nutritional requirements were also unchanged from those of the parent. It was sensitive to flagellotropic phage chi. Capillary tube method of J. Adler (Nature, **153**: 708, 1966) was applied to examine chemotaxis of the mutant: it failed to make chemotactic bands in broth or in glucose solution (2.5×10^{-4} M). From these characteristics, the mutant was inferred to be generally non-chemotactic, comparable with that designated as *che*$^-$ in *Escherichia coli* (Armstrong, J. B. *et al.*, J. Bacteriol. **93**: 390, 1967).

Transduction was carried out with phage P22 from the representative deletion mutants covering various regions of the *fla-H1* cluster to the non-

Table 1. Transductions from various deletion mutants of the *fla–H1* region to SJ4006

Donor	Region of deletion	Relative frequency of recombinant
wild	none	100.0
fla373	*flaD, B, Q, P, N, AIII, AII, AI*	98.2
fla1319	*flaAIII, AII, AI, H1, flaL*	83.6
fla1174	*flaE, K*	9.3
fla1139	*flaE, K, motA*	4.0
fla302	*flaE, K, motA, B*	1.6
fla291	*flaE, K, motA, B, flaC*	0.0
fla234	*flaC, M*	0.0
fla1140	*flaC, M*	5.9
fla1149	*flaC*	9.8

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chemotactic mutant. Chemotactic recombinant clones were identified as swarms on NGA. The results indicated that the mutant site was covered by deletions of *fla291* and *fla234*, and it was mapped between *motB* and *flaC* (Table 1). The trails developing as the result of complementation in abortive transductants were observed in transductions where recombinant swarms appeared on NGA containing 0.2% (w/v) agar. Thus it is concluded that SJ4006 is a *che*$^-$ mutant in a cistron homologous either to *cheA* or *cheB* of *E. coli* (Armstrong, J. B. & Adler, J., J. Bacteriol. **97**: 156, 1969).

The number of recombinant swarms from the mixture of 10^9 phage particles and recipient bacteria at m.o.i. 3 were scored and converted to the percentage to the number in transduction from *fla*$^+$. The order of cistrons in *fla-H1* region is, *flaD-B-Q-P-N-AIII-AII-AI-H1-flaL-E-K-motA-B-flaC-M*.

Polymerization of External Flagellin at the Ends of the Hooks Attached to Salmonella Cells

Tetsuo IINO[1], Hideho SUZUKI and Shigeru YAMAGUCHI[2]

Exogenous flagellin monomers can be reconstituted to flagellar filaments at the tip of flagella attached to the living cells of Salmonella (Iino, T. *et al.*, 1971. Ann. Rep. Nat'l Inst. Genet. **21**: 18). The reconstitution experiment was forwarded with the reaction system in which the cells of a flagellinless mutant, SJW604, derived from a phase-1 stable Salmonella (antigen gt) were mixed with flagellin monomers of the wild strain TM2 (antigens i: 1, 2). The mutant strain has a defect in the structural gene of phase 1 flagellin, *H1*, and consequently produces hooks to which flagellar filaments are not attached.

To the mixture of 5×10^{10} cells/ml of SJW604 and 8 mg/ml of TM2 flagellin in 0.03 M phosphate buffer (pH adjusted to 6.6 with 1 M KOH), 1/25 volume of 2 M K_3-citrate was added and kept at 26°C. After incubation for 1 hour, 3.7% of the cells were found to carry reconstituted normal

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flagella; 15% among them carried two flagella and others only one. Average length of the reconstituted flagella was 2.2 normal wave units. After two hours of additional incubation, the fraction of flagellated cells increased up to 6.7% of the cell population and the average length of their flagella to 2.5 normal wave units. Most of them carried a single flagellum each and 21% of the flagellated cells carried two flagella. Very rarely cells with three flagella were detected. The cells incubated in the same condition without mixing flagellin were entirely nonflagellate as many as 1000 cells being examined. Thus, the experiment demonstrated that on the mutant cells flagella are reconstituted from exogenous flagellin without intermediation of ready-made flagellar filaments. When the *fla*$^-$ mutants, which cannot produce hooks as well as flagella, were used in place of SJW604, reconstitution was not initiated.

Isolation of Flagellar Hooks from Non-flagellate Salmonella Mutants

Shigeru YAMAGUCHI[1], Tetsuo IINO[2] and Tsuneyoshi KUROIWA[3]

A bacterial flagellum is composed of a filament, a hook, and a basal body. The filament is the distal helical part of the flagellum and is a polymer of flagellin. The basal body is the proximal structure, bound to the cell wall and membrane (DePamphilis, M. L. and Adler, J., 1971. J. Bacteriol. **105**: 384). The hook connects the filament with the basal body. Both filaments and hooks can be detached from flagellated cells by mechanical shaking (Abram, D. *et al.*, 1970. J. Bacteriol. **101**: 250).

In Salmonella, the non-flagellate phenotype results from mutation in either the *H* or the *fla* genes. The *H* genes, *H1* and *H2*, are the structural genes for phase-1 and phase-2 flagellins, respectively. The precise functions of *fla* genes are not known. Fourteen *fla* complementation groups have been recognized. So-called non-flagellate mutants lack the filament, but it is not known if they possess other parts of the flagellum. In the present study the possession of hooks by non-flagellate mutants, including both

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those representative of all the known *fla* complementation groups (*flaAI, AII, AII, B, C, D, E, F, K, L, M, N, P,* and *Q*) and the *H1* mutants derived from phase-1 stable strains of *Salmonella abortus-equi* SL23, was examined by electron microscope observation of material mechanically detached from bacteria.

The procedures for the isolation of hooks were as follows. A suspension of bacteria in saline was shaken at 750 strokes/min and centrifuged at $10,000 \times g$. The supernatant was further centrifuged at $98,000 \times g$ for 1 hr and the pellet was resuspended in 0.1 M Tris-hydrochloride (pH 7.8). This suspension was then given two cycles of differential centrifugation first at $10,000 \times g$ for 10 min to sediment the bacterial debris and then at $98,000 \times g$ for 1 hr to sediment the hooks. The final pellet was resuspended in a small volume of 0.1 M Tris-hydrochloride (pH 7.8), to which lysozyme (0.02%) and Brij-58 (1%) were added to lyse the contaminating fragments of cell wall and membrane. After incubation at 30°C for 30 min, the suspension was centrifuged at $98,000 \times g$ for 1 hr. The resulting pellet was resuspended in distilled water and observed with an electron microscope.

Hooks were found in all preparations obtained from *H1* mutants but none in those from *fla* mutants. Hooks obtained from *H1* mutants were of almost uniform length (72 ± 8 nm) and diameter (18 nm). Their size and shape were indistinguishable from those of the wild strain. One end of those hooks appeared to be frayed or split and the other end to be blunt. Surface patterns of hooks suggest a structure composed of several fine coils and twisted to form a short helix of relatively small pitch.

Hook and filament are not only distinguishable morphologically but they also differ in serological specificity (Lawn, A. M., 1967. Nature, London **214**: 1151) and solubility in acid and/or alcohol (Abram, D. *et al.*, 1970. J. Bacteriol. **101**: 250). However, it is still possible that both are composed of flagellin molecules in different conformational states. But the presence of hooks in *H1* mutants clearly shows that hook formation is not under the control of the flagellin structural gene and that hooks are therefore composed of subunits other than flagellin.

It has been shown that flagellar filaments have a structural polarity and are formed by sequential polymerization of flagellin molecules at the distal end of each filament (Asakura, S. *et al.*, 1968. J. Mol. Biol. **35**: 227; Iino, T. 1969. J. gen. Microbiol. **56**: 227; Emerson, S. V. *et al.*, 1970. Science **169**: 192). The structural similarity of the distal frayed end of the hook to

that of the filament may indicate the formation of the hook by sequential polymerization of subunit molecules. Hooks are about the same length irrespective of the presence or absence of the filaments. Therefore the possibility of the addition of flagellin to those distal ends being the factor limiting their maximum length is excluded. (The details were submitted to J. gen. Microbiol.)

Ribonucleic Acid Dependent Synthesis of Salmonella Flagellin by a Cell-Free System

Hideho SUZUKI

It has been known from serological and genetic studies that two structural genes, H_1 and H_2, concerned with flagellar protein of Salmonella do not simultaneously express their genetic information, namely only either of them is active at one time (phase variation).

To develop an assay which can determine the biochemical process(es) causing the regulated expression of these genes, the cell-free flagellin synthesizing system was constructed with preincubated S–30 of *E. coli* and RNA of Salmonella, after the method used for synthesis of *B. pumilus* flagellin (H. Suzuki and H. Koffler, 1970).

RNA was extracted by phenol method from the Brij 58-lysate of EDTA-lysozyme-treated Salmonella cells. Most part of activity to stimulate flagellin synthesis was attached to the RNA fraction insoluble in 2.5 M sodium acetate. Therefore, the sodium acetate-insoluble fraction was used as a source of an informational RNA. Newly synthesized flagellin was recovered with carrier flagellin from acid-soluble (pH 2.3) fraction, purified through reaggregation with ammonium sulfate and chromatographed on DEAE-cellulose column. Chromatography of flagellin at pH 8 on DEAE-cellulose could separate at least four serologically-distinct kinds of flagellin: a, enx, i and 1.2, eluting them in this order.

In vitro synthesized flagellin, as identified by chromatography, corresponded to the serological type of the cells from which the informational RNA derived. When RNA of phase 1 monophasic strain and that of phase 2 monophasic strain were introduced simultaneously to the cell-free protein synthesizing system, both phase 1 and phase 2 type of flagellin were synthesized. RNA extracted from the cells of a diphasic strain direct-

ed synthesis of both phase 1 and phase 2 flagellin, in such a ratio as expected if the culture was at equilibrium with respect to phase variation. The cells of the diphasic strain were propagated from a single colony expressing either phase 1 or phase 2. RNA prepared from these cells stimulated *in vitro* synthesis of flagellin predominantly in the same phase as expressed by the original colony.

These results indicate non-existence of a regulatory activity causative of phase variation at a translational level, and support the notion that activity of the genes concerning phase variation is regulated by control of phase-specific mRNA production.

Phage-induced Alterations in *Xanthomonas citri*

Wen Chuan WU and Tetsuo IINO[1)]

Temperate phage PXC7 was isolated from lysogenic strain XCJ18 and propagated on sensitive strain XCJ19 of the citrus canker bacterium *Xanthomonas citri*. When it lysogenized XCJ19, the tenetic characteristics of this bacterium were modified by an alteration from smooth to dwarf colony types accompanied with the changes in some of bacterial cells occurring in chains and response to virulent phage CP_2 from sensitive to resistant. The lysogenic dwarf convertants, incubated in nutrient broth, reverted in some cells to the production of smooth colonies, among which some consisted of lysogenic cells and others of non-lysogenic cells, either resistant or sensitive. Both lysogenic and resistant smooth revertants failed to adsorb phage PXC7 and gained resistance to CP_2. Moreover, they had also grown some of their cells in the form of chains. All of these changes, however, were not found with the sensitive smooth revertant.

The lysogenic dwarf convertant, after it arose from smooth strain XCJ19, continued to liberate phage PXC7 spontaneously at the frequency considerably higher than its lysogenic smooth revertant. The resistant smooth revertant did not liberate the phage. They did so even after having entered their susceptible host. As a result, the lysogenic dwarf convertant could not grow sufficiently to developing canker lesions as its lysogenic, resistant, and sensitive smooth revertants and smooth original strain. In addition, the

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lysogenic dwarf convertant also reverted in the host tissue in some cells to produce smooth colonies, which consisted either of lysogenic or sensitive cells. Smooth revertants arisen in such a way have the advantage of survival. The lysogenic smooth revertant protects istelf from phage infections. The lysogenic and sensitive smooth revertants may grow sufficiently to achieve normal canker development, if they enter again their susceptible host. On the other hand, the sensitive smooth revertant may also be relysogenized. If this does occur, the phage-induced alterations in *Xanthomonas citri* showing in culture media might also take place under natural conditions.

The details were published in Annals of the Phytopathological Society of Japan **38** (2, 4), 1972.

Transduction by Phage Pl in *Salmonella typhimurium*

Masatoshi ENOMOTO and Bruce A. D. STOCKER

The general transducing phage P1, active on *E. coli* and *Shigella*, does not attack smooth *S. typhimurium* lines, which fail to adsorb it. However, phage P1 (grown on *S. typhimurium*, to circumvent DNA-restriction) plates with high efficiency on *S. typhimurium* LT2 mutants with some sorts of lipopolysaccharide core defect: classes *rfaH* and *rfaG*, deficient of galactosyl- and of glucosyl-LPS transferases, class *galU*, lacking UDP-glucose pyrophosphorylase, and class *galE*, lacking UDP-galactose epimerase. Transduction of nutritional characters by phage Plkc, at frequencies of 10^{-5} to 10^{-6} per plaque-forming unit, was obtained to LT2 recipients of these classes.

When motility was transduced by phage P1 from a motile LT2 *galE* donor to non-motile mutants of an LT2 *galE* line the rate of cotransduction of *H1* (phase-1 flagellar antigen gene) was 50–90% for all *fla* and *mot* loci tested (except for the unlinked *flaF*); the corresponding rates for transduction by phage P22 vary from 0.01% to 38%. *flaD* was co-transduced with *his* by phage P1. When phage P1 grown on a motile LT2 donor was applied to various *mot A fla* double mutants the number of trails (abortive transductants) produced was about the same as when the recipient was *motA*$^-$ but *fla*$^+$; when phage P22 was used the number of trails obtained from some double mutants was less than 1% of the number obtained from the *motA*$^-$ *fla*$^+$ recipient. These observations indicate that the chromosome

fragments in P1 transducing particles are longer than those in P22 transducing particles, as is expected from the known DNA contents of the phages.

Phage P1 grown on *E. coli* K12 or *Shigella dysenteriae* 16 was applied to *mot* and *fla* derivatives of the LT2 *galE* restriction-negative line. Swarms, i.e., complete transductants, were very infrequent. The appearance of trails indicated that *E. coli* K12 has (at least) genes functionally corresponding to *flaA*$^+$, *flaC*$^+$, *flaD*$^+$, *flaK*$^+$, *flaF*$^+$, *motA*$^+$, and *motB*$^+$ of *S. typhimurium* but *Shigella* has only *flaF*$^+$ or a part of it.

III. BIOCHEMICAL GENETICS

Amino acid sequence of human IgM immunoglobulins

Tomotaka SHINODA, Claudine PAUL, Akira SHIMIZU, and Frank W. PUTNAM

Amino acid sequence analysis of one IgM globulin and comparative sequence analysis data on other IgM globulins have given new evidence on the evolutionary relationships and genetic control of immunoglobulins. To assemble the sequence of the μ heavy chain, Fab and Fc fragments were prepared by tryptic cleavage at high temperature, 11 different fragments by CNBr cleavage, and more than 300 different peptides by digestion of these fragments or the whole μ chain and the whole IgM by trypsin, chymotrypsin, thermolysin and pepsin. Sequence analysis was done by conventional methods and by use of the automatic protein sequenator for CNBr and Fc fragments and large peptides. The results have permitted the ordering of the fragments and the peptides and the location of five intrachain disulfide bridges, four interchain bridges, and five glycopeptides. The effect of the disulfide bridges and of carbohydrates on IgM conformation has been studied by molecular model building. Comparative structural study of several μ chains supports the concept of the identity of the constant region (Cu) of different μ chains and the division of the N-terminal region (V_H) into variable-sequence subgroups shared by μ, α, and γ chains. (Science **173**: 629 (1971); Prog. Immunol., **1**: 291 (1971); Am. N. Y. Acad. Sci., **190**: 83 (1971)). The entire work was carried out at Zoology Department, Indiana University.

Genetic Polymorphism of Serum Transferrin in the Field Mouse, *Apodemus giliacus*

Kazuo MORIWAKI, Tamiko SADAIE and Isamu HAYATA*

Remarkable chromosomal polymorphism in the field mouse, *Apodemus giliacus*, collected in Hokkaido has been preliminarily reported by Hayata *et al.* (Proc. Jap. Acad. **46**: 567, 1970). Starch gel-electrophoretic survey

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of serum in these mice revealed an apparent transferrin polymorphism; namely fast moving band designated TfA, slow moving band TfB and the mixture of them TfAB. The identity of transferrin was proved by analysing the electrophoretic pattern of Fe^{59}-tagged serum by autoradiography. Both of them have moved between haptologin and slow-α_2-macroglobulin on the gel. Typical electrophoretic pattern of TfAB type-serum is shown in Figure 1.

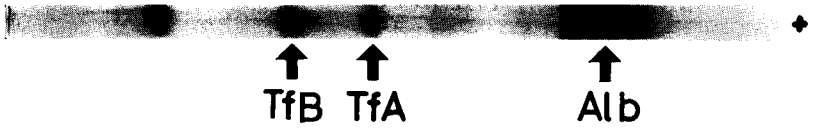

Fig. 1. Starch gel electrophoretic pattern of TfAB type serum of *Apodemus giliacus*.

Table 1. Progeny test of serum transferrin variants in *Apodemus giliacus*

Parents	Number of Litters	Progenies			
		TfA	TfAB	TfB	Total
TfA × TfA	3	18	0	0	18
TfA × TfAB	2	6	3	0	9
TfAB × TfAB	1	1	2	2	5

Among 5 mice collected in the field, 3 were TfA and 2 were TfAB. Laboratory crosses of these mice partly succeeded and demonstrated segregation of the transferrin types in the progenies as shown in Table 1. These results might suggest that the mode of inheritance is based on a pair of codominant alleles, Tf^A and Tf^B. There seems to be no linkage relationship between the biarmed chromosomes characteristic in this species and those transferrin types.

Electrophoretic Pattern of Serum Protein in Indian Brown Spiny Mouse, *Mus platythrix platythrix*

Kimiyuki TSUCHIYA, Tamiko SADAIE and Kazuo MORIWAKI

Starch-gel electrophoresis of serum protein of Indian brown spiny mouse, *Mus plathythrix platythrix*, kindly sent by Mr. Tachibana in Mysore, India,

was carried out by the routine procedure in our laboratory (Moriwaki, K. *et al.* Genetics **63**: 193, 1969). Figure 1 demonstrates the electrophoretic pattern where the sera of *Mus musclus* (BALB/c mouse) and of *Rattus rattus rattus* (Oceanian type black rat, $2n=38$) were run in parallel as references. Identification of transferrin among those electrophoretic bands

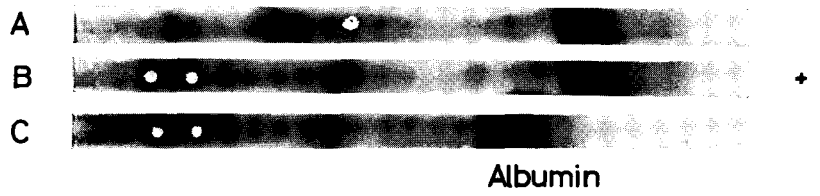

Fig. 1. Starch gel electrophoretic pattern of serum protein in Indian brown spiny mouse, *Mus platythrix playthrix*. A: *Mus musclus* (BALB/c mouse bearing γA-myeloma), B: *Mus platythrix platythrix*, C: *Rattus rattus rattus* (Oceanian type black rat, 2n=38). White dots indicate transferrin identified by Fe^{59}-labeling.

was conducted by Fe^{59}-labeling of the protein on the starch-gel plate followed by autoradiography with X-ray film. It seems to be interesting from the view point of species differentiation that the electrophoretic mobility of albumin in *Mus platythrix platythrix* was very similar to that in *Mus musculus*, whereas that of transferrin in the former species was rather close ot that in *Rattus rattus rattus*.

Androcidin: An exotoxin-like product of SR-spirochetes of *Drosophila* that kills male fly zygotes?

Kugao OISHI[1]

The SR-condition in *Drosophila* by SR-spirochetes is a phenomenon characterized by an absence of male progeny. SR fly strains with transovarially transmitted SR-spirochetes are maintained by mating them with males from normal strains. Infection with SR-spirochetes results in the selective death of male zygotes.

It has been proposed that SR-spirochetes produce an exotoxin-like substance, androcidin, which kills male zygotes (Oishi, 1971). Thus, if one

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eliminates SR-spirochetes from hemolymph of the SR flies and introduces the spirochete-free hemolymph into normal flies, it may be expected that the injected normal flies will produce only female progenies after a short lag as long as the androcidin activity remains high, and then progenies in a 1; 1 sex-ratio as the androcidin activity is diluted out.

ORNSR flies (Oregon-R strain of *D. melanogaster* carrying NSR strain of SR-spirochetes) were first injected with spv-2 (a virus strain normally associated with WSR strain of SR-spirochetes, this virus multiplies in and lyses NSR spirochetes) to eliminate NSR spirochetes from the fly. Normal Ore-R females (1–5 days old) were injected with the NSR-free hemolymph thus obtained, mated, and reared individually at 25°C. Flies were transferred into new culture bottles every two days and progenies examined for the sex-ratio according to the brood.

In a recent experiment, out of 34 flies injected 11 produced enough progenies and of which 7 produced progenies in an expected a manner.

The results strongly support the working hypothesis mentioned above. Refinement of the procedures and techniques is being made.

Migration rate of acid phosphatase isozymes controlled by a gene in *Oryza sativa* and *O. perennis*

Chiang PAI and Toru ENDO

In our previous papers (Shahi *et al.* 1969, Jap. J. Genet. **44**: Endo *et al.* 1971, *Ibid.* 46), variations in leaf acid phosphatase were examined in *sativa* cultivars including the Indica and the Japonica types. The both types each showed a specific zymogram consisting of three major bands accompanied by at least three minor bands. The difference between the two types was in the migration rate of the set of three major bands. Extensive examination of zymographic variations was continued with hybrid materials between strains of *O. sativa* and *O. perennis*. Five different zymograms on starch gel were so far detected in *O. perennis*. Two of them were identical to those of the Indica and Japonica types of *sativa*. The other three also had three major bands accompanied by at least three minor bands, and these bands appeared to migrate as a group at different rates according to genotype. The lowest migration rate was found in the Indica type of *sativa* and the second lowest in Af107 (*O. perennis* subsp. *barthii* from Africa). The migra-

tion rate was higher in the order of W628 (Asian *perennis*), Japonica types and W1426 (*barthii*). The F_1 hybrids of these strains always produced three hybrid bands and had nine major bands in total unless some of them overlapped one another. The F_1 populations examined produced only three different (two parental and F_1) zymograms. Although the action of genes controlling these isozymes remains unknown, yet the results obtained suggest the presence of a locus specifying the migration rate of a set of three major bands accompanied by minor bands.

Effect of foreign DNA on seed formation in egg plants

Toru ENDO

Repeated grafting experiments among different pepper strains strongly suggested the possibility of creating of so-called grafthybrids. Although the frequency was still very low, some characters of the strains used as stock appeared in the offspring of the scion or in the G_1 generation. Sexual F_1 hybrids of those strains were morphologically uniform, whereas the G_1 plants were variable and showed certain fruit characters which were not observed in the F_1 hybrids. Though the mechanism is not yet elucidated, graft-hybridization may be due to the translocation of decomposed nuclear fragments from the xylem tissue of the stock to the germ cells of the scion.

In order to evaluate this hypothesis, the following experiment was made on the stem 60 to 70 mm below the flower petiole of each plant by using a gimlet. The hole (2 mm) was covered with vinyl tube (10 mm diameter), rubber stopper, a mixture of lanolin-celite and aluminum foil. Foreign DNA solution (0.2 to 0.5 mg/ml SSC) was poured into the tube and was kept for 24 or 48 hours. The solution was then substituted with the same amount of SSC.

After this treatment, 5 to 10 days old fruits obtained from self-pollination were examined. The control (SSC only) produced normal fruits with 150 to 200 seeds. Treatments for 48 hrs with 0.5 mg animal DNA (calf thymus and salmon sperm) resulted in fruit abscission. Treatments with 0.2 to 0.5 mg animal DNA for 24 hr often interrupted fruit browth but seldom gave fruits half or one-third in size of the normal fruiss. They had 3 to 8 seeds only, but plants from the seeds appeared to be normal. Corn DNA

(isolated from 16 days old endosperm) was also tested. A preliminary experiment with corn DNA showed a comparatively weak retarding effect on fruit development. A continuous treatment with corn DNA (1 mg/ml SSC), however, resulted in the death of the upper part of the plant which extended toward the of the treatment. An additional treatment with plant hormone (6-benzyladenin and/or indole-3-acetic acid, 1 ppm) was ineffective.

