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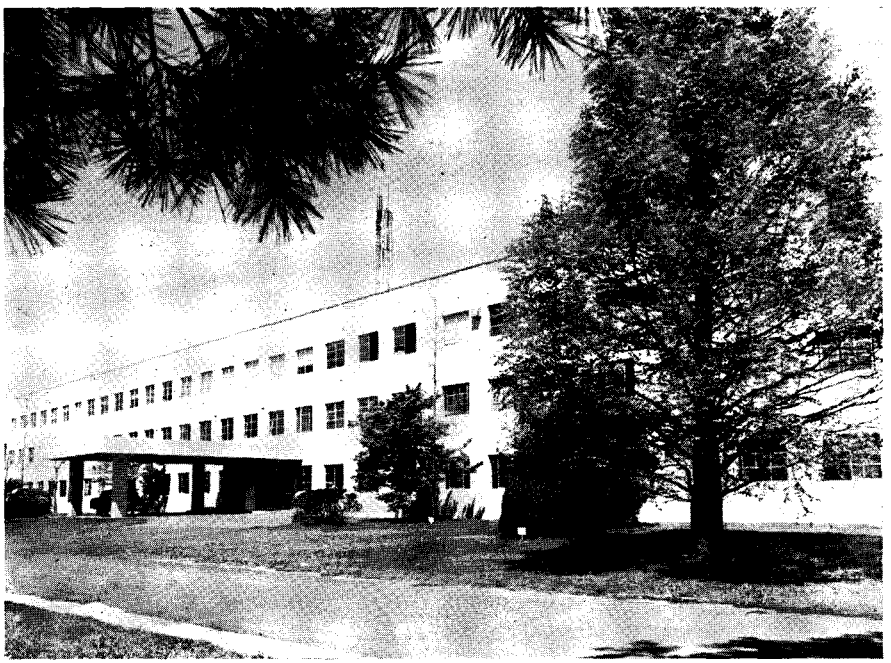

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GENERAL STATEMENT

It is with sadness that I report the deaths of two honorary members of our institute, Dr. Yoshimaro Tanaka and Dr. Taku Komai, one after another in July, 1972, and within one year since we were bereaved of our first director, Dr. Kan Oguma, in September, 1971. We greatly regret that three great leaders, not only in this institute but in Japanese genetics as a whole passed away successively in a single year. Drs. Tanaka and Komai, in co-operation with Dr. Oguma, have made invaluable contributions to the foundation of this institute, especially in the training of the young members, and their devoted efforts have borne rich fruits.

As to international exchange of researchers this year, twelve staff members traveled abroad on official business to attend international conferences, to make co-operative studies, to undertake training, and to make an exploration. Among our many foreign visitors, the two months' stay by Prof. J. F. Crow who, as a Visiting Member, collaborated with Dr. M. Kimura and others in the Department of Population Genetics, was one which we particularly remember. Dr. Marian R. Goldsmith of Harvard University stayed about one month in Dr. Tazima's laboratory in the Department of Morphological Genetics. She made this visit in order to use the facilities which are available only in this Institute, and was engaged in a study of developmental genetics, making considerable use of the special mutant strains of silkworm maintained here.

In March, 1972, Dr. Tetsuo Iino, Head of the Department of Microbial Genetics, was transferred to the University of Tokyo, as Professor of Genetics. Since he obtained a position here in 1952, he has developed elaborate microbial genetical studies using Salmonella. For the three years 1955~1958 he studied abroad at Dr. Lederberg's laboratory at Wisconsin University. During the past twenty years he has brought about many remarkable achievements on the cytomorphogenesis of flagella, the genetic analysis of flagellar antigens, and, recently, the *in vitro* reconstruction of flagellar fibres from their component flagellin molecules. Dr. Tsutomu Sugiyama, who came to us from the Central Research Department of the du Pont Institute, U.S.A., succeeded Dr. M. Tsujita as Head of the Department of Biochemical Genetics in September, 1972.

The older of our two mouseries was reconstructed this year. The new building measures 557 m^2 in floor area, containing 10 breeding rooms which are conditioned with clean air at 23°C and 60% humidity and are furnished with an automatic device to supply water to the animals.