IV. DEVELOPMENTAL GENETICS

In Vitro Studies on Spermatogenesis of *Drosophila melanogaster*

Yukiaki KURODA

Testes obtained from pupae of the Oregon-R strain of *D. melanogaster* were cultured by a previously described procedure (Kuroda, Y. (1970) Exp. Cell Res. **59**: 429). Those aseptically obtained from 48-hour pupae which were grown under sterile conditions were cut into several fragments in *Drosophila* physiological salt solution. They were found to be filled with germ cells at various maturation stages. The anterior fragments of the testes contained spermatogonia, about 5 μ in diameter. The middle fragments contained germ cells at more advanced stages of spermatogenesis, spermatocytes and spermatides, about 15–20 μ in diameter. In the posterior fragments spermatides at early stages of spermiogenesis were found.

These fragments of testes were cultured at 28°C in T–5 flasks with 0.8 ml of Medium K–17, which was slightly modified from Medium K–6′ and supplemented with 0.1 mg/ml fetuin, 5 mg/ml peptone and 15% fetal bovine serum.

After 24 hours of cultivation, none or only slight detectable changes were observed in anterior or middle fragments which contained spermatogonia, spermatocytes or spermatides, although the germ cells increased slightly in size and testicular sheath cells grew and extended on the glass surface of the culture flasks.

On the other hand, the spermatides in the posterior fragments expanded markedly toward both ends along the long axis of the sperm bundles, indicating that they attained more advanced stages of spermiogenesis. This result indicates that under culture conditions employed the process of spermiogenesis could be continuously traced under a phase microscope, whereas it is difficult to examine the early process of spermatogenesis.

Growth and Tissue-Specificity of *Drosophila* Embryonic Cells in Culture

Yukiaki KURODA

Embryonic cells of *Drosophila melanogaster* were cultured in a chemically defined medium supplemented with fetuin, peptone and fetal bovine serum. Dechorionated eggs at the stage of dorsal extension after gastrulation (4.5 hours after egg laying) and at the stage of sac-like midgut (12 hours after egg laying) were surface-sterilized, torn into small fragments and explanted on the glass surface of T–5 flasks. More active migration and division of cells were observed when tissues from the latter stage of embryos were used for cultivation.

At the early period of cultivation, fibroblastic cells and spindle-shaped cells came out from the cut end of tissue fragments, and stretched their cytoplasm on the surface of culture flasks. The fibroblastic cells strongly resembled in their morphology observed in the cultures of pupal ovaries. The slender spindle-shaped cells were specific for embryonic tissues in culture and have not been found in the cultivations of pupal ovaries and testes. Both types of cells increased gradually in number in further cultivation and formed cell sheets around the original fragments after 48 hours.

In addition to the above types of cells some nerve fibers were found to extend from fragments of nervous tissue. They were fine thread-like and branched off at their distal endings. The deposition of some small droplets was found on the nerve fibers which increased in size in further cultivation.

After 7 days of cultivation large muscle cells became noticeable. They were motile, made contact with each other and fused to form syncytial complexes, some containing more than ten nuclei. These muscle cells pulsated synchronously with other muscle cells in contact with them. Their pulsation continued for more than forty days in culture when the culture medium was changed every week.

When tissue fragments of *v* embryos were explanted in a medium which contained 0.1 mg/ml tryptophan, almost the same types of cells as those observed in cultures of wild type embryos came out from the explants and grew actively under the employed culture conditions.

An attempt was made to culture embryonic tissues from the SR strain of *D. melanogaster*. About one half of the embryos in this strain ceased their development about 4 hours after egg laying. They were assumed to

be male. When tissue fragments from presumed male embryos were cultured, a conspicuous difference was found in the types of cells that came out from the tissues. Neither growth of fibroblastic cells and spindle-shaped cells nor extension of nerve fibers were observed. After 9 days of cultivation, however, muscle cells grew abundantly and their active pulsations were observed.

Differential Inhibition by Hexosamines of Aggregation of Normal Liver and Hepatoma Cells in Rotation Culture

Yukiaki KURODA

Recently some quantitative and qualitative changes in glycolipids or glycoproteins in the surface membrane of cells have been detected following malignant transformation. In the present experiment the effects of some hexosamines and their derivatives on aggregation of embryonic normal liver cells and DAB-induced rat hepatoma cells were examined in rotation culture.

Single cell suspensions were obtained by trypsinization from 7-day embryonic quail livers and monolayer cultures of DAB-induced rat hepatoma cell lines, dRLa-74 and dRLh-84, and were rotated for 24 or 48 hours under standard conditions in the presence of hexosamines and their acetyl compounds at the concentrations of 1, 3, 10 and 30 mM.

The effects of D-glucosamine on aggregation of normal embryonic liver cells and rat hepatoma cells are shown in Table 1.

D-Glucosamine had inhibitory effects on normal liver cells at the con-

Table 1. Effects of D-glucosamine on aggregation of normal liver and hepatoma cells

Concentration of D-glucosamine	Inhibition of 24 hr-aggregation of		
	Normal liver cells	Hepatoma dRLa–74	Hepatoma dRLh–84
0 mM	−	−	−
1 mM	++	−	−
3 mM	++	−	−
10 mM	++	+	++
30 mM	++	+	++

centration of 1 mM and which increased gradually at higher concentrations. On the other hand D-glucosamine had no effect on aggregation of hepatoma cells at concentrations to 3 mM.

D-Galactosamine had more differential inhibitory effects on normal liver cells and hepatoma cells. At the concentration of 10 mM D-galactosamine affected strongly the aggregation of normal liver cells, whereas no effect was found on the aggregation of hepatoma cells.

N-acetyl-D-glucosamine was ineffective or slightly effective for aggregation of both normal liver cells and hepatoma cells at the concentrations tested up to 30 mM. Very interesting were the effects of *N*-acetyl-D-galactosamine. It had an inhibitory effect on aggregation only of heatoma dRLh-84 cells, which had a high tumor-producing activity, whereas no effects were found on the aggregation of normal liver cells and hepatoma dRLa-74 cells which had a low tumor-producing activity (Table 2).

Table 2. Effects of *N*-acetyl-D-galactosamine on aggregation of normal liver and hepatoma cells

Concentration of *N*-acetyl-D-galactosamine	Inhibition of 24 hr-aggregation of		
	Normal liver cells	Hepatoma dRLa-74	Hepatoma dRLh-84
0 mM	−	−	−
1 mM	−	−	−
3 mM	−	−	−
10 mM	−	−	++
30 mM	−	−	++

These results suggest that changes in some amino sugar composition of the surface membrane may be produced following malignant transformation.

Studies on Sorting-Out Mechanism of Animal Cells in Rotation Culture

Yukiaki KURODA

In the process of topographical heterogenization in homogeneous cell populations and formation of the highly specialized architectures in histogenesis and organogenesis of higher animals, the mutual cohesiveness and

sorting-out mechanism of constituent cells may play some extremely important role.

For analyzing the sorting-out mechanism of animal cells the time-lapse examination of the sorting-out process of HeLa cells and 7-day embryonic quail liver cells was carried out and the correlation between the sorting-out activity and aggregate-forming activity of both types of cells was examined in respect to their responses to some inhibitors for RNA and protein syntheses and to glucosamine.

When mixed cell suspension of HeLa and quail liver cells was rotation-cultured, quail liver cells started to aggregate at first, and 3 hours later HeLa cells in the same suspension started to cohere. Aggregates of two types of cells independently increased in size by cohesion of the same type of cells or cell clusters. This supports the hypothesis of "differential adhesiveness", and it is suggested that the substances responsible for the aggregate-forming activity and sorting-out activity may be qualitatively different among a variety of tissues.

Both the sorting-out activity and aggregate-forming activity of cells were inhibited by puromycin and actinomycin D. The inhibition by the antibiotics of the former activity was accompanied by that of the latter activity, suggesting that the substances responsible for both activities may be identical with or closely related to each other and produced through the processes susceptible to the inhibitors of DNA-dependent RNA synthesis and protein synthesis.

Glucosamine also affected the sorting-out activity and aggregate-forming activity of cells. Its inhibitory effects were concentration-dependent, and not reversible after the treated cells were transferred to normal medium. It was suggested that glucosamine may be substituted for *N*-acetyl-glucosamine present on the cell surface and that this process may be produced through some metabolic processes.

Further Studies on Radiation Effects on Sorting-out Activity of HeLa Cells

Yukiaki KURODA

In the previous studies the presence of a restitution process in the loss of sorting-out activity of HeLa cells following X-irradiation was revealed

when HeLa cells were exposed to fractionated X-rays. The most noticeable restitution was found when the time of interval between fractionated X-rays was 2 hours (For details, see Kuroda Y. (1971) Radiat. Res. **48**: 565).

In the present experiments the metabolic process of substance(s) which were relevant to the sorting-out activity of HeLa cells and susceptible to X-irradiation was investigated. HeLa cells which were irradiated with 2,000 R X-rays were intermixed with 7-day embryonic quail liver cells. The mixed cell suspensions were rotated by the standard procedure and sorting-out process of both cells was examined at an interval of 3 hours.

The sorting-out activity of HeLa cells for quail liver cells was retained for the initial six hours of rotation culture, then it was gradually lost in further cultivation.

When mixed suspensions of non-irradiated HeLa cells and quail liver cells were rotated in a medium containing 0.5 μg/ml actinomycin D, the essentially identical result was obtained: the sorting-out phenomenon persisted for the initial six hours, then it disappeared in further cultivation. When 10 μg/ml puromycin or 1 μg/ml cycloheximide was added to the culture medium, the sorting-out activity of HeLa cells for quail liver cells was inhibited immediately after addition of the antibiotics.

As a result of these experiments it was suggested that the substance(s) relevant to the sorting-out activity of the cells may be produced through the process susceptible to the inhibitors of DNA-dependent RNA synthesis and protein synthesis and that the damage in the sorting-out activity produced by X-rays may be in the process of DNA-dependent RNA synthesis (transcription).

Effects of High Molecular Fractions of Serum Protein on Growth of Mammalian Cells in Culture

K. MINATO

Supplementation of serum is necessary for the growth of most mammalian cells cultured in chemically defined media. It has been found in many earlier works that α-globulin and albumin fractions of serum were effective in promoting the growth of cells. It has not been known, however, whether the effect of these high molecular substances was due to their protein molecule, as carriers of some other low molecular substances such as vitamins

and hormones, or as nutrient sources for cell growth.

In the present experiment effective substances contained in the serum and their functional mechanism were analyzed in monolayer cultures of HeLa cells by adding some test substances to Eagle's (1959) chemically defined medium.

As for the concentrations of serum, 5% was the most effective for the growth of cells among concentrations tested in the range of 0.65–10%. At the concentrations lower than 5% the growth rate decreased in proportion to the logarithm of serum concentration. As for the density of cells at the inoculation, the lower the density was, the more delayed was the initiation of the logarithmic cell growth. At the cell density more than 1×10^4 cells per ml no difference was found in the growth of cells.

Effects of some fractions of serum protein on cell growth was assayed. The dialysed serum was almost as effective as whole serum, although the dialysate had little growth promoting activity. When serum was fractionated by precipitation with various concentrations of ammonium sulfate, a fraction from which low molecular substances were removed by saturated ammonium sulfate delayed the initiation of cell growth, with no detectable effect on the growth rate. A seurm fraction precipitated with 52% ammonium sulfate was ineffective in promoting cell growth.

These results suggest that the growth promoting substances in the serum may be in a fraction precipitated with ammonium sulfate at the concentrations between 52% and saturation. More detailed analysis is now under investigation.

V. CYTOGENETICS

Hybridization between several subspecies of black rats (*Rattus rattus*)

Tosihide H. YOSIDA and Koroku TAKAHASHI

The black rat (*Rattus rattus*) is divided into many subspecies, but the opinions of the taxnonomists are inconsistent. Some have claimed that some subspecies should be classified as independent species of the black

Table 1. Results of crossing experiments among several subspecies of *R. rattus*

Parents	Fertility	Breeding generation
R. r. tanezumi × *R. r. diardii*	Very good	F_4
R. r. tanezumi × *R. r. mindanensis*	Very good	F_2
R. r. tanezumi × *R. r.* Thailand	Very good	F_2
R. r. tanezumi × *R. r. rattus*	Good	F_2 (F_1 was semisteril)
R. r. mindanensis × *R. r. rattus*	Good	F_1
R. r. diardii × *R. r. rattus*	Good	F_1
(*R. r. tanezumi* × *R. r. diardii*) F_1 × (*R. r. tanezumi* × *R. r. rattus*) F_1	Good	Second generation
(*R. r. tanezumi* × *R. r. diardii*) F_1 × (*R. r. diardii* × *R. r. rattus*) F_1	Good	First generation

rat. For instance, subspecies *R. rattus diardii* in Malaya is preferred to be considered to represent species *R. diardii* by Yong (1969). To classify properly the animal groups it is important to know the result of matings between subspecies of the black rats. Therefore we collected several subspecies of them, bred them and then mated them in the laboratory.

The following subspecies were used in crossing experiment: Japanese black rats, *R. rattus tanezumi*; Malayan black rat, *R. rattus diardii*; Australian, New Guinean, and Hawaiian black rats, *R. rattus rattus*; Philippine black rat, *R. rattus mindanensis*; and Thailand black rat, *R. rattus* Thailand. The results of crossing experiments are given in the following table (Table 1).

Based on the above experiments it can be said that subspecies of black rats in Asia, namely *R. r. tanezumi, R. r. diardii* and *R. r. mindanensis*, and *R. r.* Thailand, which are all characterized by 42 chromosomes in diploid, are very similar and hybrids between them breed easily in the laboratory. Therefore, it is not necessary to classify them any independent species. Black rats in Oceania which are remarkable by having 38 chromosomes could be classified into separate subspecies, because the F_1 hybrids between the rats and Asian rats are semisterile.

Reference

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Karyotypes of hybrids between several subspecies of the black rats (*R. rattus*)

Tosihide H. YOSIDA, Tomoko SAGAI and Koroku TAKAHASHI

It was already reported in the previous papers (Yosida *et al.* 1965, 1969, 1971a) that the Asian black rats had $2n=42$ chromosomes, but pairs No. 1, No. 9 and No. 13 are remarkable by acrocentric and subtelocentric polymorphism. Pair No. 1 in almost all Philippine black rats (*R. rattus mindanensis*) was subtelocentrics, but most of the pair No. 1 in Thailand (*R. rattus* Thailand) and Japanese black rat (*R. rattus tanezumi*) were acrocentrics (Yosida *et al.* 1971b). Segregation of the acrocentric and subtelocentric chromosomes was just theoretical as was in the case of matings within the Japanese population. In the previous paper (Yosida *et al.* 1971b), the present author suggested that polymorphic chromosomes found in Japanese black

rats chould be caused by natural mating between black rats with acrocentric pair No. 1 originally had lived in Japan and those in islands of Southeast Asia characterized by subtelocentric pair No. 1. Based on the results of the present maing experiments the above suggestion seems to be correct.

Hybrids between Asian ($2n$=42) and Oceanian black rats ($2n$=38) are also easily produced in the laboratory and they had 40 chromosomes, but the F_1 hybrids were semisterile and only one F_2 hybrid was obtained from crossing between F_1 hybrids (Yosida *et al.* 1971c). The F_2 rat had 39 chromosomes which were characterized by M-1/A-4, A-7, and M-2/M-2. Acrocentric No. 4, No. 7, No. 11 and No. 12 pairs in the Asian black rat are shown by A-4, A-7, A-11 and A-12, respectively. The metacentrics arose due to Robertsonian fusion of No. 4 and No. 7 and No. 11 and No. 12 are denoted by M-1 and M-2, respectively. Three rats with the same karyotype as the above mentioned were collected in Eniwetok island, South Pacific, which seemed to be produced by natural hybridization of the rats with $2n$=38 and 42 chromosomes. One offspring obtained in the laboratory by mating between the rats with $2n$=39 which were collected in Eniwetok had also $2n$=39 and the same karyotype as the above.

In the laboratory it is difficult to obtain many F_2 rats, and therefore back crosses of the F_1 rats with $2n$=40 to Asian black rats with $2n$=42

Table 1. Chromosome constitution in 17 rats obtained by back cross (F_1 × Japanese rats)

Type	Chromosome constitution	No. of rats obtained
I	M-1/A-4, A-7, M-2/A-11, A-12	3
II	M-1/A-4, A-7, A-11/A-11, A-12/A-12	8
III	A-4/A-4, A-7/A-7, M-2/A-11, A-12	4
IV	A-4/A-4, A-7/A-7, A-11/A-11, A-12/A-12	2

were performed. From back crosses 17 rats were obtained and their chromosome types were analysed (Table 1). Production of rats with 16 karyotypes was theoretically expected from the above back crosses, but only four types were obtained. Frequencies of rats with the four karyotypes were also theoretically the same, but that of type II was the highest observed (50%). Explanation why frequency of the type II does occur so more often higher than those of the other types is difficult at the present time. It is important

to know that the above four types have the same number of chromosome arms, but the rats with a higher or a lower number of chromosome arms should degenerate in the embryonal stage.

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A new differential staining technique using sodium dodecyl sulfate and banding pattern analysis of black rat chromosomes

Tosihide H. YOSIDA and Tomoko SAGAI

Differential staining techniques revealing the banding patterns in metaphase chromosomes were reported by several investigators. A new technique which uses sodium dodecyl sulfate has been developed by us. In the present paper the procedure of the technique and the banding pattern analyses of the black rat chromosomes are reported.

Technique: The slides obtained by the conventional air drying procedure were immersed in a mixture of 2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 6.8) and 0.1% (w/v) SDS (sodium dodecyl sulfate) for a few seconds at room temperature. They were then washed with running tap water and stained for about 10 min. in Geimsa solution dilluted in Sörensen's buffer (pH 6.8).

Banding pattern: By use of the above technique the banding pattern of metaphase chromosomes of black rat was revealed. The karyotype of the Japanese black rat (*Rattus rattus tanezumi*) consists of 42 chromosomes (21 pairs), among which 13 autosome pairs are acrocentrics, 7 autosome pairs are metacentrics and X and Y are acrocentrics. In all chromosome pairs, a characteristic banding pattern was produced by the above technique. The number of segmental bands in pair No. 1 chromosomes was 7, that of pairs No. 4, 7, 9, 11 and 12 was two, and of pair No. 11 was three. Even

though the number of segmental bands is the same in pairs No. 4, 7, 9, 11 and 12, the pattern is different by each chromosome pair. Pair No. 1 is also characterized by having two constrictions, one near the centromere and the other near the distal end, and pair No. 2 by one constriction near the distal end.

Pairs No. 1 and 9 in the Japanese black rats are polymorphic with respect to acrocentric and subtelocentric form. As to the origin of the subtelocentrics the present author considered to have arisen from a pericentric inversion in the acrocentrics (Yosida *et al.* 1965). From the banding pattern analysis of these chromosomes it is strongly suggested that the breakage and the subsequent pericentric inversion have occurred near the centromere of pairs No. 1 and 9 and thereby subtelocentrics have developed. Oceanian type black rats (*R. rattus rattus*) are characterized by having 38 chromosomes, among which two pairs are large biarmed elements, which are assumed to have derived by Robertsonian fusion of pairs No. 4 and 7, and pairs No. 11 and 12 present in Asian type black rat (Yosida *et al.* 1969, 1971). From the comparative analysis of banding patterns of these related chromosomes, our assumption on the origin of the large biarmed elements in the Oceanian type black rats seems to be proved.

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Similarity of banding pattern of metaphase chromosomes in black and Norway rats

Tosihide H. YOSIDA and Tomoko SAGAI

Chromosome numbers in Asian black rat (*Rattus rattus*) and Norway rat (*R. norvegicus*) are $2n=42$, and their karyotypes are also very similar in both species. In the black rats pairs No. 1, 9 and 13 are characterized by having acrocentric and subtelocentric polymorphic pairs. Based on the similarity of karyotypes in both species it was suggested that the black rat is closely related to the Norway rats (Yosida 1971).

Banding patterns of chromosomes in black and Norway rats, which were revealed by Yosida-Sagai method, were compared (Fig. 1). As seen in the figure, banding pattern in both species is very similar. Subtelocentric No. 1 chromosomes in the Norway rat had two constrictions as similar to

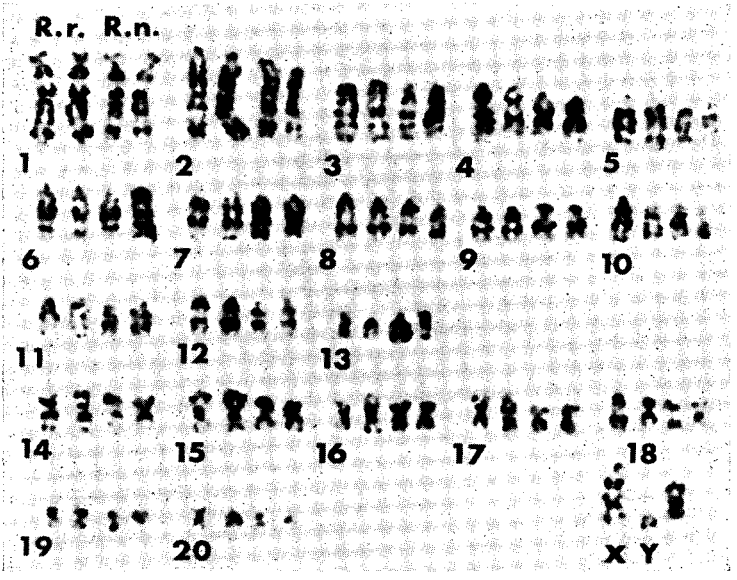

Fig. 1. Banding pattern of metaphase chromosomes in black rat (*R. rattus*) and Norway rat (*R. norvegicus*). In each pair, left two chromosomes zre of *R. r. rattus* (*R. r.* in the figure) and right two *R. norvegicus* (*R. n.* in the figure).

the subtelocentric homomorphic pair in the black rat, and their banding pattern is also similar. No.2 acrocentric chromosomes in the Norway rat showed one constriction near distal end as seen in the black rat. Banding pattern of the chromosomes in the former species is also similar to that in the latter. No. 9 chromosomes are usually subtelocentrics in the Norway rat and the banding pattern is similar to subtelocentric homomorphic pair in the black rats.

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Banding Patterns of Chinese Hamster Chromosomes Revealed by New Techniques

Hatao KATO and Tosihide H. YOSIDA

Two kinds of technique were newly developed to reveal banding patterns of the Chinese hamster chromosomes. Both techniques were essentially the same as those used for the extraction of proteins. The techniques consisted of incubation of air-dried metaphase preparations in a solution composed of 1) 2 M NaCl and 5 M urea for 20 min at 37°C, or dipping the slides for 2 seconds in 2) phosphate buffer (pH 8.0) containing 0.07% 2-mercaptoethanol, 2 M urea and 0.05% SDS, and staining them in a Giemsa solution. Banding patterns produced by these techniques appeared to be identical to those induced by the methods reported by previous workers, requiring post-fixation incubation of slides in warm saline.

The banding pattern was typical for each chromosome pair, permitting unequivocal identification of several pairs which were hardly distinguished by the conventional staining procedures. It was confirmed that these patterns had been well preserved in the chromosomes of the cultured cell line. Details are presented in Chromosoma **36**: 272 (1972).

A new technique to reveal banding pattern of human chromosomes by using urea

Yukimasa SHIRAISHI and Tosihide H. YOSIDA

The present report deals with a new technique to reveal the banding pattern of human chromosomes by the use of urea. Slides prepared by routine air drying technique were treated with urea-Sörensen buffer solution (three volume of 8 M urea and one volume of Sörensen buffer solution) for ten minutes at pH 6.8 and 37°C. Afterwards, throughly washed in tap water to avoid the precipitation of urea, and then they were stained with Giemsa solution diluted about 50–70 times with Sörensen buffer (pH 6.8) for 5–20 minutes at room temperature.

By this technique, a specific banding pattern of each pair of homologous chromosomes was revealed, permitting easy identification of all human metaphase chromosomes. The appearance and location of darkly stained bands are consistent and specific in each metaphase chromosome. How-

ever, they were not always constant as to cell stage. At prometaphase, many finer bands could be distinguished along the chromosome lengths, but supercontracted metaphase chromosomes are also disadvantageous for the observation of banding pattern, because the bands lie too close. The chromosome to be used for analysis of banding pattern should have a suitable degree of contraction. A suitable condition of the chromosome is when the length of pair No. 1 is contracted to about 10 μ. To find such suitably contracted metaphases in specimens prepared by the present technique is quite easy. The banding pattern revealed by urea-Sörensen buffer incubation showed that the patterns appear to be almost identical to those given by previous workers.

Factors Involved in the Production of Banded Structures in Mammalian Chromosomes

Hatao KATO and Kazuo MORIWAKI

It has so far been believed that the band formation in chromosomes reflects a denaturation-reassociation process of repetitious nucleotides and that a post-fixation incubation of slides for a considerably long period is indispensable. As described in the previous report, we found that the banded structure was inducible within a few seconds when freshly prepared slides were properly treated prior to staining with a Giemsa solution. This finding seemed to be imcompatible with the postulation mentioned above, and prompted us to investigate to what degree factors other than the repetition of DNA sequence can be involved in this phenomenon.

Over 70 reagents were tested for their ability to produce bands in the Chinese hamster chromosomes by incubating air-dried slides in aqueous solutions of these reagents for a definite period prior to the Giemsa staining. Acids were found to be without effect on the band production. Many of salts were able to induce bands if their pH was alkaline. Strong bases were also found to be potent band-inducing reagents. They produced bands only in a few seconds. Protein denaturants such as urea, guanidine-HCl and several surface active compounds were also effective in band production.

In the light of these results, it seems very likely that the solubilization or extraction of chromosomal proteins, probably of acid nature, would be the

primary cause of the appearance of the banded structure in chromosome arms. Details of this work are presented in Chromosoma **38**: 105–120 (1972).

Differential Responses of Several Aneusomic Cell Clones to Ultra-Violet Irradiation

Hatao KATO and Tosihide H. YOSIDA

With the use of a colcemid-reversal method (Kato, H. and Yosida, T. H. 1970. Exptl. Cell Res. **60**: 459), we have succeeded in inducing chromosomal nondisjunction in cloned Chinese hamster cells and so far obtained clonal lines with various types of trisomic and monosomic chromosomes (Kato, H. and Yosida, T. H. 1971. Cytogenetics **10**: 392). The present report deals with the sensitivity of some of these aneusomic clones to ultra-violet light in terms of the colony forming ability after irradiation.

Exponentially growing cells were trypsinized, plated at desired concentrations into 60 mm plastic dishes and exposed to UV light, 2537Å, emitted from a 15 W germicidal lamp at an incident dose-rate of 10 ergs/mm^2/sec. The cultures were then incubated for 7 days. Colonies with more than 25 cells were counted as viable.

Clones 19a and 67b were trisomic and a clone 67f was monosomic for No. 10 chromosome, respectively. All of them showed a significant difference in the sensitivity from the parental control line, D–6. D_0 values were 57.5 ergs/mm^2 for the D–6, 57.5 ergs/mm^2 for the 19a and 67b and 40 ergs/mm^2 for the 67f. The heightening of the UV sensitivity was also detected in another type of trisomic clones. No. 9 trisomy clones, 11c and 54e, showed almost the same D_0 values as that of the No. 10 trisomy. A disomic clone was isolated from a long term culture of the monosomic clone. This clone was found to be more resistant than the monomosic one, while apparently more sensitive than the control disomic line. It appears that the disomic has arisen through duplication of a monosomic chromosome in a monosomic cell by nondisjunction, thus possessing two identical chromosomes.

There was no statistically significant difference among these clones in the ability of unscheduled DNA synthesis following UV-irradiation.

It is very likely that the increased UV-sensitivity of these aneusomic clones is attributed to their altered chromosomal constitutions. The

addition or loss of a chromosome to or from the genome, irrespective of the type of the chromosome, may force cells to modify their genic expression, resulting in the alteration in the activity of various enzymes including those involved in the DNA repair process. Details of this work are presented in Exptl. Cell Res. **74**: 15–20 (1972).

Induction of Sister Chromatid Exchanges by Ultra-Violet Light and Inhibition by Caffeine

Hatao KATO

Sister chromatid exchanges on the Chinese hamster chromosomes were studied by pulse-labeling cells with ^{3}H-thymidine at various concentrations and by fixing them at the 2nd post-labeling mitosis. There was no significant change in the exchange frequency regardless of a 40-fold range of variation in the tritium dose (0.76–0.96 exchanges per No. 1 chromosome), whereas both chromatid and isochromatid deletions increased linearly with the tritium dose. This suggest that the sister chrematid exchanges are spontaneous events, not largely affected by endogenous radiation from incorporated tritium.

When cells were submitted to synchronization treatments utilizing either excess thymidine, 5-fluorodeoxyuridine or hydroxyurea, the frequency of the sister chromatid exchanges increased twice as high as the control level. Ultra-violet irradiation on cells treated with excess thymidine induced an additional rise in the exchange frequency (4.20 per No. 1 chromosome at 40 ergs/mm^2), but only in those irradiated at the early S phase. The post-irradiation treatment with caffeine caused a significant decrease in the exchange frequency, while a striking increase in aberrant chromosomes, mostly of deletion type. Further studies are in progress on the basis of a working hypothesis that the Chinese hamster cells have a repair mechanism similar to the recombinational post-replicational repair process which is known in microorganisms, its occurrence being visualized as the sister chromatid exchange at metaphase.

Induction of chromosome aberrations in human leucocytes growing *in vitro* by treatment with cadmium sulfide

Yukimasa SHIRAISHI and Tosihide H. YOSIDA

Chromosome aberration in cultured human leucocytes are induced by treatment with cadmium sulfide. Human leucocytes from a normal female were cultured according to the routine phytohemagglutinin method. After 72 hours incubation at 37°C, cadmium sulfide was added to the culture medium at the final concentration of 6.2×10^{-2} μg/ml, and then cultured for 4 and 8 hours in the medium. By treatment with the cadmium sulfide, several chromosome aberrations, such as chromatid breaks and translocation were observed. In the untreated control, no chromosome aberrations were noted in the observation of 50 cells. On the other hand, in the treated cells chromatid breaks were observed at 38% in 4 hour treatment and 42% in 8 hours treatment. Translocations were observed at 12% in only 8 hour treatment. Dicentric like chromosomes were often observed in 4 and 8 hours treatment, but it is difficult to recognize whether they are real dicentric or only a twist occuring in two doughter chromatids.

Since chromatid aberrations were found so frequently in culured cells treated for 4 and 8 hours before harvest, these aberrations should have occurred in the late S and G_2-periods. Based on the above investigations it seems highly possible that cadmium sulfide has some mutagenic action on genetic material as seen in some other radiomimetic chemicals.

Abnormalities of chromosomes found in cultured leucocyte cells from Itai Itai disease patients

Yukimasa SHIRAISHI and Tosihide H. YOSIDA

In the previous paper of this report we found that cadmium sulfide produced a marked increase in the frequencies of chromosomal aberrations. Itai Itai disease, which is a kind of diseases caused by environmental pollution, seems to be caused by cadmium. However, it was discussed by several scientists whether this compound is really related to the cause of the disease. To give an information on the cause of this disease, we examined the chromosomes of the patients.

Peripheral leucocytes obtained from 10 patients (female) and six normal

females as contral were cultured according to the routine procedures. All cultures were incubated for 72 hours at 37°C, and slides were prepared by routine air-drying methods.

The frequency of the cells with the chromosome abnormalities was assessed in 100 to 200 cells in each of the patients and normal subjects. In normal humans, the chromatid breaks were observed at 0 to 2.0%. In 7 among 10 patients, however, chromosome abnormalities such as translocation, chromatid breaks, dicentric or dicentric like chromosomes and acentric fragments, were observed at about 10 to 50%. Among the abnormalities, chromatid breaks were observed as a common type in these patients. In addition, cells with chromosome translocations were found more frequently than the dicentric or acentric fragments. On the other hand, three patients showed a lower frequency of chromosome abnormalities (2 to 3%). These frequencies are not sifinificantly different from those of control subjects. Bloods were taken three different times from the patients and chromosomes were observed sequencially. The frequencies of the chromosome abnormalities in 7 patients were always higher, but slightly different by the time obtained the blood was taken. There is a question that why 7 patients had so high frequencies of chromosome abnormalities. Should not the other three patients without higher chromosome abnormalities be a real Itai Itai disease? Another question is that the state of chromosome abnormalities are similar to the Fanconi's syndorome as observed by Bloom *et al.* (1966) and Dosil *et al.* (1970). Therefore, we have to examine whether the chromosome abnormalities found in the 7 patients were not caused by the other disease. It is remainsed as a further important problem to solve the relation between chromosome abnormalities and Itai Itai disease.

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Relation between karyotype alteration and aging of tumor stemline cells

Tosihide H. YOSIDA

Based on the extensive cytogenetical studies of the Yoshida rat sarcoma, Makino (1952, 1957) has established a concept of tumor stemline cells for the transplantable tumors. According to him, the most important character of tumor stemline cells is the constancy of stemline idiogram through many transfer generations. The constancy, however, is not always absolute as Makino (1957) later has recognized it himself. Change of tumor stemline karyotypes has been studied by many investigators in several tumors. Based on the cytogenetical studies of several experimental tumors the present author (Yosida 1966, 1968) explained the karyotype alteration of tumor stemline cells to be depending on a sequential event of mutation and selection of tumor cells. Here is a question why transplantable tumors have a stemline each consisting of a certain karyotype through many generations, in spite of the continuous and random occurrence of mutations in the cell population.

To consolidate the different opinion on the behaviour of tumor stemline cells, constancy and variability, the idea of the "aging" of the tumor cells will be proposed. In human diploid cell strain, the diploid karyological character is limited to about 50 serial passages (Hayflick and Moorhead 1961). The diploid cells are the first stemline of the cell population. In this case the first 50 passages seems to be the age of the diploid stem cells. By proliferation of aneuploid cells occurred after diploid cells have degenerated the second stemline cells developed. The second stemline cells are also limited by "aging" like as the first diploid stem cells, and then the third and fourth stemline cells develop sequencially.

In karyotype alteration in the tumor stemline cell could be explaned by the same mechanism as above. As examples of the karyotype alteration in tumor cells four cases will be described here. In the Yoshida rat sarcoma the karyotype change was observed sometimes during the tumor transplantations (Yosida 1959, Matsushima and Yosida 1971). In the MY mouse sarcoma drastic change of karyotype from diploidy to tetraploidy was observed about the 90th transplant generation, which corresponds to about 6 years (Yosida *et al.* 1960). In the case of the Shay's rat chloroma the karyotype alteration occurred sequentially with a period of about 2 years

(Yosida and Sakai, unpublished). A rapid karyotype alteration has been observed in mouse plasma cell tumors. In this case MSPC-1 mouse plasma cell tumor which established by us the karyotype change from diploidy to tetraploidy occurred within one to two transplant generations (Yosida 1968,

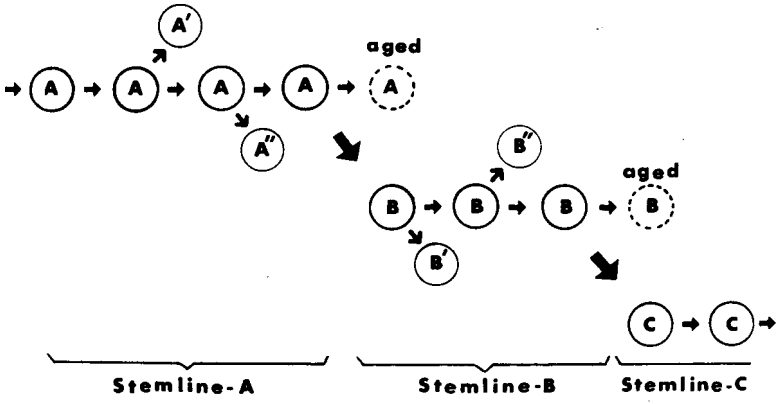

Fig. 1. Scheme of karyotype alteration in tumor stemline cells by aging. A, B and C show tumor cells in each stemline. A′, A″, B′ and B″ show mutant cells which are not developed as a new stemline. Broken circle denotes the cell degeneration by aging.

Yosida *et al.* 1970). Thereafter the stemline karyotypes of the tumor changed sequentially one to another in the period of about several transfer generations (Moriwaki *et al.* 1971). Karyotype alterations in the tumor stemline cells will be explained by the "aging" of the tumor cells (Fig. 1). Stemline karyotype "A" degenerates after a certain transfer generations by age, and the new cells with karyotype "B", which occurred as result of mutation and selection from the stemline "A", increase in the cell population, and the tumor is occupied by the new stemline "B". The stemline "B" is replaced by stemline "C" by aging after a certain number of transfer generations.

The concept of aging in tumor stemline cells has been reported in 1971 Annual Meeting of the Society of Chromosome Research in Japan, by Matsushima and Yosida (1971), and by Yosida (1972).

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Karyological studies in Japanese ants (Hymenoptera, Formicidae) III. Karyotypes of nine species of Ponerinae, Formicinae, and Myrmicinae

Hirotami T. IMAI and Masao KUBOTA[1]

The karyotypes of nine Japanese ants in three subfamilies (Ponerinae, Formicinae, and Myrmicinae) were successfully analysed by the improved squash technique. Three ponerine species had $2n=7$ and $n=4$ (*Ponera scabra*), $2n=22$ (*Brachyponera sinensis*), and $2n=28$ and $n=14$ (*Cryptopone sauteri*). Four formicine species had $2n=18$ and $n=9$ (*Camponotus* sp. and *C. tokioensis*), $2n=26$ and $n=13$ (*Camponotus japonicus*), and $2n=30$ and $n=15$ (*Lasius niger*). Two myrmicine species had $2n=18$ and $n=9$ (*Leptothorax congruus*), and $2n=37$, 38, 39, and $n=17$, 18, 19, 20 (*Pheidole nodus*). It was found that the variation of chromosome number observed in *P. nodus* was caused by Robertsonian type polymorphism. For details, see Chromosoma (Berl.) **37**: 193–200 (1972).

A new criterion for classification of mammalian chromosomes[2]

Hirotami T. IMAI

Using the Chromosome Atlases by Hsu and Benirschke (1967, 1968, 1969), a search was made for a quantitative criterion for discriminating the "acro-

[1] 13 Nakasone, Odawara.

[2] The author indebted to Drs. E. Matsunaga and K. Moriwaki for their valuable advice and encouragement.

or telocentrics" from the "meta-, submeta- and/or subtelocentrics". Examination of some measurements for each chromosome with reference to the classification made by these authors showed that, although they had taken into account the arm ratio and/or the size of the short arm, there was

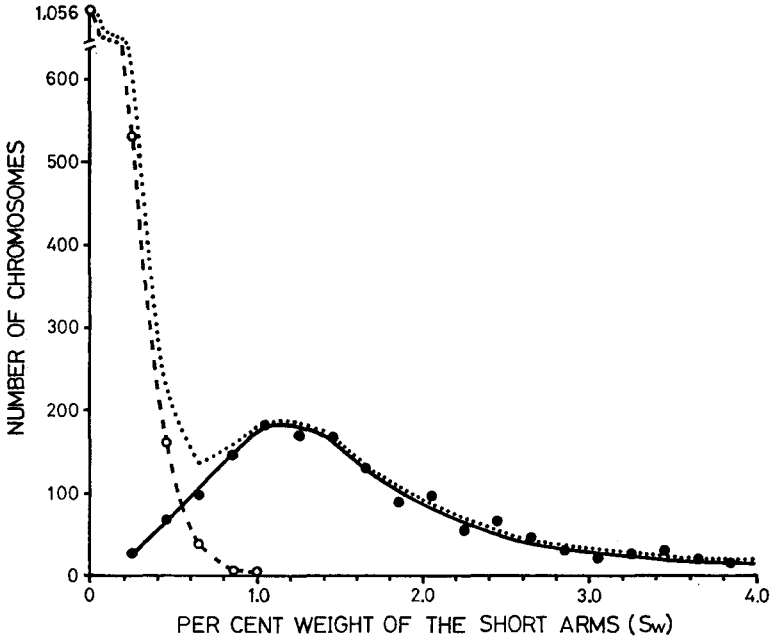


Fig. 1. Frequency distribution of chromosomes according to the size of the short arms (S_w). Open circle and broken line; the "acro- or telocentrics" identified by Hsu and Benirschke. Solid circle and solid line; the "meta-, submeta- and/or subtelocentrics" identified by these authors, where the chromosomes showing S_w 4.0 were omitted. Dot line; the total chromosomes examined.

no consistent rule in their classification that could be defined quantitatively. On the other hand, the frequency distribution of the size of the short arm (measured in wieght relative to the X-containing haploid set) of all the chromosomes revealed an apparent bimodality (Fig. 1), which made it possible to classify the chromosomes into two distinct categories, one with the short arm smaller than 0.6 (acro- or telocentrics) and the other with the short arm larger than 0.6 (meta-, submeta- and subtelocentrics). This simple

rule would give betwetr agreement with the Hsu and Benirschke's classification as compared with the arm ratio system; it would further enable us to score the fundamental number (NF) more objectively than before.

Karyotypes of four species of bats collected in Japan

Kimiyuki TSUCHIYA, Masashi HARADA and Tosihide H. YOSIDA

Karyotypes of greater horseshoe bat (*Rhinolophus ferrumequinum mikado*), Hosono's whiskered bat (*Myotis hosonoi*), Japanese pipistrelle (*Pipistrellus abramus*) and large noctule (*Nyctalus lasiopterus aviator*) belonging all to the Chiroptera were analysed.

Five greater horseshoe bats were collected from Numazu in Shizuoka Pref. (1 female), Takine in Fukushima Pref. (2 males) and Akiyoshi in Yamaguchi Pref. (1 female and 1 male), a male Hosono's whiskered bat from Mt. Fuji, 2 male Japanese pipisterelles from Takasaki in Gunma Pref. and a female large noctule from Misima in Shizuoka Pref. The karyotypes were analyzed in the femoral and fumerus bone mallow cells using a rutine air-drying technique and staining with acetic orcein.

Table 1. Karyotype formulae of 4 Chiropteran species

Species	2n	Karyotype formulae					
		M	SM	ST	A	X	Y
Rhinolophus ferrumequinum mikado	58	4			52	SM	A
Myotis hosonoi	44	10			32	SM	A
Pipistrellus abramus	26	12	8		4	A	m
Nyctalus lasiopterus aviator	42	8	2		30	M	

The diploid chromosome number in greater horsehoe bat, Hosono's whiskered bat, Japanese pipistrelle and large noctule were 58, 44, 26 and 42, respectively. Karyotypic formulae of these species are listed in Table 1. Karyotype of the greater horseshoe bat (*R. f. mikado*) was identifiable from that of *R. f. ferrumequinum* already observed by Capanna and Civitelli (Caryologia, **17**: 361, 1964). Karyotype of Hosono's whiskered bat (*M. hosonoi*) was almost indistinguishable from that of *M. macrodactylus* observed by Sasaki (Mamm, Chrom. Newsletter, **11**: 22, Figs. 8, 1970). The present data on the karyotype of Japanese pipistrelle (*P. aburamus*) have

confirmed the previous observation by Takayama (Jap. J. Genet., **34**: 107, 1959). The karyotype of *N. l. aviator* was similar to that of *N. noctula* reported by Dulic *et al.* (Experientia, **23**: 945, 1967).

Indian brown spiny mouse, *Mus platythrix*, having the lowest fundamental chromosome number

Kimiyuki TSUCHIYA and Tosihide H. YOSIDA

Ellerman has reported that the Indian brown spiny mouse, *Mus platythrix platythrix* Bennett, is distributed in the Deccan district of India (The Fauna of India, **3**: 771, 1961). One male was sent from Mysore, South India, by

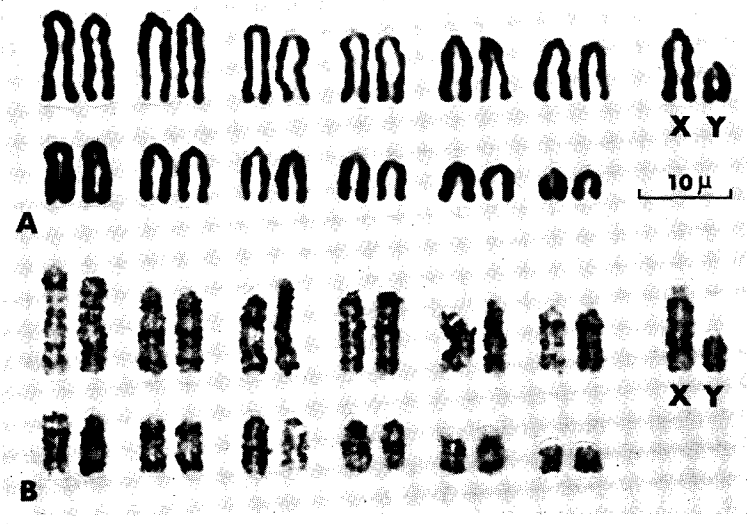


Fig. 1. Karyotype (A) and banding pattern, (B) revealed by NaOH treatment of a male Indian brown spiny mouse.

courtesy of Mr. Tachibana in Octobber, 1971. The external measurements of this specimen were as follows: Length of head and body 120.0 mm, hind foot without claw 17.5, ear from notch 17.0, and body weight 59.5 g. Chromosomes were observed in bone marrow cells by routine air-drying technique. Diploid chromosome number was 26 in 20 cells, and all autosome pairs and the X and Y chromosomes were acrocentrics (Fig. 1).

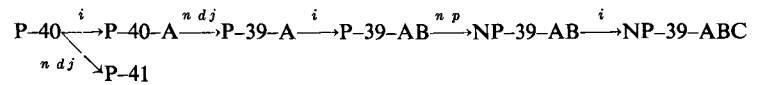
According to Gropp *et al.* (Experientia, **25**: 875, 1969) the tobacco mouse, *Mus poschiavinus*, has 26 diploid chromosomes, but the karyotype is obviously different from that of *Mus platythrix platythrix*, namely the former has 7 pairs of metacentrics and the fundamental number (FN) 40, whereas FN of the latter is 26. Diploid chromosome number in the African pigmy mouse, *Mus minutoides*, was 18, but all chromosomes were metacentrics, and FN was 36 (Matthey, Rev. suisse Zool., **73**: 585, 1966). Fundamental number in the Indian brown spiny mouse is the lowest for the genus *Mus*, so far as the present knowledge of their karyotypes is concerned.

Possibility of Clonal Senescence in MSPC–1 Mouse Myeloma Cells during Serial Transplantation

Kazuo MORIWAKI and Tamiko SADAIE

The present author had already made his opinion known of the possibility of cell population change in MSPC–1 mouse myeloma due to the senescence of a cell clone followed by the proliferation of a new variant cell clone during serial transplantation (Moriwaki, K. and H. T. Imai, Acta Haem. Jap. **33**: 67, 1970; Moriwaki, K. *et al.* J. Nat. Cancer Inst. **47**: 623, 1971). For supporting further this hypothesis, quantitative comparison of the growth rate between 2 cell populations obtained from an earlier transplant generation and from the later one, was undertaken in this study.

MSPC–1 myeloma has exhibited at least 5 times of cell population change during about 50 *in vivo* serial passages. Three of them were detected by the appearance of the marker chromosomes, A, B, and C, probably due to isochromosome formation, and one of the remaining was found to be a loss of the capability for γA-globulin production. Fifth change was a single non-disjunction, which seems to have occurred close to one of the isochromosome formations mentioned above. It has been visualized by the banding pattern analysis of chromosomes (On the analytical procedure, see this Annual Report p. 53). These changes are summarized in the following flow chart.



(P: γA producer; NP: γA non-producer; Number: modal chromosomes;

A.B and C; marker chromosomes; *i*: isochromosome formation; *n dj*: non-disjunction; *n p*: loss of an ability of γA production)

In order to reveal the mechanism of those cell population changes, the quantitative comparison of growth rates between P–41 subline instead of P–40–A in this case from the earlier transplant generation and NP–39–ABC subline from the later generation, from each 10^4 viable cells were inoculated subcutaneously. After 2 weeks the total cell numbers of the individual tumors were counted; P–41 was 0.8×10^8 and NP–39–ABC 2.4×10^8. *In vivo* growth rate of NP–39–ABC seems to have become larger than that of P–41 by 3 times after 4 changes in the tumor cell population. This may imply that the ratio of increase in *in vivo* growth rate per single population change is approximately 1.32, because $1.32^4=3.0$. Provided that one can detect new variant cells by karyotype analysis when the minor population exceeds approximately 2% of total cells and that their growth rate is larger than that of the major cell population surrounding them by 1.32 times, further 14 transplantations are needed before the tumor cell population is occupied mostly by the variant cells, because $1.32^{14}=50$. In fact, however, the tumor cell population changes have been completed more rapidly. For instance, at generation 21 the tumor cell population consisted of more than 98% P–40–A cells, whereas at generation 27 more than 98% P–39–AB cells occupied that tumor. Such rapid population change has to be explained not simply by the larger growth rate of the new variant cells but also by possible senescence of aged cell clones. So far nobody considered the concept of clonal senescnce in transplantable tumor cells based on the quantitatively valid evidence which can exclude the possibility of cell population shifts by the selection of fast growing variant cells. The present study seems to have successfully demonstrated the possible clonal senescence as such.

Simplified Alkaline Phosphate Buffer-Giemsa Method for Inducing Banded Structure in the Mouse Chromosomes

Kazuo MORIWAKI and Tamiko SADAIE

The recent advance in cytogenetical methods for producing banding patterns in the metaphase chromosomes has made it possible to identify clearly each pair of mouse chromosomes which was almost impossible for a long time. The present study attempted to modify those procedures re-

commended by several workers (Sumner, A. T. *et al.* Nature New Biology **232**: 31, 1971; Schnedl, W. Chromosoma **35**: 111, 1971: Miller, O. J. *et al.* Proc. Nat. Acad. Sci. **68**: 1530, 1971) to more simiplified forms. We used bone marrow cells of normal BALB/c mouse and MSPC–1 mouse myeloma cells as materials.

Table 1. Effects of alkaline pretreatment and solvent for Giemsa solution on the stained features of metaphase chromosomes MSPC–1 mouse myeloma cells

NaOH pretreatment	Solvent for Giemsa solution	Features of staining		
		Whole chromosome	Dots	Bands
−	Distilled water	+	−	−
+		−	+	−
−	0.25 M Sucrose	+	−	−
+		−	+	−
−	0.35% NaCl	+	−	−
+		−	−	+
−	M/15 Sörensen buffer	+	−	−
+		−	−	+

The chromosome preparations of those cells have been made following routine procedures for fixation and air-drying. Those preparations were treated with 0.005 N sodium hydroxide for 1–2 seconds at room temperature followed by staining with Giemsa solution for 5 minutes diluted 25 times by M/15 Sörensen phosphate buffer (pH 6.8). This simplified procedure was good enough to induce clear banded structure on the metaphase chromosomes of those cells. In order to simplify this method further, Giemsa solution was diluted with distilled water or 0.25 M sucrose but neither of which could induce definite bands. Giemsa solution diluted with 0.35% sodium chloride could give a reliable bnading pattern. The data of those experiments are summarized in Table 1. The details of further survey of suitable conditions for inducing band patterns was reported elsewhere (Kato, H. and K. Moriwaki, Chromosoma **38**: 105, 1972).

Callus induction and organ redifferentiation of *Triticum*, *Aegilops* and *Agropyron* by anther culture

Mikio KIMATA and Sadao SAKAMOTO

Callus induction and organ redifferentiation were studied by the anther culture of 17 *Triticum*, 11 *Aegilops* and 8 *Agropyron* species. Out of three different culture media used for callus induction, the best result was obtained by the Miller's medium supplemented with 2.21 mg/l 2, 4-D. Callus formation from pollen grains was observed in four *Triticum*, two *Aegilops* and two Agropyron species. On the contrary, a high frequency of callus formation from anther filaments was obtained in emmer wheats. In order to induce organ redifferentiation, calluses were transferred to the same medium as mentioned above omitting 2,4-D. Root formation was observed in several species. Sprouting appeared only from calluses derived from pollen grains of C C C^u C^u, an artificially synthesized amphiploid ($2n=28$) between diploid *Ae. caudata* and *Ae. umbellulata*, and many plantlets took shape. However, all were haploid albino plants ($2n=14$). 117 days after transplanting an albino plant with seven leaves flowered. However, the spike of this plant included only a single spikelet without pistil and stamens. The details were published in Japan. J. Palynology **8**: 1–7 and Japan. J. Genetics **47**: 61–63.

VI. MUTATION AND MUTAGENESIS IN ANIMALS

Effect of protein synthesis inhibitor on repair of X-ray induced premutational lesions in silkworm spermatids

Y. TAZIMA and Y. FUKASE

Our previous experiments (this Report No. 16: 100–102) showed that post-irradiation treatment of silkworm spermatogonia with many metabolic inhibitors increased mutation frequency to a considerable extent. Although it was surmised that the treatment with those chemicals suppressed the repair of premutational lesions, it was impossible at that time to exclude another possibility that those treatment might intensify the blocking of the cell cycle progression caused by the X-ray irradiation thus increasing the mutation frequency. Since DNA synthetic cycle does no longer exist in the spermatids, the latter possibility could be rejected only if the irradiation experiment had been carried out with these cells. Furthermore, repair of radiation induced premutational lesions has since been demonstrated by irradiating the spermatids with two split doses at an appropriate time interval (Tazima, 1959, Jap. J. Genet. **44** Suppl 1: 123–130).

An experiment has, therefore, been carried out in which a treatment with a protein synthesis inhibitor was combined with fractionated irradiation given to spermatids.

Wild type males of strain C108 were irradiated at mature larval stage with two split doses of 500 R each given three hours apart. Immediately before the initial 500 R exposure, chloramphenicol and/or puromycin in physiological salt solution were injected interperitonealy. The injection was 0.02 ml per capita with various concentrations, *i.e.*, 0.01% (2γ) and 0.1% (20γ) for chloramphenicol and 0.005% (1γ) and 0.02% (4γ) for puromycin. Control group was also irradiated but was injected with physiological saline only. Both treated and control males were crossed to *pe re* females for the measurement of induced mutation frequency. The results are given in Table 1.

The results showed that those chloramphenicol and puromycin treated males exhibited significantly higher mutation frequencies than saline treated control. Since in this case the possibility of blocking the progression of cell

cycle was excluded, it seemed more likely that inhibition of protein synthesis increased the mutation frequency. That is to say, the process of protein synthesis seems to be necessary for the repair of premutational lesions.

Table 1. Mutation frequencies obtained after fractionated X-ray irradiation of silkworm spermatids with or without a protein synthesis inhibitor

Treatment	Observed	Number of mutants				Mutation frequency ($\times 10^{-5}$)				
	nnumber	*pe*	*pe*, +	*re*	*re*, +	*pe*	*pe*, +	*re*	*re*, +	total
Saline only	18.242	7	22	3	4	38	121	16	22	197
CMP 0.01%	17.726	9	21	3	8	51	119	17	45	232
CMP 0.1%	17.217	9	33	4	6	52	192	23	35	302
Purom 0.005%	18.260	11	32	4	7	60	175	22	38	295
Purom 0.02%	19.824	3	39	8	8	15	197	40	40	292

Studies on strain differences in radiosensitivity in the silkworm XIV. Further data on dominant lethals

Yataro TAZIMA and Yosoji FUKASE

In the previous issue of this Report (No. 21: 61–62), we have reported that mature sperm were rather insensitive to radiation in the induction of dominant lethals and that the differences in sensitivity was not clear among strains at this cell stage but became marked when spermatogonia has been irrdiated.

Since only two strains, rb as a sensitive and Kansen as a resistant one, and their hybrid were used in the previous experiment, the irradiation experiment was continued using another sensitive strain Sekko and a less sensitive strain Kojiki in addition to rb. F_1 hybrids between rb and either Sekko or Kojiki were also irradiated. Those hybrids were fairly resistant to radiation. In contrast, the parental strains were much more sensitive and became sterile when irradiation doses were higher than 2000 R for Sekko and 3000–4000 R for Kojiki and rb, thus invalidating the irradiation with appreciably high doses that ensure a reasonable yield of dominant lethals. The data thus obtained sometimes did not represent good regression curves for parental strains, although the difference became more or less marked among strains. The radiosensitivity in F_1 again seemed to fall between both parents.

With the purpose of avoiding so far as possible the disturbance due to the sterilizing effect, comparisons have been made by irradiating spermatids in mature larvae. Strains used were again both sensitive and less sensitive strains, *i.e.*, rb, Sekko, Kojiki, Aojuku and Kansen, together with their

Table 1. Frequencies of dominant lethals induced in male germ cells of the silkworm

Irrad. cell stage	Strain	Exptl season	Dose (kR)	Hatchability decline (%)	Freq. of dom. lethals per kR (%)
S-gonia	Kansen	703	4	2.5	0.62
	Kojiki	711	4	4.3	1.10
	Sekko	711	1	3.3	3.3
	rb	703	2	10.0	5.0
	"	711	2	15.5	7.7
	rb×Ka	703	4	5.0	1.25
	Se×rb	711	4	24.0	6.00
	Ko×rb	711	4	7.3	1.82
S-tids	Kansen	713	4	4.6	1.15
	Sekko	712	3	3.0	1.00
	Aojuku	713	4	5.0	1.25
	Kojiki	712	4.5	10.0	2.20
	"	713	4	10.0	2.50
	rb	712	4	10.0	2.50
	"	713	4	15.0	3.75
	Ao×Ka	713	4	7.0	1.75
	ub×Se	712	4	5.0	1.25
	Se×rb	713	4	8.0	2.00
	rb×Ko	712	4.5	8.0	1.77
	Ko×rb	713	4	15.0	3.75
Sperm	Kansen	701	10	10.0	1.00
	Aojuku	701	10	10.0	1.00
	Sekko	701	10	10.0	1.00
	rb	701	10	10.0	1.00

hybrids, rb×Kojiki, rb×Sekko and Aojuku×Kansen. Selection at this stage permitted to deliver radiation doses up to 4000 R in all parental strains and to 6000 R in all hybrid combinations guaranteeing more reliable data than irradiation to spermatogonia. The induction rates per unit dose were reduced. The differences among strains also became smaller but seeemed

to represent more reliable data showing good regression curves.

The data so far obtained are summarized in Table 1. As is shown in the table, induction rates of dominant lethals are very low in the silkworm. After the irradiation of mature sperm the rate was about two figures lower than for the mouse and about one figure lower than for *Drosophila.*

It was also confirmed in these experiments that with regard to the induction of dominant lethals the sensitivity in F_1 was intermediate between both parents, as has been already observed for recessive visible mutations at marked loci.

Studies on strain differences in radiosensitivity in the silkworm XV. Comparison of recombination values among different sensitivity strains.

Yataro TAZIMA and Kimiharu ONIMARU

It has been known for several microorganisms that many radiation sensitive mutants not only lack the capacity to repair their damaged DNA but are also uanble to undergo genetic recombination. Those mutants were mostly discovered as UV-sensitive but some were known to be sensitive also to X-rays. Conversely, in some other cases recombination deficient mutants were observed to be more sensitive to radiation than the wild type. It is therefore of great interest to examine if there is in the silkworm any close relationship between radiosensitivity and recombination properties. In this work a comparison of recombination values has been made among groups with different genetic background in F_1 and BF_1 of crosses with strains of different radiosensitivity.

For the measurement of recombination values the section between $+^{pe}$ and $+^{re}$ of the Vth linkage group was chosen. Strains utilized for the comparison were Aojuku, Kansen, Kojiki, Sekko and rb. All of them had $+^{pe}+^{re}/+^{pe}+^{re}$ genotype. Females of each strain were mated to double recessive *pe re* males. F_1 males were then back-crossed to *pe re* females and more than 250 batches of each cross were subjected to the examination of recombination values in F_1 males (Cross I). F_1 females were crossed again with males of their parental strain of wild type, *i.e.*, Aojuku, Kansen etc. Those BF_1 males were then crossed to *pe re* females. About one half of the batches thus obtained comprised wild type eggs only. Another half

were segregating three types of egg color, whose male parents were assumed to be heterozygous *pe re*/++. They were also assumed to comprise both hetero- and homo-zygotes with respect to the major sensitivity gene(s) and/or their modifiers, if any were present. Those bactches were subjected to the examination of recombination values in BF_1 males (Cross II).

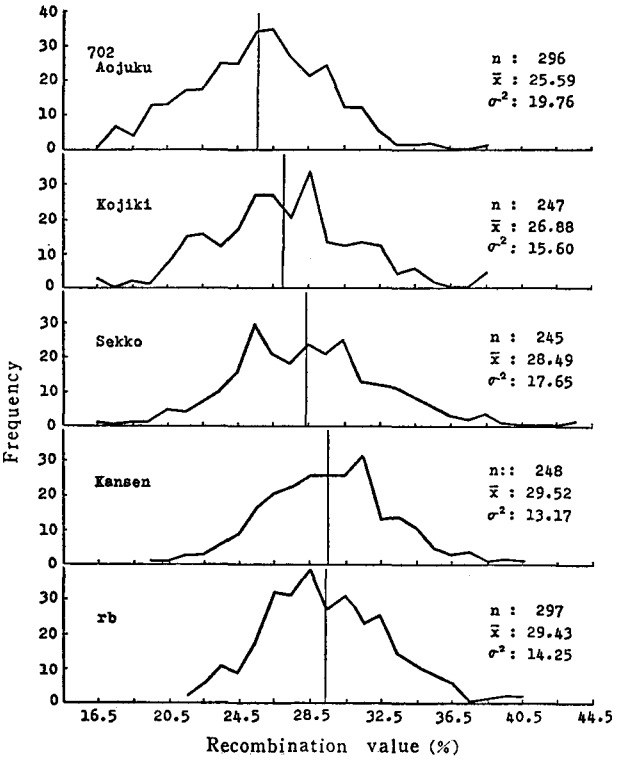


Fig. 1

The results of Cross I are given in Fig. 1. As is seen from the figure, difference was clearly observed among strains, being the lowest 25.59% for Aojuku and the highest 29.43% for rb. The differences were significant by t-test analysis among strains except for that between Kansen and rb. In Cross II more distinct differences were observed among strains, although the tendency was almost similar to that of Cross I. The lowest recombina-

tion frequency was 25.03 for Aojuku and the highest was 30.30 for rb. The array of recombination values among strains was almost in parallel with that of radiosensitivity with exception of Kansen, but in the reverse order.

The result that the most sensitive strain rb showed highest recombination value was indeed against our expectations from the findings in microorganisms. The phenomenon might perhaps be related to the activity of a relevant enzyme(s) existing in those silkworm strains. Kada and his collaborators recently revealed that activity of A type DNase in rb strain is ten times as high as in Aojuku.

The finding that recombination values were clearly different among the groups even in F_1 indicates that the modifying factor(s) for recombination properties are almost dominant.

RBE of 14 MeV fast neutrons for visible recessive mutations in silkworm oocytes

Akio MURAKAMI

This communication shows some preliminary data on the relative mutagenic effects of 14 MeV neutrons as compared with ^{137}Cs gamma-rays for prophase I oocytes of the silkworm. Prophase I oocytes of the wild type strain *C108* were irradiated either with fast neutrons or gamma-rays in 13 day-old pupae. The pupae were exposed to neutrons of 250, 540, and 1,000 rads (at dose-rate of 2–7 rads/min) and gamma-rays of 500, 1,000, 1,500, and 2,000 R (at dose-rate of 100 R/min). The gamma-ray of the neutrons was negligible. Mutation frequencies were estimated by the specific locus method using egg-color genes, *pe* and *re*.

The dose-response relations for both radiations slightly deviated from the linear relation so that the RBE varied according to the dose comapred: at lower doses the RBE was about 2.3 and at higher doses it was slightly reduced to about 2.0. This may indicate that there is no drastic change in the RBE value for the cell before and during meiosis, as found in male silkworm. It may also indicate that the RBE of 14 MeV neutrons for the specific locus mutation in silkworm oocytes is lower as compared with that of 2.5 MeV neutrons, which had a higher vaerage LET (Machida and Nakao, 1969).

In addition to the study mentioned above, the RBE of 14 MeV neutrons to gamma-rays has been compared in oocytes among four different X-ray

sensitive strains (Kojiki and Sekko as representing the sensitive strain and Aojuku and Kansen as representing the resistant strain). These four strains were irradiated with a single dose of neturons (400 rads) and ^{137}Cs gamma-rays (1,000 R) in late pupal stages. In this experiment, the ratio of mutation rate per rad of the neutrons to that of gamma-rays was roughly regarded as the RBE of the neutrons. The fast neutrons have shown RBEs of about 2.3 and 2.4 at *pe* and *re* loci, respectively. But no appreciable difference was found in RBE among four strains regardless of their sensitivities.

The nature of specific locus mutations induced by fast neutrons in silkworm spermatocytes

Akio MURAKAMI

The analysis of the nature of fast neutron-induced mutations may provide useful information for the understanding of the change in RBE (relative biological effectiveness) with the progression of gametogenesis. From this view point, mutants at egg-color specific loci which were obtained in the study on the relative genetic effectiveness of 14 MeV neutrons have been investigated with regard to their lethality in homozygous condition.

Whole-body mutants for either *pe* or *re* locus were picked up from the F_1 eggs. To discard the non-irradiated *pe*: *re* chromosome, mutants, *pe* and *re*, were crossed to, $+re/+re$ and pe+/pe+, respectively. Thus 22 mutants at *re* locus and 6 at *pe* locus were analyzed as to their segregation ratios in the F_2 and lehtality in homozygous condition for the muation bearing chromosome. The majority of them showed segregation into three different phenotypes, expected wild and marker phenotypes as well as a newly induced mutant phenotype. The segregation ratio in the F_2 was very close to 2: 1: 1 throughout for all cases.

Among 28 mutants analyzed, mutants associated with embryonic lethals were 21 and the remaining 7 mutants were viable. This figure does not seem to be lower in the frequency of viable mutants than that for low LET radiations reported by Tazima and Onimaru (this Report, No. 10 (1960): 115–117).

From these results, it could be said that specific locus mutations obtained after fast neutrons-irradiation of spermatocytes are in a broad sense of a point mutation, including small deficiency or deletion. But it should be

noted that mutations associated with gross chromosome aberrations would frequently be subjected to selection during the course of spermatogenesis.

Mutagenesis of the silkworm by acridine orange

Akio MURAKAMI

It has been known that certain acridine compounds are mutagenic in microorganisms when supplemented to the culture media. As a plausible interpretation it was assumed that (1) the reaction between acridine dye and DNA would result in a frameshift mutation as due to the intercalation of the chemical between DNA bases and (2) the complex of DNA which stacked with acridine dye could be unstabilized in their molecular state under light conditions leading to mutations such as copy errors.

In male Drosophila, there are many reports that monofunctional quinacrine mustard ICR–170 which has two main reactive parts, one being an acridine dye and another an alkylating mustard, is mutagenic for the post-meiotic cells, while one of acridine dyes, proflavine, which is a powerful mutagen for T_4 phages, is not mutagenic for mature sperm (Carlson *et al.*, 1967). Each reactive portion in the ICR–170 seems to have the mutagenic action on germ-cells. Accordingly, it seems unlikely to exclude a possibility of mutagenesis of the ICR–170 as an alkylating agent.

Such being the case, it would seem to open the question whether acridine dyes have mutagenic action on higher organisms as well as microorganisms. The present communication reports a positive mutagenic action of acridine orange (AO) on silkworm oocytes, but no such action in mature sperm. Eight-day old silkworm wild-type, *C108* strain, pupae were injected with 0.5 mg per pupa in 0.85% saline. Germ-cells of this stage pupae are mature sperm and prophase I oocytes, respecively, for male and female. They were mated after emergence of moths from pupae to marker strain moths having recessive egg-color genes, *pe* and *re*. The F_1 eggs were scored on the visible phenotype as either whole or mosaic (fractional) mutant.

Results indicated that the AO is not an effective mutagen for mature sperm, while the AO it induces significantly high mutational incidences for prophase I oocytes. In addition, the recovered mutants were overwhelmingly

mosaic types. A test for transmissibility and gonadal mosaicism is in progress.

The mechanism by which the AO induces mutations in the silkworm is not yet known, but the mechanism proposed for microorganisms could be, in part, extended to the present finding. The preponderance of mosaic mutants would be induced when the AO in egg-plasm were intercalated between DNA bases during meiotic and cleavage stages and the final mutational event were fixed by delayed effect within the first few divisions of the zygotes after syngamy. But it could not exclude the possibility when DNA which stacked with the AO in newly deposited oocytes (or eggs) was received sufficient amount of visible light to induce mutations as the photodynamic effect.

No increase in mutation frequency of pupal mature sperm was observed with the treatment of the AO. It is well known that the DNA molecule in the sperm is crystallized and its structure may remarkably differ from that of meiotic and cleaving nuclei. Thus it could be said that the AO may be mainly incorporated into non-crystallized DNA, but not crystallized DNA. This thought seems to be compatible with the idea proposed by Streisinger *et al.* (1964) that the acridine ring insertion would be observed during DNA replication.

We are carrying out further studies and we hope to test which hypothesis is applicable to the present finding by using other acridine dyes either under light or dark condition.

Induction of mutations by EMS in female germ-cells of the silkworm

Akio MURAKAMI

Ethyl methanesulfonate (EMS) has widely been used as a mutagen for intragenic changes in phages as well as in higher organisms. There are many reports as to the EMS mutagenesis in the male silkworm. Little is known, however, about the mutagenesis of this chemical for the female silkworm. The present experiment was intended to obtain the information of EMS mutagenesis for female germ-cells of this insect. In the female silkworm, the post-meiotic stage begins about 2 hours after oviposition and lasts only 10 min. The syngamy takes place about 30 min thereafter.

EMS in 0.85% NaCl solution was injected into the ventral abdomen of the *C108* wild-type female silkworm in 13 day-old pupae (or 1–2 days before emergence). The germ-cell at this stage is in prophase I oocytes. For the detection of the mutants the egg-color specific locus method using *pe* and *re* genes was employed. After emergence of moths from the treated female pupae, they were crossed with the double recessive marker male moths. F_1 eggs were scored for egg-color mutations and classified as whole (complete) or fractional (mosaic) mutants.

Results indicated that a high mutation induction was observed for the late stage pupae following treatment of the EMS. Almost all mutants were fractionals containing both mutant and non-mutant cells, while whole mutants were very rare, as was found for mature sperm in pupae and moths. In the present study, it is of much interest that some complex mosaic eggs were found possessing three different phenotypes, +, *pe*, and *re*, in one individual.

Fractionals may originate from the alteration by EMS in one of double stranded DNA by the production of two different cell lines of wild and mutant phenotypes in the mosaic egg. Explanation for rarely observed whole body mutants is difficult since a single-hit event on double-stranded DNA in one chromosome may be more probable than a simultaneous double-hit event. A tentative interpretation is that two independent mutational events on both strands in oocytes might have occurred by the reason of a high frequency of fractional-type mutations. However, it would seem unlikely to exclude the possibility that the fractional mutation changes into the whole mutations prior to syngamy because DNA replication may take place in pronuclear cells.

It has been known that in the male silkworm EMS is less mutagenic for pre-moiotic germ-cells than for post-meiotic germ-cells (Tazima and his associates, this Report, No. 18 (1968): 87–88). The present finding indicated, however, that EMS is a powerful mutagen for pre-meiotic oocytes in pupae. For reference, no remarkable increase in mutation frequency of larval oocytes was observed with the treatment of EMS. If EMS, which had been incorporated into egg-plasm, were mutagenic for post-meiotic and cleavage nuclei in eggs, alteration on DNA (or chromosome) would be produced during these cell stages. This thought seems to applicable to the production of complex mosaic mutants.

Analysis for transmissibility of mosaic eggs and gonadal mosaicism of

the moths obtained from the mutant eggs are in progress by mating these mutants again to the marker strain. The data obtained so far indicated that mutant characters of about one-third of mosaic eggs were transmitted to the F_1 generation either as wild or mutant eggs. It should be noted, however, that a secondary mosaic egg was rarely observed among some desendants of the primary mosaics. The origin of such type mosaics may be interpreted as due to the replicating instability as observed in fission yeast (e.g., Nasim, Mutation Res. **4** (1965): 753–763) and in the male silkworm (Onimaru and Tazima, this Report No. 19 (1968): 63).

Instability of p^M-allelic genes locating on the second chromosome piece translocated to the W chromosome

Yataro TAZIMA and Toshio OINUMA*

$\widehat{W.II}^M$ is a stable translocation involving p^M gene on the second chromosome piece and the female determining gene(s) on the W chromosome part. Although very seldom a detachment of the translocated piece occurrs giving rise to p^M males, p^+ females and p^M-mottled females.

The p^M-mottled females, when crossed to p males, gave birth to daughters of four kinds of phenotype belonging to the same multiple allelic group, *i.e.*, p^B, p^M, p^+ and p in addition to mottlings for those traits. In contrast, their sons comprised only one phenotype, p. The newly arisen four phenotypes were all transmitted to their descendant as if each of them were a complete mutation that had occurred on the translocated chromosome. Namely, when they were mated to p males, p^B females produced p^B daughters and p sons and p^+ females yielded p^+ daughters and p sons showing about 1 : 1 sex ratio. These findings suggested that those newly arisen seggregates were not the products of detachment of the $\widehat{W.II}^M$ translocation, but kind of genetic change, perhaps a mutation, had occurred in chromosome whose transmission was confined to the female line. Since $\widehat{W.II}^M$ was the only chromosome that satisfies such requirement, it was assumed that all those segregates were the products of mutation that occurred at the p^M locus.

The $\widehat{W.II}^M$ translocation was discovered in 1949 as a mosaic of larval marking among $\widehat{W.II}^P$ bearing individuals, which had $\widehat{W.}p^+$ *y oα* constitu-

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tion, presumably due to a mutation that occurred spontaneously from p^+ to p^M. Accordingly, it follows that the mutation occurred repeatedly at this locus, first from p^+ to p^M and then from p^M to p^B or p^+.

Mottlings were also transmitted *per ce* from generation to generation mostly through the female line. When the male was a carrier, though it occurred less frequently, their mode of transmission was quite unstable.

Mottlings have been obtained with regard to three mutant chracters, *i.e.*, p^B, p^M and p^+. They always produced more than two kinds of allelic phenotype among their daughters in addition to a few mottled individuals. As mentioned above, complete type mutants never showed such kind of genetic behavior in their descendants. They differ greatly in this respect from the mottlings. When mutation occurred, it was only to one direction from dominant to recessive, except for the case from p^M to p^B, when the discrimination between two phenotypes was sometimes ambiguous. Since the genetic change occurred so fequently in this case, it could be regarded as a kind of ever-sporting gene. Although the mechanism is not yet known, it seems almost certain that the instability is caused by some sort of chromosome rearrangements which leads to misreplication of the *p*-locus gene as well as to mechanical breakage of the chromosome.

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN MICROORGANISM AND PLANTS

Cellular factor involved in repair of DNA damage induced by ionizing radiation

Takehiko NOGUTI and Tsuneo KADA

We have recently shown, for *Bacillus subtilis* cells irradiated with γ-ray and made permeable by toluene-treatment, that rejoining of DNA single-strand breaks as well as recovery of transforming activity was taking place when the permeable cells were subsequently incubated in the presence of precursors and cofactors for DNA-polymerase and DNA-ligase (Noguti, T. and Kada, T., 1972, J. Mol. Biol. **67**: 507). Because 3′-OH chain termini are required for the function of the Kornberg-type DNA-polymerase and 3′-OH end groups of chain breaks of DNA irradiated with X- or γ-ray may be complicated due to damage of deoxyribose residues (Kapp, D. S. and Smith, K. C., 1970, Rad. Res. **42**: 34; Bopp, D. S. and Hagen, U., 1970, Biochim. Biophys. Acta, **209**: 320), certain "cleaning factor" might have worked prior to the repair process involving DNA-polymerase and DNA-ligase. Existence of such a factor is suggested in the present studies.

When cells of *Bacillus subtilis* strain 17A were irradiated with 30 KR of γ-ray, treated with 1 % toluene at 0°C and incubated at 37°C in potassium phosphate buffer containing 4dXTP, Mg^{2+} and ^{3}H-TTP, incorporation of the label in the DNA fraction increased remarkably during the incubation period. Such an increase was very mild if irradiation was not given. Addition of N-ethylmaleimide (1.5 mM), an inhibitor for semi-conservative DNA synthesis in toluene-treated *E. coli* cells, had no effect on the result. When DNA was extracted from γ-ray irradiated and toluene-treated cells and was used as primer for purified *Micrococcus* DNA-polymerase (Zimmerman, B. K., 1966, J. Biol. Chem., **241**: 2035) for *in vitro* DNA synthesis, we found that the incorporation rate of the radio-active precursor was several fold greater with DNA extracted from cells irradiated with 30 KR γ-ray than with that from unirradiated cells.

Because the priming activity of cellular DNA was enhanced by γ-ray-irradiation of the cells, the situation is completely reversed from the case

of *in vitro* irradiation as shown by Harrington, H. (1964, Proc. Nat. Acad. Sci., U.S., **51**: 59; 1966, J. Mol. Biol., **15**: 152). It is supposed that DNA chain termini required for DNA-polymerase function may have been produced enzymatically in the cell. In order to examine the presence of such a factor in cellular proteins, following experiments were performed. Toluenized cells were treated with lysozyme and Brij-58 and centrifuged at 12,000 g for 20 minutes; the supernatant fraction was used as "cellular extract".

Table 1. Effect of Pronase treatment and heating on the "cleaning" activity of cellular extract. Pronase treatment was done at a concentration of 3 mg/ml for 20 minutes at 37°C. Heating of cellular extract was done in boiling water for 10 minutes

Treatment	γ-ray dose (R)	Minutes of incubation period	Incorporation rate (cpm/60 min./tube)
Brij-lysozyme solution	0	40	498
	1000	40	164
Cellular extract	0	0	452
	0	40	918
	1000	0	210
	1000	40	2140
Pronase-treated cellular extract	0	40	461
	1000	40	181
Heated cellular extract	0	40	316
	1000	40	180

DNA was extracted from phage T7, divided into three parts and treated in following different ways. In the first, the priming activity for *Micrococcus* DNA-polymerase was determined directly on the DNA sample. The second portion was combined with the cellular extract of *B. subtilis*, incubated and then the DNA extraction procedure was followed. The third portion, which served as a control for the second, was combined with the Brij-lysozyme solution containing no cellular extract, and the DNA extraction procedure was performed exactly as was done for the second portion. The rate of *in vitro* DNA synthesis with *Micrococcus* DNA-polymerase was reduced in the first or third treatment, as radiation dose increased, while it was enhanced by the second treatment where the irradiated DNA samples were

combined with the cellular extract.

Adjoining experiments were performed using T7 DNA to determine whether the above cellular factor is an enzymatic protein or not. Results given in Table 1 show the following points: (1) *In vitro* irradiation of T7 DNA with 1000 R γ-ray reduced considerably its priming activity for *Micrococcus* DNA-polymerase. A similar radiation effect was obtained even if the irradiated DNA was incubated with the Brij-lysozyme extract not containing the cell and the DNA isolation procedure was followed as usual. (2) The priming activity increased remarkably compaired to the controls by incubating the irradiated DNA with the cellular extract, but only slightly if the DNA was not irradiated. (3) Such an increase was not found when the cellular extract was treated with Pronase or heated before incubation with the irradiated DNA.

From the above results it is clear that the cellular factor enhancing the priming activity of DNA following γ-ray-irradiation is a protein. We therefore suppose that this factor may "clean" radio-lesions in DNA and produce 3′-OH chain-termini required for DNA-polymerase function. Further purification studies of the above factor are in progress.

Adenosine triphosphate-dependent deoxyribonuclease activities in *Bacillus subtilis*

Yoshito SADAIE and Tsuneo KADA

Deoxyribonucleases requiring ATP for their function have been detected in wild strains of *Escherichia coli* and several other microbial species but were lacking in *rec B* and *rec C* mutant strains of *E. coli*, and its possible role in genetic recmbination has been discussed (Tsuda, T. and Strauss, B. S., 1964, Biochemistry **3**: 1678; Winder, F. G. and Coughlan, M. P., 1967, Biochim. Biophys. Acta, **134**: 215; Buttin, G. and Wright, M., 1968, Cold Spring Harbor Symp. Quant. Biol., **33**: 259; Oishi, M., 1969, Proc. Nat. Acad. Sci., U.S., **64**: 1292; Anai, H., Hirahashi, T., Yamada, M. and Takagi, Y., 1970, J. Biol. Chem., **245**: 775; Barbour, S. D. and Clark, A. J., 1970, Proc. Nat. Acad. Sci., U.S., **65**: 955; Goldmark, P. J. and Linn, S., 1970, Proc. Nat. Acad. Sci., U.S., **67**: 434). Recently, Vovis, G. F. and Buttin, G. (1970, Biochim. Biophys. Acta, **224**: 42) showed that a recombination-deficient mutant strains of *Diplococcus pneumoniae* lacked in the ATP-

dependent deoxyribonuclease activity. We found these enzyme activities in wild and recombinationless strains of *Bacillus subtilis*.

The deoxyribonuclease activity in the cell lysate was assayed by measuring the radioactivity in the acid-soluble product released from native or heat-denatured ^{3}H-DNA. Cells of *Bacillus subtilis* 168 *thy* were labelled with ^{3}H-thymidine. DNA was extracted and purified by the phenol method.

Table 1. ATP-dependent and non-dependent nuclease activities of *Bacillus subtilis*

Min. of incubation for reaction	c.p.m. in acid soluble material			
	Native DNA		Denatured DNA	
	−ATP	+ATP	−ATP	+ATP
0	50.8	79.4	110.6	42.6
30	114.5	793.4	748.6	837.6
60	101.0	1274.2	1306.0	1545.6

Heat-denaturation of DNA was performed by heating the SSC solution (0.15 M NaCl–0.015 M sodium citrate) of ^{3}H-DNA at 100°C for 10 min. and cooling rapidly in ice-water. Preparation of Brij-58 cell lysate was based on the method described by De Lucia and Cains [Nature **224**: 1164 (1969)]. Cells of *B. subtilis* H17 grown in 100 ml TF-medium supplemented with 10 μg/ml arginine and 10 μg/ml tryptophane were collected by centrifugation at their late log phase and were resuspended in 5 ml of 10% sucrose-0.1 M Tris-HCl (pH 8.3). After addition of lysozyme and EDTA (finally 50 μg/ml and 5 mM respectively), the mixture was kept at 37°C for 30 min. Further lysis of cells was carried out by supplementing Brij-58 and $MgSO_4$ (finally 0.5% and 0.05 M respectively) and incubating at 37°C for 5 min. The lysate was centrifuged at 19,500 g for 30 min. and the supernatant was collected and used immediately for the enzyme assay. The reaction mixture for measurement of ATP-dependent deoxyribonuclease activity contained, in 0.5 ml of Tris-HCl buffer (62 mM, pH 8.7), 0.1 ml of the cell extract, 20 μg ^{3}H-DNA and 0.1 mM ATP (neutralized previously with NaOH). This mixture contains some amount of Mg^{++} derived from the cell extract. The mixture was incubated at 37°C and the reaction was stopped, at indicated time, by cooling to 0°C and adding 2 drops of 0.2% bovine albumin and 2 ml of 10% TCA. The sample was finally centrifuged at 12,500 g for 15 min. and the supernating 0.2 ml fraction was used for

counting the radioactivity of acid-soluble material with Beckman Scintillation Counter Model 225.

Results obtained with cells of strain H17 possessing wild recombination capacity are shown in the Table. It is shown that the strain has two kinds of deoxyribonuclease activities: the one requiring ATP and acting on native DNA; the other specific to denatured DNA and independent of ATP. These enzyme activities were also found in several typical recombinationless mutant strains of *Bacillus subtilis* so far studied.

Effect of ultraviolet light on DNA degradation in wild and recombinationless cells of *Bacillus subtilis* with or without toluene-treatment

Yoshito SADAIE and Tsuneo KADA

In some typical mutants of *E. coli* K12 carrying single or multiple *rec* genes, positive correlations have been pointed out between cellular possession of ATP-dependent nuclease and degree of DNA-degradation following UV-irradiation. Strains carrying a *rec A* gene alone exhibit "reckless" DNA-degradation, on the other hand those carrying a single *rec B* or *rec C* gene, or both, in addition to the *rec A* show "cautious" DNA-degradation which is the character shown by *rec B* or *rec C* mutant strains (Willetts, N. S. and Clark, A. J., 1969, J. Bacteriol., **100**: 231). The *rec* B^+ or *rec* C_+ gene determines ATP-dependent DNase which, therefore, seems to be engaged in the degradation of DNA after UV-irradiation (Buttin, G. and Wright, M., 1968, Cold Spring Harbor Symp. Quant. Biol., **33**: 259).

To examine these relationships in *B. subtilis* recombinationless derivatives which we isolated, cells of different strains were labelled with ^{3}H-thymidine and the release of acid-soluble radioactive materials was measured following UV-irradiation. We found that the cellular degradation of DNA was initiated by UV-irradiation and that, in spite of high UV-sensitive characters, the extent of DNA degradation of two *rec* strains (M45 and L43) was slightly higher, but not "reckless", than that of the wild strain (H17). The situation is not therefore comparable to the case with *E. coli* strains.

Toluene-treatment makes the cellular membrane permeable and accessible to small molecules such as co-factors for cellular nucleases. The treatment at 0°C was effective in *Bacillus subtilis* (Noguti, T. and Kada, T.,

1972, J. Mol. Biol., **67**: 507). Cells labelled with ^{3}H-thymidine were exposed to UV of different doses, toluenized and washed at 0°C. Cells were then incubated in potassium phosphate buffer with various supplements such as ATP, Mg^{++} or 4XTP, and radioactivities in TCA soluble materials were determined.

Following were main results obtained.

(1) When toluene-treated cells were incubated in phosphate buffer without supplements at 37°C, no detectable degradation of cellular DNA was observed. However, if the incubation was done with Mg^{++}, a considerable DNA-degradation took place. Similar results were obtained using the wild strain (H17) as well as the recombinationless strains (M45 and L43).

(2) In these wild and recombinationless cells of strains H17, M45 and L43, the presence of ATP promoted further the above Mg^{++}-stimulated degradation. However, ATP alone without Mg^{++} was not effective enough to provoke the DNA degradation. This degradation may be independent of DNA synthesis in the cell, because addition of 4dXTP (together with ATP and Mg^{++}) to the incubation buffer did not modify the degradation pattern.

(3) UV-irradiation of the cells before toluene-treatment did not promote the DNA-degradation described above.

This observation was similar in both the wild (H17) and the two recombinationless strains (M45 and L43) studied.

These observations lead us to assume that, either in wild or recombinationless cells, toluene-treatment impaired the integrity of cellular membrane which may be required for manifestation of UV-induced DNA-degradation and made cells susceptible to certain endogenous nucleases including the ATP-dependent one.

Cell culture of *Haplopappus gracilis*

Etsuo AMANO

A single cell suspension culture of a plant could be useful in UV and other mutagenesis studies. For this purpose, *Haplopappus gracilis* ($2n=4$) seemed to be promising as the callus tissues derived from it is soft and fast-growing. Small chromosome number would make the cytological analysis easier and might help to stabilize the ploidy of the cell.

The surface of the seeds was disinfected with 1: 1 mixture of 95% ethanol and 3% H_2O_2 for 10 min. After rinsing the seeds twice with distilled water they were sown on a mineral supplemented agar medium in glass capped test tubes, and were placed under continuous illumination by a white fluorescent lamp and kept at 25°C. From the germfree seedlings the tissues were dissected and placed on a slant medium, which contained inorganic salts (T. Eriksson (1965) Physiol. Plant. **18**: 976–993) and organic supplements including 2.4.D (2 ppm), Vitamines, yeast extract (0.3%) and sucrose (2%). The medium was adjusted to pH 5.8 by 1 N-NaOH and was used either as liquid or solidified by agar (0.8%). For induction of callus, 10 ppm of 2.4.D was effective, but standard concentration of 2 ppm for callus culture also worked well.

Three callus lines were derived from leaf (HL), stem (HS) and root (HR). A line of callus (HSe) was induced directly from a seed placed on 2.4.D containing medium. Another line (HNC) was rather spontaneous in origin. A cutting of stem, placed on mineral supplemented agar medium without 2.4.D ,produced fine powdery cells from the stem surface near the base. These cells were transferred to slant medium and became a callus line. All of the five callus lines were soft and grew well and fast. But some differences among them were observed.

1) Hs tended to show a granular organization, especially at low (0.5 ppm) 2.4.D concentration, but the others did not. 2) HSe, HS and HR turned to dark brown by aging but HNC and HL kept their fresh light color longer. This coloration was remarkable by its progressive widening of the colored area when developed in a culture on an agar plate in a Petri dish. 3) Microscopical observation revealed rather homogeneous cell populations in HNC and HL in contrast to the mixture of variously shaped cells in HSe.

Liquid culure could be made in 100 ml Erlenmeyer flasks on a rotating stand (2 rpm). The suspension of cells, however, was not fine enough and visible aggregates were present. We were not yet successful in developing good size colonies from a filtered cell suspension. Cytological observation by aceto-carmine staining of the liquid cultured materials (HSe) showed a pair of meta-centric and a pair of submeta-centric chromosomes. No poly- or aneuploid cells were observed.

Grafting test of seedling mutants in *Caspicum annuum*

Etsuo AMANO

Modification of genetic traits of scions by grafting has been known in *Solanaceae*. To study the presence and, if present, the characteristics of the phenomenon, with comparable genetic background, seedling mutants have been induced in *Capsicum annuum* var. Sapporo Wase. Among the mutants induced by ethyl methanesulfonate, seven chlorophyll mutants were tested for survival by grafting.

Table 1. Results of grafting of seedling color mutants of *Capsicum annuum*, var. Sapporo Wase. Scions were grafted on normal (+/+) plants, at the stage when the cotyledons were opening

Scion	Mutant			Normal plants segregated	
	Viability	Grafting	Survival*	Grafting	Survival*
alA_1	lethal	10	0	13	13
alA_2	"	5	0	2	2
ygA_1	"	17	0	4	4
ygA_2	"	26	0	4	4
lgA_1	weak	9	8	—	—
lgA_2	"	2	2	—	—
lgA_3	"	9	7	—	—

*: Survival after two weeks.

Two albinos and two yellow mutants among the seven mutants tested, were lethal and died before producing the first leaf. These lethal mutants could not grow even on a sucrose supplemented nutrient agar medium. As shown in the table, these lethal mutants could not survive on normal green (+/+) stocks, although the sister green plants segregated from the same fruit i.e. mutant/+ and +/+) could be grafted successfully.

The remaining three mutants, yellow- or light-green, were all weak but viable. Most of the grafts obtained from these viable mutants were successful and the scions grew further. Although all of the three mutants could be distinguished from the normal green sisters segregated in seedling stage, they got greener and in one mutant mottling on the new leaves developed, so that somatic reverse mutation sector would be difficult to score.

Graftings of very distinct color mutants, albino and yellow, were not successful. But their heterozygotes will be useful for a somatic mutation

test of mutagenes, as the inactivation of normal gene in mutant/+ heterozygous tissue can be scored as mutant colored sectors. Experiments of double mutagenesis in search of a reverse mutation sector in a mutated tissue are being tried using the yellow mutants.

Radiosensitivity in stored wheat seeds

Taro FUJII

In this study, an attempt was made to obtain some information about the relationship between storage and radiosensitivity of *Triticum monococcum* var. *flavescens*. Seeds harvested in July 1970 were stored in a low temperature storage room (12°C with 40% humidity) until the sowing time which was October 27, 1971. One lot of stored seeds was irradiated with ^{137}Cs source of 6 kCi strength, during January 1971 and again stored under similar conditions until sowing time. A second lot of stored seeds as well as freshly harvested seeds were irradiated with the same source on October 27, 1971. All three lots were sown simultaneously on October 27, 1971. One hundred seeds were used for each treatment and the doses administered were 0, 3, 5, 7, 10, 15 and 20 kR.

Germination percentage and seedling height of 20 days old seedlings were used as an index of radiation injury. In general, germination rate of stored seeds was lower than that of fresh seeds. There was no dose effect on germination in the fresh and non-post irradiation stored seeds. Drastic effect of radiation was observed in the 9 months post-irradiation storage series beyond 15 kR treatment.

Radiation damage in relation to seedling height was noticed at 20 kR in fresh seeds while with post and non-post irradiation storage seeds it was at 7 kR. The seedling height reduction was however more drastic in post-irradiation storage as compared to non-post irradiation storage seeds, the percentage seedling height at 20 kR being 5.3 and 36.2, respectively. At low doses viz., 3 and 5 kR, stimulation effect was noticed in fresh as well as stored seeds. From the above data it may be inferred that post-irradiation storage has a definite radiation damage effect unlike irradiated seeds sown immediately.

Fractionation effect of neutrons on mutation frequency in miaze

Taro FUJII

Recovery of premutational damage or decrease of mutation frequency in relation to *Bz*-gene, after maize pollen grains irradiated by gamm-rays, fractionated at 2 h interval, was already reported (Jap. J. Genet. **46**, 243–251). To obtain further information about the mutational response at the same locus with high energy fractionation radiation treatments, two

Table 1. Relationship between mutation frequency and fractionation treatments

Dose (rads)	Number of seeds	Mutation frequency (%)
First experiment		
Single treatment		
250	5951	2.07
389	5615	2.87
641	6353	5.10
Fractionation treatment (2 h)		
148+167	6375	2.31
205+193	6494	3.11
271+250	6411	4.35
Second experiment		
Single treatment		
323	2723	1.29
432	2447	2.98
680	1732	4.50
Fractionation treatment (3 h)		
154+158	5107	1.94
198+240	3553	2.56
316+330	5613	3.53

experiments were conducted with maize pollen grains, using the 14 MeV neutron source. The fractionation time intervals in these experiments were 2 and 3 hours. Three dosages ranging from 250 rads (minimum) to 640 rads (maximum) were selected in the first experiment. In the second experiment, the three doses ranged between 323 and 680 rads. The results are presented in Table 1.

The differences observed between the total dose in single and fractionation

treatments are due to the differential output of energy emitted by the neutron source at different dates of treatments. Mutation frequency increased linearly in relation to dose both in single and in 2 h fractionation treatments. Results of the second experiment with 3 h interval treatment, on the other hand, showed some differences in mutation frequency between single and fractionation series. Although there was a dose relationship response in both single and 3 h fractionation treatments the mutation frequency in general was less in the fractionation treatment.

From the above results it may be assumed that the mutational effect of fast neutrons could be repairable but the extent of recovery is smaller than in the case of gamma-ray treatments, as indicated in the earlier studies where a significant recovery or lowering of mutation frequency was observed. Further, the environmental conditions which are known to modify greatly the effect of gamma-rays, however do not appear to do so in the case of neutron treatments.

VIII. POPULATION GENETICS (THEORETICAL)

Theoretical foundation of population genetics at the molecular level

Motoo KIMURA

A theoretical framework based on the diffusion equation method was used to treat some problems of population genetics at the molecular level.

To represent the mode of production of molecular mutants, two models were considered. One is the "model of infinite sites" and the other is the "model of infinite alleles."

In the model of infinite sites, it is assumed that the number of nucleotide sites making up the genome is so large while the mutation rate per site is so low that whenever a mutant appears it represents a mutation at a new site. This was used to investigate such problems as the behavior of mutants in a finite population, the rate of mutant substitution in evolution, and the amount of heterozygosity and linkage disequilibrium under steady flux of mutations.

In the model of infinite alleles, it is assumed that the number of possible allelic states of a cistron is so large that whenever a mutant appears it represents a new, not preexisting allele. This was used to investigate the number of alleles and probability of polymorphism in a finite population.

A hypothesis that mutants are selectively neutral at the majority of sites but are selected at a relatively small number of sites was examined, and the roles of associative overdominance and subdivided population structure in maintaining genetic variability were considered. For details, see Theor. Population Biology **2**: 174–208.

Linkage disequilibrium between two segregating nucleotide sites under steady flux of mutations in a finite population

Tomoko OHTA and Motoo KIMURA

Linkage disequilibrium or nonrandom association of mutant forms between two segregating nucleotide sites in a finite population was studied

using diffusion models, assuming that the number of nucleotide sites making up the genome is so large while the mutation rate per site is so low that whenever a mutant appears, it represents a mutation at a homoallelic site, i.e., a site in which no mutant forms are currently segregating in a population. It was shown that under steady flux of molecular mutations in a finite population, if we measure the amount of linkage disequilibrium between two segregating sites by

$$\sigma_d^2 = E\{D^2\}/E\{x(1-x)y(1-y)\},$$

where D is the ordinary coefficient of linkage disequilibrium, and x and y are the frequencies of mutants at the two sites, then we have

$$\sigma_d^2 \approx (5+2R)/(11+26R+8R^2),$$

where $R=N_e c$ in which N_e is the effective size of the population and c is the recombination fraction between the two sites. It was pointed out that if multiple alleles in a random mating population are generated through segregation at two or more nucleotide sites within a cistron, a strong linkage disequilibrium is usually expected between those sites, even in the absence of selection. For details, see Genetics **68**: 571–580.

Behavior of neutral mutants influenced by associated overdominant loci in finite populations

Tomoko OHTA and Motoo KIMURA

The behavior of isoallelic mutants in finite populations was investigated with special reference to nonrandom association of neutral isoallelic mutants to overdominant loci by random genetic drift. The overdominant loci are assumed to be sparsely distributed along the whole chromosomes and the neutral loci are distributed more densely among them. The behavior of neutral mutants is influenced by the surrounding overdominant loci and the apparent selective force takes the form of "associative overdominance". This was treated theoretically using a model assuming that the overdominant loci are equally spaced and have equal selection coefficients. For this model the approximate magnitude of associative overdominance was estimated. Mont Carlo experiments proved the validity of the theoretical prediction, although the estimated degree of associative overdominance is not as effec-

tive as true overdominance. For the set of parameters used in the experiments, (effective population size 100 or 200, 21 linked overdominant loci with heterozygote advantage of 0.01 in chromosome segments of 0—0.66 recombination fraction) it was about 1/4 as effective as true overdominance in retarding gene fixation. The associative overdominance at intrinsically neutral loci, will contribute, at least partly, to bring about a constant distribution of neutral alleles, by preventing fixation of these alleles in local populations. For details, see Genetics **69**: 247–260.

Genetic load due to mutations with very small effects

Tomoko OHTA and Motoo KIMURA

A unified treatment of substitutional and mutational loads was presented assuming a steady flux of molecular mutations in a finite population. The case of genic selection for diploid populations ("no dominance") was analysed in detail. We restrict our consideration to segregating loci, disregarding the part of the load coming from fixed deleterious mutants.

An important point which emerged from the analysis is that mutations with very small effects (having selection coefficients of the order of the reciprocal of the effective population number) can create considerable genetic load. This suggests that slightly detrimental mutations constitute a real threat to eventual extinction for a species having small population number. If N_e is the effective population size and s is the selective disadvantage of the mutant, the mutational load as a function of N_es may be used to define the "cline" between neutral and deleterious mutations. The load depends on $|N_e s|$ rather than $|s|$ alone. The importance of mutations with very small effects for variation and evolution of the species was discussed. For details, see Jap. J. Genet. **46**: 393–401.

Associative overdominance caused by linked detrimental mutations

Tomoko OHTA

Associative overdominance due to linked detrimental mutations was investigated using the method of moment equations based on diffusion mo-

dels. The expectation of the apparent selective value at the marker (neutral) locus has been evaluated. Assume two linked loci, at one of which the steady flux equilibrium is reached under constant mutational input of deleterious mutations (with rate v) having disadvantages hs in heterozygote and s in homozygotes. At another locus, the neutral alleles are segregating with frequencies near 0.5. Let N_e be the effective size of the population and c be the recombination fraction between the two loci. Then the coefficient of associative overdominance at the neutral locus can be obtained by taking the expectation with respect to chromosome frequencies at steady flux equilibrium. It becomes approximately

$$s' \approx \frac{L_I - L_0}{2N_e(c + hs) + c/2hs},$$

where $(L_I - L_0)$ is the inbreeding depression caused by deleterious mutations under complete inbreeding, and $N_e hs \gg 1$ and $hs \gg v$ are assumed.

More generally, if the inbreeding depression of a chromosome segment with a length of recombination fraction C is $(L_I - L_0)$ then s' at the neutral marker at the edge of the segment is

$$s' \approx \frac{L_I - L_0}{2N_e C} \log \frac{C + hs}{hs},$$

where hs is the average heterozygote disadvantage of detrimentals.

The significance of the associative overdominance is discussed in relation to actual observations. It is proposed that the most of the observed heterozygote superiority including inversion chromosomes of Drosophila, isozyme alleles in Avena and ABO blood group genes in man could be explained by the associated detrimentals. For details, see Genet. Res., Camb. **18**: 277–286.

Pattern of neutral polymorphism in a geographically structured population

Motoo KIMURA and Takeo MARUYAMA

In a two-dimensional stepping-stone model of finite size, if a pair of alleles happen to segregate in the whole population, marked local differentiation of gene frequencies can occur only if migration between colonies is sufficiently rare so that $Nm < 1$, where N is the effective size of each colony

amd m is the rate at which each colony exchanges individuals with four surrounding colonies each generation. On the other hand, if $Nm \geq 4$, the whole population behaves as if it were panmictic and the allelic frequencies become uniform over the entire distribution range unless mutation is unusually high. Tendency toward local differentiation is much weaker in two-dimensional than in one-dimensional habitats. For details, see Genet. Res. Camb. **18**: 125–131.

Some methods for treating continuous stochastic processes in population genetics

Takeo MARUYAMA and Motoo KIMURA

The purpose of this note is to give methods of calculation of quantities associated with the genetics of natural populations. The methods are based on diffusion approximation.

Consider a random mating population of constant effective size and a locus at which two alleles, say A_1 and A_2, are segregating. Then the change in the frequency of A_1 follows a Markov chain. Let us denote by $x(\omega, t)$ the gene frequency of A_1 in sample path ω at time t, with $p=x(\omega, 0)$. Let $f(x)$ be an arbitrary function defined on the open interval (0, 1). We define the following quantity

$$F_1^{(n)}(p) \equiv u(p)E\left\{\left[\int_0^{\tau(\omega)} f(x(\omega, \xi))d\xi\right]^n \middle| x(\omega, \tau(\omega)) = 1\right\}$$

where $E\{\ \}$ indicates the expectation with respect to the paths, $\tau(\omega)$ is the fixation time, and $u(p)$ is the fixation probability of A_1. Then $F_1^{(n)}(p)$ represents the fixation probability times the expectation of the n-th moment of the sum of the quantity given by $f(x)$ from time 0 until fixation, given that the initial frequency is p. Now let

$$\phi(\lambda, p) \equiv \sum_{n=0}^{\infty} \frac{\lambda^n}{n!} F_1^{(n)}(p) .$$

Then $\phi(\lambda, p)$ satisfies the conditions $\lim_{\lambda \to 0} \phi(\lambda, p) = u(p)$ and

$$L\phi(\lambda, p) + \lambda\phi(\lambda, p)f(p) = 0, \tag{1}$$

where

$$L = \frac{V_{\delta p}}{2}\frac{d^2}{dp^2} + M_{\delta p}\frac{d}{dp}$$

and where $M_{\delta p}$ and $V_{\delta p}$ are respectively the mean and variance of the change of gene frequency per generation.

The above treatment gives the sum of the quantity, but it is also desirable to know the expectation of the quantity at a specified time. This can be done by solving for the transition probability explicitly. In doing this we need to solve a partial differential equation. An alternative way is to use the Laplace transform. Let

$$g_1(t, p) \equiv u(p)E\{f(x(\omega, t))\,|\,x(\omega, \tau(\omega)) = 1\}$$

and let

$$G(\lambda, p) \equiv \int_0^\infty e^{-\lambda t} g_1(t, p)dt.$$

Then $G(\lambda, p)$ satisfies the ordinary differential equation

$$LG(\lambda, p) - \lambda G(\lambda, p) = -f(p)u(p).$$

Here the mutation in one or both directions is allowed.

Example. We shall calculate the expectation of the gene frequency of a neutral gene (A_1) in the course of fixation. For mathematical simplicity, instead of calculating the frequency of A_1, we calculate the frequency of A_2. Then we need to solve

$$\frac{p(1-p)}{4N}\frac{d^2G(\lambda, p)}{dp^2} - \lambda G(\lambda, p) + p(1-p) = 0.$$

The appropriate solution is $G(\lambda, p) = (p(1-p))/(\lambda+(1/2N))$ and its inverse Laplace transform is

$$g_1(t, p) = p(1-p)e^{-\frac{t}{2N}}.$$

Therefore the mean gene frequency of the successful gene (A_1) is given by

$$1 - (1-p)e^{-\frac{t}{2N}}.$$

Similar analogues of the above treatments for calculations including both cases of fixation and loss of A_1 can be constructed easily.

Time-transformation of a diffusion model of a geographically structure population

Takeo MARUYAMA

Consider a population consisting of a series of colonies and consider a locus at which two alleles (A_1 and A_2) are segregating. Let $x_{\omega,t,i}$ be the gene frequency of A_1 at time t in sample path ω, and let $N_{t,i}$ be the size of colony i at time t. Let $N = \sum_i N_{t,i} =$ a constant, and $X_t = 1/N \sum_i x_{\omega,t,i}$. Let the relative fitnesses of A_1A_1, A_1A_2 and A_2A_2 be $2s$, s and 0 respectively. We assume that mating and selection occur independently in each colony and that only migration connects the colonies. Let

$$\tau_\omega \equiv \int_0^t \frac{1}{N} \sum_i 2x_{\omega,\xi,i}(1 - x_{\omega,\xi,i})N_{\xi,i}d\xi$$

and let $P(\tau, X, Y)$ be the probability that the overall gene frequency of A_1 is X when $\tau_\omega=0$ and is Y when $\tau_\omega=\tau$. Then irrespective of the geographical structure of the population, $P(\tau, X, Y)$ satisfies the following Kolmogorov backward euqation:

$$\frac{\partial P}{\partial \tau} = \frac{1}{8N} \frac{\partial^2 P}{\partial X^2} + \frac{s}{2} \frac{\partial P}{\partial X},$$

and therefore we have the solution for the transition probability:

$$P(\tau, X, Y) = 2e^{-\frac{SX}{2}} \sum_{k=1}^{\infty} \frac{e^{-\frac{1}{2}\left(k^2\pi^2 - \frac{S^2}{4}\right)\tau} \sin k\pi X \sin k\pi Y}{\left(k^2\pi^2 - \frac{S^2}{4}\right)},$$

where $S = 4Ns$.

The process governed by $P(\tau, X, Y)$ is a standard Brownian motion over (0, 1) with absorbing states at $Y=0$ and $Y=1$.

IX. POPULATION GENETICS (EXPERIMENTAL)

Evidence for the neutral hypothesis of protein polymorphism

Tsuneyuki YAMAZAKI and Takeo MARUYAMA

A question that is currently much discussed is the mechanism for maintenance of polymorphisms in natural populations. There are two main hypotheses: One is that the polymorphism is mainly due to neutral, favored, or harmful alleles that are in the process of increasing to fixation in the population or are being lost by drift. The other hypothesis is that these are maintained by recurrent mutation of alleles that are maintained by balanced selective forces, such as heterosis or frequency dependent selection. The purpose of this note is provide evidence to distinguish the two possibilities. Consider an arbitrary number of polymorphic loci in a Mendelian population of arbitrary geographical structure. Then classify the polymorphisms into equally spaced classes according to their gene frequencies in the entire population (not local gene frequency), e.g. ten classes of 0.1 interval. Say genes (polymorphisms) A_{1_1}, A_{1_2}, ..., A_{1_n} belong to the first class, A_{2_1}, A_{2_2}, ..., A_{2_n} to the second class and so on. Let $H(A_{i_j})$ be the actual number of heterozygotes formed between gene A_{i_j} and any other in the entire population. Then if all the alleles are neutral, the sum of $H(A_{i_j})$ over all j is equal for all i, *i.e.*

$$(1) \quad H_1 \equiv \sum_j H(A_{1_j}) = H_2 \equiv \sum_j H(A_{2_j}) = H_3 \equiv \sum_j H(A_{3_j}) = \cdots = H_n.$$

This is true regardless of whether the population is divided into wholly or partially isolated subgroups or is geographically continuous with isolation by distance.

We should note here that the number of polymorphisms which belong to a particular class is influenced by the population structure and it can not be known unless we know the details of the structure. The same is true for the distribution of gene frequencies of polymorphisms. Therefore it is quite remarkable that when we assess the amount of heterozygotes, given that the gene frequencies are specified, the total amount is invariant under the population structure. This invariance holds true for the selective case of additive effect. When there is dominance between alleles, the invariant property itself breaks down. However the sum of heterozygotes, the H_i,

can be calculated for a random mating population under otherwise the same assumptions.

The absolute values of the H_i are not so much informative for they depend on mutation rate and population size. But the relative values of H_i are of our interest. Four different situations, in which all the mutant genes are 1) neutral, 2) additively advantageous, 3) additively deleterious and 4) overdominant, are illustrated by the curves in Figure 1. The cases 1) and 2) assume somewhat similar pattern while the other two are entirely different.

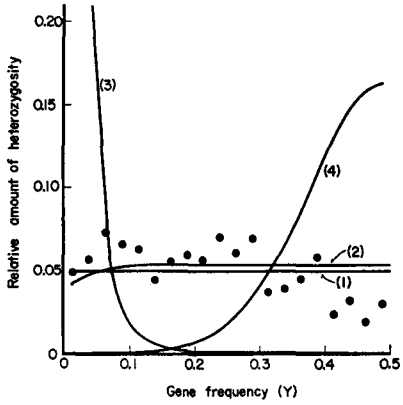

Fig. 1. Distribution patterns of heterozygosity. The curves indicate the theoretical expectations: (1) neutral; (2) advantageous ($Ns=10$); (3) deleterious ($Ns=-10$); (4) overdominance ($Ns=10$). The dots indicate the observed results. (The total area under each curve and the dots is unity.) From Yamazaki and Maruyama (1972).

We note that when we wish to compare the pattern of distribution of the H_i, we can combine data, from different Mendelian populations and even from different species. We have done this by collecting data for some 400 polymorphisms in Drosophila, mice, sparrow and man. The result is shown by the dots in Figure 1, (Yamazaki and Maruyama, 1972, Science **178**: 56–58).

Detection of single-gene effect by inbreeding

Tsuneyuki YAMAZAKI

The technique of inbreeding is often used in detecting the effect of single-gene. However, it is rather difficult to interpret the results of experiments correctly unless the experiments are carefully planned. For example, in a recent issue of Nature, C. Wills and L. Nichols (Nature **233**: 123 (1971)) published a short article claiming evidence for single gene heterosis at the octanol dehydrogenase (ODH) locus of *Drosophila pseudoobscura*. In brief their methods and results are as follows; In each generation, pairwise brother-sister matings were made using only heterozygotes for the locus. The expected ratio of progeny from this mating is 1: 2: 1. An excess of heterozygotes was observed under the stressed media containing octanol, the substrate of ODH, after the 12 generations of inbreeding, although no deviation from Hardy-Weinberg proportion was observed under the same medium in outbred flies. Thus, they concluded that the heterosis was pronounced under the stressed media when the background genes were made homozygous by inbreeding and that this heterosis was attributed to the effect of ODH locus alone.

However, there is one serious shortcoming in their method which makes this conclusion unwarranted. Namely, the rate of increase of inbreeding coefficient by this inbreeding method is much quicker in the homozygotes than in the heterozygotes. To clarify this point, I have performed simulation experiments, assuming the length of the chromosome to be either 50, or 100 morgans. The method of inbreeding was exactly the same as that used by Wills and Nichols. The rates of the decrease of heterozygosity in both genotypes are shown in Figure 1. Even after 12 generations of inbreeding, approximately 28% of linked genes remain heterozygous in the heterozygotes with respect to the marker locus when the chromosome length is 50 morgans. On the other hand, only 3% of linked genes remain heterozygous in the homozygotes with respect to the marker. (17% in the heterozygotes, and 4% in the homozygotes in case of 100 morgans.) The excess of the heterozygotes will naturally arise due to the difference in the amount of heterozygosity in the background of the two genotypes, considering a large amount of genetic variability concealed in chromosomes. Nevertheless, there is tendency that an excess of heterozygotes were more pronounced under the octanol medium than under the KCl medium, at

each locus. It has been found from our experiment that octanol medium is more harmful to the survival of Drosophila than KCl medium. Therefore, the results obtained by Wills and Nichols can be interpreted as follows: (1) an excess of heterozygote at the marker locus was observed after inbreeding, because a sufficient difference in the amount of heterozygosity at the

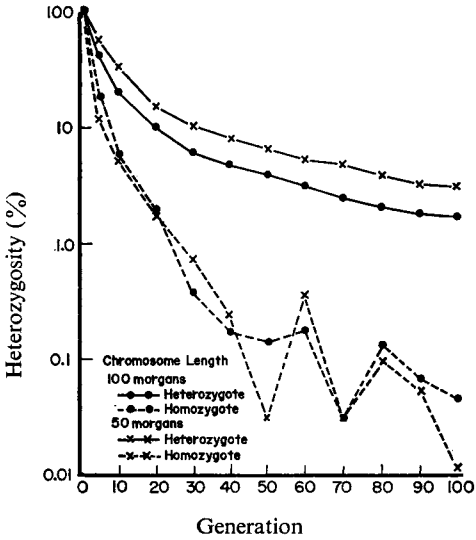

Fig. 1. Rate of the decrease of heterozygosity of the linked genes by inbreeding, in both heterozygote and homozygote with respect to the marker gene. The values are the average of 10 experiments.

genes linked to the marker was produced between the two genotypes in the course of inbreeding. (2) An excess of heterozygote was observed under the octanol medium but not under the KCl medium because octanol is more harmful to the survival of flies than KCl and that the homozygotes were preferentially killd in the octanol medium.

Deleterious and sterility genes in Japanese and Korean natural populations of *Drosophila melanogaster*[1)]

Chozo OSHIMA and Jong-Kil CHOO[2)]

Many flies of *Drosophila melanogaster* were collected simultaneously in the middle of September, 1971 at two sites of Anyang, the suburbs of Seoul, in Korea, and on the other hand, many flies of the same species were collected simultaneously in November at Katsunuma and Shikishima in Yamanashi prefecture. The distances between two sites of Anyang and between two sites of Yamanashi were about 8 km and 20 km respectively.

Many second chromsomes, extracted from each male fly, were analyzed by Cy/Pm method, and the results are shown in Table 1.

Table 1. Frequencies of deletrious and normal second chromosomes isolated from Korean and Japanese natural populations of *D. melanogaster*

Country	Population	No. of tested chromosomes	Frequency (%)			
			lethal	semilethal	subvital	normal
Korea	Anyang- I	80	19 (21.6)	5 (5.7)	11 (12.5)	53 (60.2)
	Anyang-II	107	24 (22.4)	6 (5.6)	12 (11.2)	65 (60.7)
	Total	195	43 (22.1)	11 (5.6)	23 (11.8)	118 (60.5)
Japan	Katsunuma	208	53 (25.5)	35 (16.8)	27 (13.0)	93 (44.7)
	Shikishima	202	68 (33.7)	16 (7.9)	22 (10.9)	96 (47.5)
	Total	410	121 (29.5)	51 (12.4)	49 (12.0)	189 (46.1)

The total frequency of lethal and semi-lethal chromosomes in Japanese populations was about 42 percent, and that in Korean populations was about 28 percent. The frequencies of delterious chromosomes in Kofu and Katsunuma natural populations during several years (1965–1968) have been maintained to be a about 22 percent, but the frequency of deleterious chromosomes was found to be about 40 percent in both summer and autumn populations in 1970. The cause of such an increase in the last two years remains unexplained.

The mean viabilities of homozygotes for all second chromosomes and

1) This work was supported by a grant (50801) from the Ministry of Education.

2) Visiting researcher from Department of Biology, Chung-Ang University, Seoul, Korea.

also for quasinormal chromosomes were represented in Table 2. The mean viability of all homozygotes of Japanese populations was lower significantly than that of Korean populations.

Table 2. Mean viabiliies of homozygotes for all second chromosomes and also quasinormal chromosomes of *D. melanogaster*

Country	Population	All homozygotes		Quasinormal heterozygotes	
		No. of tested chromosomes	viability	No. of tested chromosomes	viability
Korea	Anyang- I	88	22.46±1.45	64	30.35±0.56
	Anyang-II	107	22.18±1.33	77	30.38±0.50
	Total	195	22.31±0.98	141	30.36±0.37
Japan	Katsunuma	208	18.35±0.96	120	29.37±0.44
	Shikishima	202	18.09±1.03	118	29.84±0.45
	Total	410	18.22±0.66**	238	29.66±0.32

** Highly significant at 1% level.

Table 3. Frequencies of sterility second chromosomes in Korean and Japanese natural populations of *D. melanogaster*

Country	Population	No. of tested chromosomes	Frequency (%)			
			Female	Male	Both sexual	Total
Korea	Anyang- I	67	15 (22.4)	6(9.0)	0 —	21 (31.3)
	Anyang-II	81	8 (9.9)	6 (7.4)	2 (2.5)	16 (19.8)
	Total	148	23 (15.5)	12(8.1)	2 (1.4)	37 (25.0)
Japan	Katsunuma	152	13 (8.6)	28 (18.4)	4 (2.6)	45 (29.6)
	Shikishima	128	10 (7.8)	21 (16.4)	2 (1.6)	33 (25.8)
	Total	280	23 (8.2)	49 (17.5)	6 (2.1)	78 (27.9)

A total of 428 second chromosomes, excluding lethal chromosomes, was examined to carry recessive sterility gene. The results were shown in Table 3.

Total frequencies of sterility second chromosomes in natural populations of both countries were not so different, but the frequency of male sterility chromosomes in Japanese populations was much higher than that of female sterility chromosomes, and the frequency of female sterility chromosomes in Anyang-1 population was especially high. The frequency of sterility second chromosomes in Katsunuma locality increased in the last two years as deleterious chromosomes.

X. EVOLUTIONARY GENETICS

On the Rate of Molecular Evolution

Motoo KIMURA and Tomoko OHTA

There are at least two outstanding features that characterize the rate of evolution at the molecular level as compared with that at the phenotypic level. They are; (1) remarkable uniformity for each molecule, and (2) very high overall rate when extrapolated to the whole DNA content.

The population dynamics for the rate of mutant substitution was developed, and it was shown that if mutant substitutions in the population are carried out mainly by natural selection, the rate of substitution is given by

$$k = 4N_e s_1 v,$$

where N_e is the effective population number, s_1 is the selective advantage of the mutants, and v is the mutation rate per gamete for such advantageous mutants (assuming that $4N_e s_1 \gg 1$). On the other hand, if the substitutions are mainly carried out by random fixation of selectively neutral or nearly neutral mutants, we have

$$k = v,$$

where v is the mutation rate per gamete for such mutants.

Reasons were presented for the view that evolutionary change of amino acids in proteins has been mainly caused by random fixation of neutral mutants rather than by natural selection.

It was concluded that if this view is correct, we should expect that genes of "living fossils" have undergone almost as many DNA base replacements as the corresponding genes of more rapidly evolving species. For details, see J. Molecular Evolution **1**: 1–17.

On the constancy of the evolutionary rate of cistrons

Tomoko OHTA and Motoo KIMURA

The variations of evolutionary rates in hemoglobins and cytochrome *c* among various lines of vertebrates are analysed by estimating the variance. The observed variances appear to be larger than expected purely by chance.

If the amino acid substitutions in evolution are the result of random fixation of selectively neutral or nearly neutral mutations, the evolutionary rate of cistrons can be represented by the integral of the product of mutation rate $v(s)$ and fixation probability $u(s)$ in terms of selection coefficient s around the neutral point. Namely,

$$k = 2N\int_{-2/N_e}^{2/N_e} u(s)v(s)ds,$$

where N and N_e are respectively the actual and effective sizes of the population. This integral is called the effective neutral mutation rate.

The influence of effective population number and generation time on the effective neutral mutation rate is discussed. It is concluded that the uniformity of the rate of amino acid substitutions over diverse lines is compatible with random fixation of neutral or very slightly deleterious mutations which have some chance of being selected against during the course of substitution. On the other hand, definitely advantageous mutations will introduce significant variation in the substitution rate among lines. Approximately 10% of the amino acid substitutions of average cistrons might be adaptive and create slight but significant variations in evolutionary rate among vertebrate lines, although the uniformity of evolutionary rate is still valid as a first approximation. For details, see J. Molecular Evolution **1**: 18–25.

Serological Crossreaction of the Ascidian Liver Extract with Anti-Rat Transferrin Rabbit Serum

Kazuo MORIWAKI and Tamiko SADAIE

There have been numerous reports on the existence of iron-binding protein, transferrin, in the sera of various classes of vertebrates as extensively reviewed by Lush (The Biochemical Genetics of Vertebrates Except Man, North-Holland, 1966). For the purpose of surveying the phylogenical step in which the transferrin molecule had emerged, phylum protocordata was firstly investigated in this study.

Liver of the ascidian, *Halocynthia roretzi* collected at Kesennuma in Miyagi Pref., was homogenized with 3 volumes of 5 mM Tris-buffer (pH 8.6) followed by centrifuging at 10,000 rpm for 30 min at 0°C. As the crude

extract indicated a detectable crossreaction with anti-black rate transferrin rabbit serum in the Ouchterlony test, further purification of the transferrin-like substance has been undertaken employing DEAE-cellulose and Sephadex G–150 column chromatography. Elution from DEAE-column following gradual increase of sodium chloride concentration up to 0.1 M in 5 mM Tris-buffer (pH 8.6) introduced 2 peaks, one of which designated peak 2 contrained a certain substance reacting with the anti-transferrin serum. Though an ability of binding iron was demonstrated by Fe^{59}-radioimmunoassay in the crude extract above obtained, it was shown to be involved in peak 1.

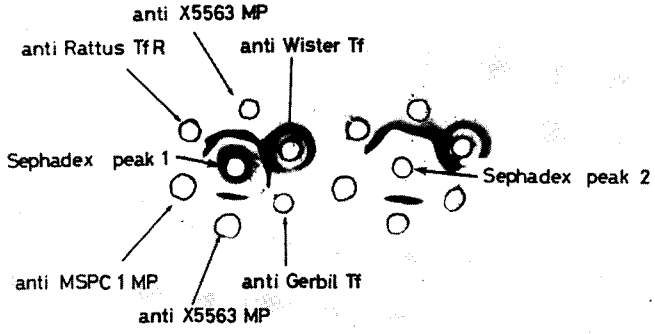

Fig. 1. Cross-reaction of the ascidian liver extract with anti-transferrin and anti-globulin rabbit sera in the Ouchterlony test.

The peak 2 was re-chromotographed on Sephadex G–150 using 5 mM Tris-buffer and 2 protein peaks, approximate molecular weight 40,000 and 150,000, have come out. Both fractions, however, equally had positive cross-reactions with various rabbit antisera; anti-black rat transferrin, anti-norway rat (Wistar rat) transferrin, anti-mongolian gerbil transferrin, anti-mouse γ-chain (H-chain of $\times$5563 γG myeloma globulin). Whereas they had negative reaction with anti-mouse α-chain (H-chain of MSPC–1 γA myeloma globulin). These data are demonstrated in Figure 1. It is of great interest that the ascidian liver extract seems to share a certain antigenic structure not only with serum transferrins of black rat, norway rat and mongolian gerbil but also with γG class heavy chain of mouse immunoglobulin.

XI. HUMAN GENETICS

An estimate of the effective number of loci for total finger ridge-count

Ei MATSUNAGA

In the previous report (Matsuda and Matsunaga, 1971, Ann. Rep. Nat. Inst. Genet. **21**: 101) it was noted that the frequency distributions of total finger ridge-count from an unrelated sample of 313 males and 313 females were more or less negatively skewed in both sexes, with the mean and the variance 151 and 2,124 for males and 142 and 2,008 for females, respectively. The parent-child correlation estimated from 1,048 pairs was 0.44 ± 0.03 and the sib-sib correlation from data on 188 sibships was 0.46 ± 0.05, while the parent-parent correlation was 0.02 ± 0.07. The regression of child on midparent was 0.82 ± 0.05, and a linearity test gave no sign of dominance. These results indicate that the great majority of variation in total ridge-count in the Japanese are to be attributed to genes with additive effect.

From the non-normality of the frequency distribution of total ridge-count in population, Holt (Ann. Human Genet. **20**: 159, 1955) conjectured that only a small number of genes having appreciable effect is involved, since a large number of additive genes would give a nearly normal distribution. A simple method is suggested here to give a rough estimate of the effective number of loci for this character.

It is assumed that 1) the variation in total ridge-count is determined by n pairs of alleles with perfectly additive effect, 2) there are only two alleles, A and a, at each locus with frequencies p and q ($p+q=1$) respectively, 3) the effect in single dose of A is α and that of a is zero in terms of ridge-count, and 4) the genes are segregating freely in the population. Then $\bar{x}=2n\alpha p+\mu$, $V_g=2n\alpha^2pq$ and $d=2n\alpha$, where $\bar{x}$ is the population mean, μ the base value assumed by the genotype homozygous for the gene a at all loci, V_g the genetic variance and d the maximum range of the frequency distribution. In the case of total ridge-count, the value of μ is obviously zero since individuals with zero total ridge-count have actually been observed. It follows that

$$p = \frac{\bar{x}}{d},$$

$$\alpha = \frac{dV_g}{\bar{x}(d - \bar{x})} \quad \text{and}$$

$$n = \frac{\bar{x}(d - \bar{x})}{2V_g}.$$

V_g can be estimated from the observed variance mutiplied by the heritability, or twice the parent-child correlation, which corresponds to about 0.9 in our data. The value of d cannot be estimated with certainty. The observed range of frequency distribution in our sample was about 260 in both sexes, so that d must be greater than this. On the other hand, because the distribution is negatively skewed, the value of p must be larger than 1/2, and from $p=\bar{x}/d$, d cannot exceed twice the value of $\bar{x}$. Assuming $d=290$ and applying the observed values for the mean and the variance in our data, we obtain the following estimates for males: $p=0.52$, $\alpha=26.4$ and $n=5.5$. The corresponding values for females can be obtained in the same way, but in this case, allowing for the sex difference in the mean, the same value for $\bar{x}$ as in males was used. The estimates are essentially the same as for males: $p=0.52$ $\alpha=25.0$ and $n=5.8$. If $d=280$, this would bring only a slight reduction of the estimate of n.

It is to be noted that, if the value of α differs from locus to locus, the estimate of n gives its lowest limit. Therefore, the minimum number of loci may roughly be estimated as about six, which must be taken as a first approximation because of the many assumptions involved.

Determination of the exchange points in a human ring chromosome

Yasuo NAKAGOME and Kazuso IINUMA

Ring chromosomes have been described in all 7 groups of human chromosomes. In none of these rings so far reported, the breakage points were determined. The only example of determination of exchange points in structural abnormality was that reported by Bobrow and Pearson ((1971) J. Med. Genet. **8**: 240). They determined the exchange points in a translocation involving no. 4 and no. 18.

A ring chromosome was observed in a 4 month-old boy with cri-du-chat syndrome. His karyotype was 46,XYq+, 5r, based on either of the quinacrine fluorescence and the Giemsa-banding techniques. The ring seemed to be very stable. Out of 184 cells karyotyped, rings were observed in 181 cells, 2 of them being dicentric and 179 monocentric.

The points of breakages in the process of ring formation were determined by the comparison of G-banding patterns of the ring with those of the normal no. 5 chromosome. It was shown that the normal no. 5 chromosome had a narrow light band at the end of each arm, followed by a dark band proximal to it. The light and the dark bands were designated as p 15 and p 14 bands for the short arm, and q 35 and q 34 bands for the long arm. The lengths of the two light bands, amounted to 11.4 and 8.5% of the length of the normal no. 5 chromosome (L) respectively. In every ring, where individual bands were identified, either of the p 14 and the q 34 bands could be recognized. Therefore, two points of breakages in the formation of the ring must be within either of the p 15 and the q 35 bands. The length of a light band between the two dark bands, p 14 and q 34, in the ring was 14.4% of the L. The size of deletion in the process of ring formation was estimated to be 5.5% of the L. The fluorescence technique seemed to be less efficient than the Giemsa-banding technique used here as far as the pattern in a ring is concerned. (For details, see Nakagome et al. Cytogenetics, in press)

G group chromosomes in satellite associations

Yasuo NAKAGOME

Either of satellite associations and separation difficulties of chromosomes at anaphases are likely to result from persistence of nucleoli (Heneen and Nichols, (1966) J. Cell Biol. **31**: 543). It was established that 3 pairs of D group chromosomes were involved in satellite associations randomly (Nakagome, (1969) Cytogenetics **8**: 296). However, in some of the cases with translocation involving D group chromosomes, non-random association pattern was demonstrated (Nakagome and Bloom, (1970) J. Med. Genet. **7**: 371).

The present study dealt with two pairs of G group chromosomes, nos. 21 and 22, in satellite associations. Metaphases were derived from normal individuals. The Giemsa banding technique was used for the purpose of

identifying each pair. A total of 152 G group chromosomes in satellite associations were identified. Eighty seven of them were no. 21s and 65 were no. 22s. There was a slight excess of 21s, however, the difference was not significant ($x^2=3.18$ d.f.$=1$). The study is still in progress and additional cells are being scored to elucidate the difference, if any, between 2 pairs of G group chromosomes.

Polyploidy in cells cultured from amniotic fluid

Yasuo NAKAGOME, Kazuso IINUMA and Ei MATSUNAGA

Up to 100% polyploidy has been described in amniotic fluid cultures of pregnancies with normal diploid embryos (Walker *et al.* (1970) Lancet **2**: 1137 and Milunsky *et al.* (1971) J. Pediat. **79**: 303). If in fact this discrepancy frequently happens, the prenatal diagnosis of polyploid embryos would be impossible. It was reported that prolonged culture of amniotic fluid cells increased the incidence of polyploid cells.

The present report describes a technique by which polyploidy was successfully kept below a few percent. In addition, abundant mitoses were obtained within a short period of time. One and a half to 2.0 ml of amniotic fluid was placed in each of a few Falcon Petri dishes (35 mm diameter) and incubated overnight in a 5% CO_2 atmosphere. Next morning the supernatant was replaced with 1.5 ml of fresh medium (MEM supplemented with 20% fetal calf serum). At this time a few fibroblasts were observed in most of the dishes. Cultures were fed every other day. Some dishes were ready for harvest on 4th day of culture. Usually several countable metaphases were then obtained from each dish when harvested. In the present series, cultures were harvested between 10th and 14th day of culture to ensure the presence of abundant mitoses. Before harvesting, usually in the evening, the culture medium was replaced with fresh medium. Next morning, colcemid (final concentration 0.06 γ/ml) was added. After 4 to 5 hours, cultures were trypsinized and harvested. Special care was taken to avoid to lose cells in the process of harvest.

A total of 482 metaphases were obtained from 6 different cultures. There were 11 tetraploid and 471 diploid metaphases, the incidence of tetraploid being 2.3%. In 3 cultures, some of the dishes were cultured further, up to 54 days and then harvested. Cells in mitosis were few in these cultures.

All 76 metaphases observed were diploid.

The number of cultures and cells observed after prolonged period of culture is still limited. However, the present study suggests that the overwhelming increase of polyploid cells may be avoided by improving of culture technique.

Y-chromatin in aged males

Kazuso IINUMA and Yasuo NAKAGOME

Oral smears from 102 males between the ages from 8 months to 87 years were examined in order to clarify whether ageing has any effect on the incidence of Y-chromatin, a highly fluorescing segment of Y chromosome stained with quinacrine derivatives, in the epithelial cells. Slides were prepared by the conventional technqiue including 20 minute staining in 0.005% quinacrine mustard dihydrochloride in McIlvaine's buffer at pH 7.0. One hundred well preserved cells per person were examined and scored for Y-chromatin positive cells under a Nikon-FL fluorescence microscope. Cells with Y-chromatins were further divided into three classes: (a) single body, showing a single distinct fluorescing spot, (b) duplex body, showing two similar spots adjacent to each other and (c) multiple bodies, multiple spots other than those scored as duplex.

The scores for total Y-chromatin positive cells (a+b+c) ranged from 43 to 99% with an average of 79.1±11.1% (Standard deviation). The incidences of cells with duplex and multiple spots were 11.5% and 2.9% respectively. The regression equation upon age based upon individual records was given by $Y=-0.056X+81.85$ for total Y-chromatin positive cells. There seems to be a slight and insignificant decrease with advancing age ($t=1.17$, d.f.$=100$, $0.3>p>0.2$). When cases were divided into two groups, one containing 42 subjects over 65 years of age and the other containing 60 subjects of younger age, then the mean scores were 77.6 and 80.1%, respectively, the difference between the two groups was small but significant ($x^2=9.24$, $p<0.005$). It is noteworthy that the incidence of Y-chromatin positive cells above 75 years of age exhibited the lowest score, and that this was the group contributed to the decrease of Y-chromatin positive cells in the older males. Of 102 subjects, one case with normal

phenotype had an unusually large Y-chromatin, suggesting the presence of a large Y chromosome. For details, see Iinuma and Nakagome (1972), Jap. J. Hum. Genet. **17**: 1.

Parental age in translocation Down's syndrome

Ei MATSUNAGA and Akira TONOMURA*

In continuation from the preceding year's work, data on the parental age at the birth of patients with translocation Down's syndrome have been collected from various cytogenetic centers in Japan. The number of cases available for analysis amounts to 102. Their distribution by the type and the origin of translocation is as follows: 23 t(DqGq), 29 t(GqGq) and one G/G tandem translocation of sporadic origin; 20 t(DqGq) and 4 t(GqGq) with maternal inheritance, and 6 t(DqGq) with paternal inheritance; and 9 t(DqGq) and 10 t(GqGq) of unknown origin. Examination of the data using vital statistics as controls revealed no evidence for paternal age effect, while maternal age was shown to be of importance in the etiology of the sporadic t(DqGq) or t(GqGq) trisomy. The mean maternal age was decreased for the t(DqGq) (25.5 years) but increased for the t(GqGq) (28.5 years), both decrease and increase being significant when compared with the control (27.1 years); the mean difference between paternal and maternal age was significantly greater in the former (4.4 years) than in the latter group (2.3 years). When the data are combined for the sporadic and unknown origin, the relative risk increases with advancing maternal age for the t(GqGq) while it decreases for the t(DqGq). These findings, together with the greater variance in maternal age for the t(GqGq) than for the t(Dq-Gq), suggest that the majority of the sporadic t(GqGq) are either t(21q21q) or 21qi and the rest t(21q22q), and that non-disjunction, isochromosome formation and centric fusion between non-homologous acrocentrics may all be correlated, in the first two instances positively and in the last one negatively, with maternal age. On the other hand, no alteration of maternal age was found for the t(DqGq) inherited from the mother. Detailed data will be published elsewhere.

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Location and neighboring amino acid sequence of inter- and intrachain disulfide bridges of human IgM

Tomotaka SHINODA

To determine the amino acid sequence and position of the disulfide bridges in IgM obtained from patients with macroglobulinemia, purified material was digested with pepsin and the peptides were separated by high voltage paper electrophoresis. Peptides containing cysteine were also isolated after tryptic, peptic, or thermolysin digestion of the μ heavy chain of IgM and of Fab and Fc fragments. The bridge peptides were sequenced after oxidation or alkylation followed by purification by paper electrophoresis or column chromatography. The results showed that human IgM has five intrachain disulfide bridges, and four interchain brideges. Two of the interchain bridges link μ heavy chains together, one interchain bridge joined each light chain to a heavy chain, and one bridge holds five monomeric units together to form a pentamer of one million molecular weight. The sequence and the location of the bridges in the μ heavy chain were as follows: Thr-Cys-Thr-Phe-Ser-Gly/Tyr-Cys-Ala-Arg-Val-Val-Asn, Fd variable; Gly-Glu-Cys/Val-Ser-Cys-Glx-Asx-Ser, L–H interchain; Val-Gly-Cys-Leu/Val-Cys-Lys (Glx, His, Asx, Asx, Gly, Pro, Lys, Glx), Fd constant; Ile-Cys (Glx, Ala, Thr, Gly, Phe, Ser, Pro, Arg) Gln/Thr-Cys (Arg, Val, Asx, His, Arg, Gly, Leu, Thr) Phe, Fd-Hinge; Cys-Val-Pro-Asx-Glx-Asx-Thr-Ala-Ile-Arg, Fc μ; Thr-Cys-Leu/Thr-Cys-Thr (Val, Thr, His, Thr, Asx, Pro, Ser, Leu, Pro, Leu) Lys-Gln, Fc μ; Ile-Thr-Cys-Leu/Tyr-Thr-Cys-Val-Val-Ala, Fc μ; Ser-Asp-Thr-Ala-Gly-Thr-Cys-Tyr, C-terminal.

XII. BEHAVIORAL GENETICS

Phototaxis and fecundity of *Drosophila virilis*.[1)]

Chozo OSHIMA and Jong-Kil CHOO

Many flies of *Drosophila virilis* were collected from the warehouse of a brewery company in Tokyo, in April 1970, and a cage population was set up with them. Approximately after three generations, about 200 females and 200 males were sampled randomly from the population. The sexes were run separately through the maze apparatus and they chose either light or dark pathway eleven times when they came to the fork of a way. About twenty pairs of flies having the similar extreme photoscores, positive or negative, were mated, and their offspring were subjective to the same selection. These both photo-positive and negative populations were inbred separately in a milk bottle containing culture medium at 25°C. Such selections were repeated over for 30 generations. The mean photoscores and variances for every ten generations were shown in Table 1.

The mean photoscores and variances of the photo-positive population have not changed remarkably throughout the experiment, but the mean photoscores of the photo-negative populations increased gradually and on the contrary its variances have reduced.

The regression coefficient of differences in photoscores between the photo-positive and negative populations was calculated to be 0.2382 for the former 15 generations and to be 0.3542 for the latter 15 generations of selection.

The fecundity of flies in these phototactic populations at the 20th and 30th generations was examined. Six pairs of F_1 flies of each four isoline extracted from these populations respectively were cultured separately in a small vial and transferred everyday to a new vial under the three kinds of light condition: 1) constant light, 2) constant dark, 3) periodical light and dark (9: L, 3: L→D, 9: D, 3: D→L). These eggs laid by each female on the cultural medium were scored everyday for ten consecutive days. The experimental results are shown in Table 2.

The mean number of eggs laid by photo-negative flies was about 20 percent

1) This work was supported by a grant (4050) from the Ministry of Education.

Table 1. Mean photoscores and variances for every ten generation of *Drosophila virilis* populations

Generation of selection	Mean photoscore and variance of both sexes of the photo-positive population						Mean photoscore and variance of both sexes of the photo-negative population					
	Female		Male		Both sexes		Female		Male		Both sexes	
	$\bar{X}$	σ^2	$\bar{X}$	σ^2	$\bar{X}$	σ^2	$\bar{X}$	σ^2	$\bar{X}$	σ^2	$\bar{X}$	σ^2
0–10	4.15	9.94	4.67	11.37	4.51	10.61	6.22	11.51	6.16	10.91	6.20	11.35
11–20	4.28	10.31	4.56	10.04	4.43	10.19	7.09	9.04	7.15	9.41	7.10	9.29
21–30	4.37	10.74	4.44	10.53	4.41	10.71	8.51	6.52	8.73	6.25	8.62	6.36

Table 2. Mean number of eggs per day per female for 10 consecutive days under three kinds of light conditions, which is F_1 fly of four isoline extracted from the photo-positive and negative populations at 20th and 30th generation

Population	Light condition	Number of eggs/day/female		
		Gen. 20	Gen. 30	Combind
Photo-positive	Constant light	45.21±3.52	38.87±2.72	39.54±3.12
	Constant dark	35.23±2.28	32.62±3.26	33.92±2.77
	Periodical L: D	48.21±2.91	35.52±1.93	41.87±2.42
Photo-negative	Constant light	67.67±4.49	48.31±2.19	57.99±3.34
	Constant dark	56.13±4.17	51.26±2.56	53.70±3.37
	Periodical L: D	56.30±2.47	49.55±2.37	52.93±2.42

more than that laid by photo-positive flies, but no significant difference was observed among these different light conditions within each population. The mean number of eggs per day per female and also their standard errors showed a tendency to decrease for ten generations.

Selection and hybridization analysis on the phototactic behaviour of *Drosophila melanogaster*

Chozo OSHIMA and Jong-Kil CHOO

From female flies of *Drosophila melanogaster* collected at Nago-cho, Okinawa in April 1970, eleven iso-female lines were established and total 452 females and 365 males of F_1 fles were collected from these iso-female lines and introduced into a population cage. The procedure of selection by using the maze apparatus in this experiment was similar to that in the experiment of *Drosophila virilis*. The effects of selection in both photo-positive and photo-negative directions are summarized in Table 3.

Table 3. Mean photoscores and variances of every five selected generation of both photo-phostive and negative populations of *D. melanogaster*

Generation of selection	Photo-positive population		Photo-negative population	
	Score	Variance	Score	Variance
0	5.04	12.17	5.04	12.17
1– 5	4.37	8.94	5.50	9.84
6–10	4.32	9.11	6.29	10.87
11–15	4.14	8.00	6.62	10.99
16–20	3.22	6.47	7.27	10.72
21–25	3.44	6.37	7.66	8.72

The photoscore of the foundation population was 5.04, and it has diverged gradually and fairly by the repeated selection for 15 generations and the photoscores of both photo-positive and photo-negative populations would reach to the equilibrium states respectively at around 20th generation. On the other hand, the variances of the photo-positive population have decreased gradually, but those of the photo-negative population have not so changed.

The heritability (h^2) could be calculated by the following formular:

$h^2=(\sigma_0^2-\sigma_g^2)/\sigma_0^2$ where σ_0^2 was the wild type variance of the foundation population and σ_g^2 was the isogenic variance of the selected population. (N. M. Hadler, 1964, Genetics **50**: 1269–1277). After 25 generations of selection, the heritabilities of the photo-positive and negative populations were obtained to be 0.597 and 0.368 respectively, and the combined heritability of both populations was 0.483.

The photoscore and variance of hybrid populations between the photo-positive and negative populations at 21st generation were examined and the results were shown in Table 4.

Table 4. Photoscores and variances of hybrid populations between photo-positive and negative populations at 21st generation

Generation 21	Female		Male		Combined	
	Photo-score	Variance	Photo-score	Variance	Photo-score	Variance
Photo-positive	3.19	6.37	3.73	7.28	3.48	6.92
Photo-negative	7.93	9.66	7.97	8.03	7.95	8.80
Average	5.56		5.85		5.72	
F_1 (posi × nega)	5.40	11.14	5.67	8.96	5.53	10.06
F_1 (nega × posi)	6.10	10.18	5.47	11.11	5.81	10.61
Average	5.75		5.57		5.67	

The photoscores of the hybrid populations were close to the average ones of these photo-positive and negative populations. As expected, the variances of the hybrid populations were larger than those of all parental populations. The phototactic behaviour was assumed to be dependent a polygenic system.

Circadian rhythm of fecundity of *Drosophila virilis* under various light environments[1)]

Chozo OSHIMA and Jong-Kil CHOO

A cage population of *Drosophila virilis* was set up with 123 females and 137 males which were collected from the warehouse of a brewery company in Tokyo on April 7, 1971. The population has been kept in an incubator,

1) This work was supported by a grant (91265) from the Ministry of Education.

whose temperature fluctuated diurnally between 20 and 30°C by a program controller. After two months, 20 pairs randomly sampled from the population were mated separately in small vials and they were allowed to lay eggs for 24 hours on culture medium and they were transferred into a new vial everyday. F_1 flies of four strains among twenty parental strains were used in this experiment. Twelve pairs (F_1) isolated from each strain were divided into three groups and the number of eggs laid everyday by these pairs were scored as maintained under three kinds of light environments at constant 25°C. These light environments were as follows: 1) constant light: 2000 lux by fluorescent lamp, 2) Periodical light and dark: 9 hours 2000 lux, 9 hours 0 lux and 3 hours changing from light to dark reciprocally by program controller, 3) constant dark: 0 lux, in this case, the flies were transferred under a red lamp (3 lux). The experiment continued for twenty days.

Table 5. Linear regression coefficients for the fecundity curves of four strains of *D. virilis* from 3rd to 20th days under three kinds of light environments and the results of analysis of variance

Strain	Constant light	Periodical light & dark	Constant dark	Average
A	−2.772	−2.041	−3.292	−2.702
B	−0.905	0.565	−0.531	−0.290
C	−4.761	−0.230	−2.776	−2.589
D	−4.913	−0.896	−1.090	−2.299
Average	−3.338	−0.651	−1.922	

	d.f.	S.S.	M.S.	F
Environ.	2	57.8333	28.9166	5.69**
Strain	3	46.1699	15.3899	2.49*
En. × St.	6	32.3224	5.3870	
Error	36	222.1121	6.1697	
Total	47	358.4374		

The mean numbers of eggs per day per female were 62.5 under the constant light, 61.4 under the periodical light and dark and 62.4 under the constant dark environemtnt. No significant difference was detected among the total eggs poroduced under the three kinds of light environments.

Linear regression coefficients for the fecundity curves of four strains from

3rd to 20th days were calculated and the average values of four strains under the constant light, constant dark and periodical light and dark environments were −3.338, −1.922 and −0.651 respectively. The analysis of variance for these regression coefficients was performed and the difference among three light environments was highly significant at 1 percent level and also significant difference among four strains was found at 5 percent level. The results were presented in Table 5.

The circadian rhythm of fecundity was assumed to be markedly influenced by the light environment and the rhythm could be recognized to be maintained under rhythmically fluctuated light environment. However, B strain showed the similar responses for these light environment.

Bad effects of urbanization and noise environments of a great city on behaviour of Drosophila[1)]

Chozo OSHIMA and Jong-Kil CHOO

Drosophila flies and relative species were collected simultaneously by a sweeping net at two places in November 1971. One place was a rural district, Tamazawa in Misima city and another place was the National Park for Nature Study in Tokyo city. Collected flies were classified, and the number of genera, species and specimens at two places were compared as shown in Table 6.

Table 6. Drosophila flies collected at two places in November 1971

	Genera	Species	Specimens
National Park for Nature Study (Tokyo city)	7	31	287
Tamazawa (Misima city)	4	20	316

Any bad urbanization effect of Tokyo could not be recognized on the result. The *montium* species group (*D. auraria* and *D. rufa*) and the *immigrans* species group (*D. immigrans*) were dominant species at Tamazawa, and on the other hand, *Scaptomyza pallida* was found to occupy about 60 percent in total specimens collected at National Park for Nature Study.

It was examined whether the oviposition of two strains of *D. melanogaster*

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

(Anyang and Katsunuma strains) and of one strain of *D. virilis* (Azumabashi strain collected in Tokyo) would be affected under the noise environment or not. The noise, 3000 cycle/sec. and 100 phone, was produced by a speaker of the Audio Signal Generator (SNR-11) in an incubator KOITOTRON having program controller of photo and temperature conditions.

Fourty sib-pairs of newly emerged flies from five lines of each strain were divided into two groups. The one group was subjected to the noise environment and the other group was cultured under the quiet environment. Each pair of flies was put in a small vial which was covered with a thin paper cap and contained the cornmeal-agar-medium. They were transferred to a new vial everyday. After transferring flies, eggs laid on medium, were scored. Such procedures were continued for 12 or 16 days. The photo-condition was a constant light (2000 lux) and the temperature was kept at 24°C constantly. The results are shown in Table 7.

Table 7. Mean number of eggs per female per day during the first period (6 or 8 days) and the second period (6 or 8 days) under the noise and quiet environments

Environment	Period	*D. melanogaster*		*D. virilis*
		Anyang strain	Katsunuma st.	Azumabashi st.
Quiet	1st	72.19±8.67	62.73±7.73	67.69±3.01
	2nd	61.54±2.77	68.65±1.69	62.84±0.98
	Total	66.87±4.61	65.69±3.89	65.27±1.65
Noise	1st	35.59±6.31	34.55±5.86	52.66±2.45
	2nd	55.22±4.46	69.08±2.11	46.95±1.51
	Total	45.41±4.51	55.15±4.87	49.81±1.57

The state of oviposition of *D. melanogaster* was remarkably depressed during the first period, but such depressions were relieved at the second period. On the other hand, the state of oviposition of *D. virilis* under the noise environment was about 20 percent depressed consistently during the whole period.

XIII. APPLIED GENETICS

Genetic variation in a naturally reproducing sapling-cluster in *Thujopsis dolabrata* Sieb. et Zucc.

K. I. SAKAI, S. IWAGAMI and S. MIKAMI

Reproduction in natural forests is one of the most interesting problems in silviculture and has been investigated ecologically as well as physiologically. Little attention, however, has been paid from the standpoint of genetics. The present study deals with genetic analysis of sapling clusters of *Thujopsis dolabrata* spontaneously growing in a cut-over area of a natural forest of *Cryptomeria japonica* D. Don. The sapling cluster is of an echinus form with a diameter of a few meters, each consisting of more than several "individuals" which were terrestrially saparated from the others. Sapling clusters form a stretch of a crowd on the area. From each of eight clusters selected at random, samples of needle-leaves were collected from five "individuals". Juice squeezed from collected leaves was investigated for isoperoxidase variation in starch-gel electrophoresis. Isoperoxidases were compared among saplings within each cluster in order to find if they were vegetatively propagated or from seed. Needless to say, propagules from the same clone will exhibit a very similar, not necessarily the same due to technical variation, pattern of isoenzyme bands. Isoenzyme similarity between saplings was estimated by measuring the disagreement count between azygous bands or by difference in staining intensity between paired bands. It was found from this study that, of eight clusters, two were found to be groups of vegetative propagules from single clones, five mixtures of clones and seedlings, while the remaining one was a mixure of seedlings. Investigation by analysis of variance of needle characters gave additional evidence of the genetic constitution of sapling clusters. It is expected in future that only one or two trees within each cluster would grow to maturity at the cost of the other. What selection would be responsible for the differential survival among saplings constituting a cluster is an interesting problem.

Isoperoxidase variation among island populations of *Pinus Thunbergii-densiflora*

Y. G. PARK, S. IYAMA and K. I. SAKAI

The Seto inland sea surrounded by Japan Proper, Shikoku and Kyushu islands is dotted with numberless islets, on which the Japanese pine species, *Pinus Thunbergii* and *P. densiflora* are naturally growing. These two pine species, though they have been defined taxonomically as separate species, are often hard to discriminate one from another. Thus, in the present study, we treat them collectively as a single species, *Pinus Thunbergii-densiflora.*

We have collected needle-leaves from approximately 30 trees in each of 20 populations growing on eleven islets. They were investigated electrophoretically for isoperoxidase variation. The maximum number of isoperoxidase bands detected so far in *Pinus Thunbergii-densiflora* was thirteen, but observations were made on four of them: H, K, M and N. The average frequencies of these four bands were 21, 17, 77 and 2%, respectively. By statistical comparison among populations of frequencies of these four bands, it was found that some populations were significantly different from others in the frequencies of one or two isozymes. On the basis of such a comparison, populations not significantly different from each other were collected in one group, taking also into consideration their locations. Thus, twenty populations investigated in the present study were classified into six groups. It is interesting to find that some groups consisted of only one population while other involved six populations which were distributed on different islets. How do such groups of populations come into existence on separate islets will be left for further enquiry.

Effect of mixing genotypes of barley on the performance of a population

Shinya IYAMA and Kan-Ichi SAKAI

This experimental study has been carried out in the past three years and is still under way. The experiment consisted of 12 cultivars of barley grown in single stands and 60 mixtures of four cultivars selected at random from the twelve. Attention was paid to make the entry of each cultivar in mix-

tures equal. It was found from the experiment conducted in 1968–1969 that some mixtures yielded better than that expected on the basis of performances of component cultivars, or were overcompensatory, while other yielded similarly to the means of their components (compensatory) or less than that (undercompensatory). Selection was conducted for and against overcompensatory effect of mixtures and six overcompensating and six undercompensating mixtures were grown in 1969–1970. The result of this study was, however, contrary to our expectation: the overcompensatory as well as undercompensatory effect of mixtures was not always recurring. It was then suspected if some or other environmental conditions including the year effect might have played a role in the overcompensating mechanism of the mixtures. Thus, another experiment was conducted in 1970–1971 with the same mixtures but grown on variable fertilizer levels. Then, it was found that poor fertilizer application caused the overcompensatory effect or the cooperative effect of component cultivars, while higher level of fertilizers showed negative cooperation or undercompensation. Further experimental study is now in progress.

Genetic changes occurring in wild quails due to "natural selection" under domestication

Takatada KAWAHARA

One of the most interesting problems in the breeding of avian species would be to enquire into the biological changes in birds which take place during domestication. For such an investigation, Japanese quails are useful. It should be used because they are being domesticated in Japan since the first decade of this century, on the one hand, and their wild relatives still propagate and migrate between the northern districts and the central parts of Japan proper.

The present paper reports the result of comparative observations on some physiological changes occurring in wild *Coturnix coturnix japonica* reared in cages for three generations. The materials for this study have originated from 268 wild birds captured in the field during the past four years. Wild birds are hardly distinguishable from the domestic ones in their appearance, but are distinctly different in some physiological aspects, for instance, occurrence of non-layers in cages at least up to 20 weeks after hatching, in

number of days to first egg or sexual maturity under continuous light, egg production during 60 days after first egg and body weight at maturity (20 weeks of age).

The present observations have revealed that the frequency of non-layers in wild birds in a cage for the three successive generations, F_1, F_2 and F_3, of propagation within the flock was 50.0, 30.9, and 23.4 percent, respectively, while in the domestic strain none was a non-layer. In the wild strain, there was a great individual variation among birds, which laid egg before the age of 20 weeks, in respect to the number of days required for attaining sexual maturity. The average age at sexual maturity in the first generation offspring of wild birds was 110.0 ± 2.7 days in contrast to 50.1 ± 0.3 days of domestic ones. The second and third generation of the wild strains showed an improvement toward earlier maturity and their mean ages were 82.8 ± 2.3 days and 81.1 ± 1.5 days, respectively.

The hen-day egg production rate was $44.5 \pm 3.7\%$ in the first generation offspring of the wild strain, whereas it was $88.3 \pm 0.5\%$ in the domestic strain. The second and third generations of the wild birds showed a slight improvement of the rate, i.e., $49.6 \pm 2.2\%$ in the second and $52.8 \pm 1.8\%$ in the third generation.

When wild brids were captured in the field, body weight of the female was 97.6 ± 1.0 g., and the first generation offspring weighed 95.3 ± 1.3 g. The second and third generation offspring of the wild strain, however, gained weight during domestication, their body weight being 100.9 ± 0.8 g. and 104.0 ± 0.8 g. The body weight of the domestic female bird was 133.5 ± 0.5 g., on the average.

It is thus concluded that wild strains can show a rapid improvement in growth as well as in egg production as a result of natural selection in domestic environment.

Multiple ovulation and egg defects in Japanese quail

Takatada KAWAHARA

In a domestic strain of the Japanese quail, it was noticed that some families tended to produce more defective eggs than the other. Among the defective eggs observed were double-yolked eggs, shell-less (membrane) eggs and soft-

shelled eggs. Progeny test showed that, of the three defects mentioned above, the occurrence of double-yolked eggs was significantly different among full-sib families. The heritability value calculated from the analysis of variance was 0.49. Investigation indicated that the correlation between double-yolk and egg production was −0.14 when measured on individual bird basis, while it was −0.29 when measured on family basis. Birds which laid double-yolked eggs tended to show earlier sexual maturity, the correlation coefficients being −0.12 and −0.14 on individual bird and family basis. The same correlation coefficients between double-yolk and ovary weight or oviduct weight were 0.33 for individual birds and 0.11 for families or 0.19 and 0.25, respectively. It is of interest to find that the egg productivity and weight of the sexual organs, however, were not correlated. In order to find out the mechanism for such an unusual behavior, a post-mortem examination was conducted with female birds immediately after oviposition of a double-yolked egg. It was then found that double-yolk was caused by simultaneous release of ova instead of successive occurrence of single ovulations.

Estimation of heritability from analysis of variance in a population with related parents

Tohru FUJISHIMA

Conventional method for estimating heritability, i.e. $h^2 = 4\sigma_s^2/(\sigma_s^2 + \sigma_d^2 + \sigma_e^2)$ etc., where σ_s^2, σ_d^2 and σ_e^2 are the components of variance for sire, dam and full-sib in the analysis of variance, bases on the assumption that no genetic relationship exists among any of the parents. However, such an idealized population where there is no relationship among parents is seldom encountered in practice.

The purpose of this paper is to present general formulae for estimating heritabilities based on analysis of variance in a population where genetic relationships may exist among sires, among dams and/or between sires and dams, including mates.

Heritabilities based on sire families, dam families and sire and dam families can be estimated as follows;

$$h_s^2 = \frac{S}{(W+D)(r_2-r_3)+Sr_2}, \qquad h_d^2 = \frac{D}{(W+D+S)(r_1-r_2)+Dr_3}$$

and

$$h_{s+d}^2 = \frac{S+D}{(W+D+S)r_1-Wr_3},$$

where r_1, r_2 and r_3 are Wrigtht's coefficents of relationship between full-sibs, between half-sibs and between non-sibs, and S, D and W are the components for sire, dam and full-sib in analysis of variance, respectively.

Only when there is no relationship among parents, $r_1=1/2$, $r_2=1/4$ and $r_3=0$, the equations reduce to the conventional one.

It was found that heritability estimates must be corrected according to the genetic relationship among members in the population and that the conventional method, ignoring the genetic relationship, may lead to both over- and under-estimation of the heritability depending on the parental relationships.

Estimation of heritability from parent-offspring regression in a population with related parents

Tohru FUJISHIMA

The conventional method for estimating heritability from the parent-offspring regression is strictly applicable only to a population where there are no genetic relationships among the parents, but populations commonly encountered in animal breeding are seldom free of such a genetic relationship.

The purpose of this paper is to present the procedure for estimating heritability applicable to the population where genetic relationships exist among the parents.

1) Heritability estimated from intra-sire dam-offspring regression:

The heritability can be estimated as;

$$\hat{h}^2 = \frac{2b_1}{1-(1-2b_1)m_2'},$$

where b_1=the coefficient of regression of offspring on dam within sire family,

m_2'=Wright's coefficient of relationship between dams within sire family.

It is clear from the equation that $\hat{h}^2=2b_1$ only when $m_2'=0$.

2) Heritability estimated from mid-parent - offspring regression:

$$\hat{h}^2 = \frac{2b_2}{2 + [2(m_1' - m_1) - m_2 - m_3](1 - b_2)},$$

where b_2=the coefficient of regression of offspring on the average of parents' phenotypic values in a pair-mating population,

m_1', m_1, m_2 and m_3=Wright's coefficients of relationship between mates, between sires and dams mated to different sires, between dams mated to different sires, and between sires, respectively.

It should be noted that $\hat{h}^2=b_2$ only when $m_1'=m_1=m_2=m_3=0$.

Variations in growth pattern among rice varieties

Hiroko MORISHIMA and Hiko-Ichi OKA

Variations in growth pattern among rice varieties were studied for better understanding of their "general adaptability". As a part of "International Rice Adaptation Experiment" (under UM/IBP), about 50 varieties from various temperate countries were evaluated at two different fertilizer levels for 3 years (1968–1970) in the Sizuoka Agricultural Experiment Station. From the dry weight data taken during the growing period (3 times) and at maturity, logistic growth curves were computed for each variety in each plot to obtain parameter values showing growth pattern and yielding capacity: maximum growth rate, days from maximum growth to heading, days from maximum growth to the time when growth rate is reduced to half of the maximum (growth persistence), growth rates at 10 different stages, total spikelet number per unit area, grain yield, percentages of grain yield attributable to the growth before and after heading, etc. Studies of variations in these parameter values indicated the following:

1) Analysis of variance showed that the varieties significantly differed in most parameter values; annual variations were significant in growth persistence, total spikelet number and grain yield; fertilizer application affected maximum growth rate, days from maximum growth to heading, grain

yield, etc.; and genotype-environmental interactions were significant in many parameters.

2) To examine the pattern of sequential change in growth rate, correlation coefficients were computed among the estimates of growth rate at 10 different stages and were subjected to principal component analysis. The results showed that the changing pattern in growth rate would be oscillatory. However, varieties with smaller amplitude tended to give higher yield.

3) Major variation trends found among the varieties were: i) early *vs.* late vigor type (maximum growth around the time of floral initiation or at later stages), and ii) short-period rapid growth *vs.* sustained slow growth types. These two variations were independent of each other. The later the maturity of a variety, the more it tended to early vigor and sustained slow growth type. Such varieties appeared to have a high yielding capacity. The variations in growth type caused by fertilizers were similar to those due to varietal genotypes.

The distribution and effects of genes causing F_1 weakness in *Oryza breviligulata* and *O. glaberrima*

Yaw-En CHU and Hiko-Ichi OKA

Weakness of F_1 plants is frequently found in hybrids between strains of *Oryza breviligulata* (wild) and *O. glabberrima* (cultivated rice) endemic to West Africa. A set of two complementary dominant weakness genes, W_1 and W_2, was found to control the observed F_1 weakness. Many *breviligulata* strains had W_1, while most of the *glaberrima* and semi-wild strains had W_2 or were free of both. In the weak F_1 plants, tissue differentiation in adventitious roots seemed to be disturbed. Modifier genes affecting the expression of the weakness genes appear to be also present. (Published in Genetics **70**: 163–173)

Preliminary observations on the establishment of perennial ryegrass and orchard grass varieties

Hiroko MORISHIMA

In order to investigate differential survival of two grass species under different conditions, an experiment was conducted using three varieties of

perennial ryegrass (*Lolium perenne*) and also three of orchard grass (*Dactylis glomerata*). After a germination test, 300 viable seeds per plot (1 m × 1 m) in size) were sown in 1970 in September in pure stand (6 varieties) and species mixture (1: 1, 9 varietal combinations). The 15 populations were tested in two environments: i) road side (2 replications, with and without white clover respectively) and ii) experimental field (fertilized and weeded, 4 replications differently managed in 1971 for another observation). The number of surviving plants was counted two months after seeding and then every three months.

In the road side plots, the number of surviving plants in pure stand two months after seeding significantly differed between species and among the varieties. It ranged from 20% to 28% of viable seeds in perennial ryegrass and from 5% to 16% in orchard grass, and the differences among species and varieties persisted untill next year. In the experimental field, the surviving rates two months after seeding were about 40% in all plots and were reduced to 10–15% next spring showing no significant differences among species and varieties. In the rate of establishment, competition between species was not apparent at least in the first several months. Perennial ryegrass had a relative frequency of about 75% in road side and about 60% in experimental field, and the relative frequencies did not change much for one year. The data suggested that the rate of establishment in an environment could be genotypically controlled.

Collection and preliminary observation of cultivated cereals and legumes in Ethiopia

Sadao SAKAMOTO and Katsuyoshi FUKUI[1)]

Observations and collections of various cultivated plants in Ethiopia were carried out, from December, 1967 to March, 1968, by the Botany Team of the Kyoto University Scientific Expedition to the Sahara and the surrounding areas. About 1,000 samples from the field and 750 samples from 21 local markets were collected during the expedition. Several random samplings from the fields were also made.

Preliminary observation and analysis of variations found in the collected samples of the following cereals and legumes were carried out.

[1)] Institute for the Study of Languages and Cultures of Asia and Africa.

cereals: wheat, barley, teff, sorghum, finger millet and maize
legumes: field pea, chick pea, horse bean, lentil, *Vigna, Phaseolus* and grass pea

An abundant variation in various characters, such as morphology and coloration of spikes and seeds, was observed in the samples of wheat, barley, sorghum, field pea, chick pea, *Vigna* and *Phaseolus*. It was specially characteristic that the occurrence of colored forms was observed commonly in the present collections of the most cereals and legumes. A close association of weedy *Lolium* and *Avena* with wheat and barley cultivation in Ethiopia was clearly demonstrated. Importance of Ethiopia as one of the most important world centers for the origin and differentiation of cultivated plants was again confirmed from the present study. The details were published in Kyoto University African Studies 7: 181–225.

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ABSTRACTS OF DIARY FOR 1971

January	20	186th Meeting of Misima Geneticists' Club
	21	94th Biological Symposium
March	5	187th Meeting of Misima Geneticists' Club
April	22	95th Biological Symposium
May	7	188th Meeting of Misima Geneticists' Club
June	4	189th Meeting of Misima Geneticists' Club
	25	96th Biological Symposium
	25	190th Meeting of Misima Geneticists' Club
July	16	191st Meeting of Misima Geneticists' Club
September	23	192nd Meeting of Misima Geneticists' Club
October	13	193rd Meeting of Misima Geneticists' Club
	15	97th Biological Symposium
	28	98th Biological Symposium
December	17	194th Meeting of Misima Geneticists' Club

FOREIGN VISITORS IN 1971

January	20–21	LEIGHTON, J., Univ. of Pittsburgh, School of Medicine, U.S.A.
February	8	BIRNBAUM, H. National Science Foundation, Tokyo Office, U.S.A.
July	10	KRISHNASWAMI, S., Indian Sericulture Institute, India
July	13	GRAHAM, K. M., Univ. of Malaya, Malaysia
October	4	COOK, R. C. National Parks and Conservation Association, U.S.A.
October	28	CANTAVIEJA, M. H., Agricultural Supervisor, The Philippines
October	30	WALLACE, J., Hy-Line Poultry Comp., U.S.A.
November	20	DEVARAJURS, D., Central Silk Board, India
December	7	DUNHAM, C. L., NAS–NRC, U.S.A. EDINGTON, C. W., USAEC, U.S.A. NEEL, J. V., Univ. of Michigan, U.S.A.

ACKNOWLEDGEMENT

The editor wishes to express his sincere gratitude to Dr. F. A. LILIENFELD for her kindness in reading the original manuscripts. (K. MIURA)

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国立遺伝学研究所欧文年報　第22号
昭和47年12月20日 印刷
昭和47年12月25日 発行

発行者　森　脇　大　五　郎
国立遺伝学研究所内
編集者　三　浦　謹　一　郎
国立遺伝学研究所内
印刷者　笠　井　康　頼
東京都新宿区山吹町184
印刷所　株式会社 国際文献印刷社
東京都新宿区山吹町184
発行所　国立遺伝学研究所
〒411　静岡県三島市谷田 1111
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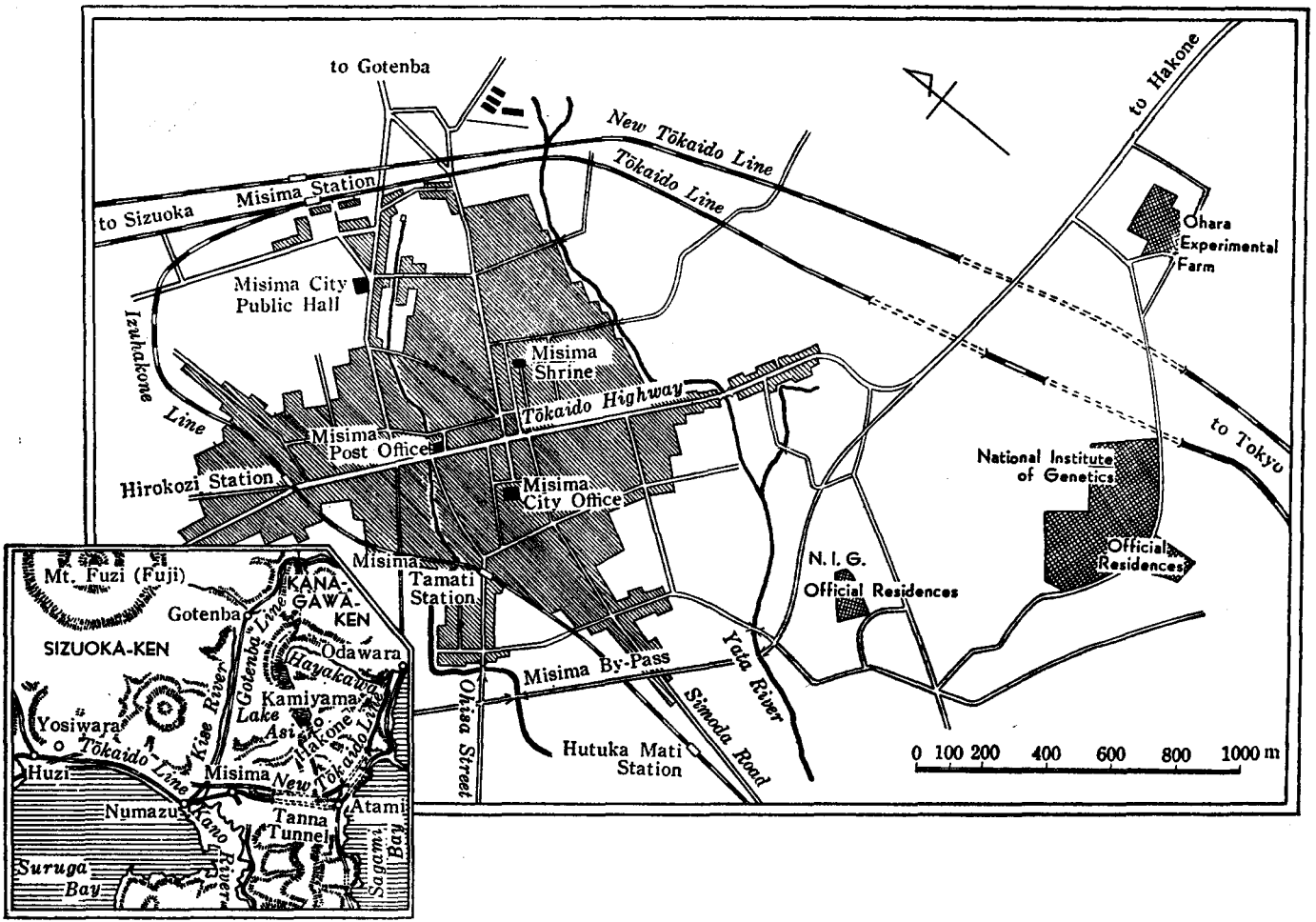
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