STAFF

Director

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

Members

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SHIMOTOHNO, Kunitada

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FUKUI, Teijiro, Chief of the Finance Section

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UEDA, Masao, Director of Institute of Population Problems

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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 1972

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)
Genetic studies on insect cells in tissue culture (KURODA and MINATO)
Developmental genetic studies on carcinogenesis in tissue culture (KURODA)
Genetics of somatic mammalian cells in culture (KURODA and MINATO)

Department of Cytogenetics

Studies on chromosomal polymorphism in *Rattus rattus* (YOSIDA and SAGAI)
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, T. and TSUCHIYA)
Experimental breeding and genetics of mice, rats and other wild rodents (YOSIDA, MORIWAKI, K., TSUCHIYA, SAKAKIBARA and TAKAHASHI)
Cytogetnetical study of ants (IMAI)

Department of Physiological Genetics

Population genetics of deleterious genes in natural populations of *Drosophila melanogaster* (OSHIMA, WATANABE and CHOO)
Analysis of inversion chromosome in natural populations of *Drosophila melanogaster* (OSHIMA and WATANABE)
Behavior genetics of phototaxis, diurnal rhythmicity of adult emergence and oviposition in *D. melanogaster* (OSHIMA and CHOO)

Studies on urbanization and noise environments for the habitat and development in *Drosophila* (OSHIMA and CHOO)
Ecological genetic studies on the differentiation of *Chrysanthemum* species (NAGAMI)

Department of Biochemical Genetics

Studies on transformation in higher organisms (NAWA and YAMADA)
Genetical and biochemical studies of pteridine metabolisms in insects (NAWA)
Analysis of gene action on cell differentiation in higher organisms (NAWA and YAMADA)
Biochemical studies on the differentiation of muscle proteins in animals (OGAWA)
Genetical and biochemical studies of human serum proteins (OGAWA and ODAKI)
Genetical and biochemical studies on Japanese middle size dog (OGAWA)
Genetics of isozymes in plants (ENDO and PAI)
Effects of exogenous DNA on plant seed formation (ENDO)
Genetics of fresh water hydra and other lower invertebrates (SUGIYAMA)

Department of Applied Genetics

Quantitative genetic studies in poultry (SAKAI, KAWAHARA and FUJISHIMA)
Genetic studies in wild populations of Japanese quails (KAWAHARA)
Theoretical studies on breeding techniques (SAKAI and IYAMA)
Behavioral genetic studies in mice (FUJISHIMA)
Studies on competition in plants (SAKAI and IYAMA)
Genetic studies in natural stands of forest tree species (SAKAI, IYAMA and KUDO)
Simulation studies on artificial selection (IYAMA)
Evolutionary studies in wild and cultivated rice species (OKA and MORISHIMA)
Analysis of genetic variations in growth pattern and phenotypic plasticity in rice (MORISHIMA and OKA)
Ecological genetic studies in some grass species (MORISHIMA)
Genetic analysis of isozymes in rice plants (PAI and ENDO)

Department of Induced Mutation

Molecular mechanisms of spontaneous, chemical- and radiation-induced mutations (KADA, SADAIE and NOGUTI)
Environmental mutagens and carcinogens (KADA, TUTIKAWA, SADAIE and NOGUTI)
Radiation genetics in mice (TUTIKAWA)
Biochemical factors involved in cellular repair of genetic damage (NOGUTI and KADA)
Mechanisms of recombination repair (SADAIE and KADA)
Mutation and differentiation studies of plant tissue culture (FUJII, AMANO, SHAMA RAO and KADA)
RBE and dose rate effects in higher plants (FUJII and AMANO)
Genetic fine structure analysis in maize (AMANO)

Department of Human Genetics

Genetic effects of family planning and population planning (MATSUNAGA)
Cytogenetics in man (NAKAGOME and IINUMA)
Molecular hybridization studies of human chromosomes (NAKAGOME)
Studies on human chromosome variants (IINUMA, NAKAGOME and MATSUNAGA)
Structural studies on human immunoglobulins (SHINODA)
Prenatal detection of genetic disorders (NAKAGOME, IINUMA and MATSUNAGA)
Biochemical genetics in man (SHINODA and MATSUNAGA)

Department of Microbial Genetics

Genetics of bacterial flagella (ENOMOTO and SUZUKI)
Genetics of motility in bacteria (ENOMOTO and WU)
Transduction mechanism of phages (ENOMOTO)
Flagellar synthesis and its regulation in a cell-free system (SUZUKI)

Deparment of Population Genetics

Theoretical studies of population genetics (KIMURA, MARUYAMA and OHTA)
Studies on molecular evolution from the standpoint of population genetics (KIMURA and OHTA)

Mathematical studies on the genetics of structured populations (MARUYAMA)
Linkage disequilibrium in finite populations (OHTA and KIMURA)
Experimental studies on protein polymorphism in *Drosophila* (YAMAZAKI)
Simulation studies on linkage disequilibrium in artificial populations (YAMAZAKI)

Department of Molecular Genetics

Studies on the chemical structure of genome of viruses containing double-stranded RNA (MIURA, SUGIURA, FURUICHI, SHIMOTOHNO, WATANABE, YAZAKI, SUZUKI and KIMURA)
RNA polymerase in a virion containing double-stranded RNA (MIURA, SHIMOTOHNO, FURUICHI and HORI)

RESEARCHES CARRIED OUT IN 1972

I. MOLECULAR GENETICS

The 5′-Terminal Structure of Cytoplasmic Polyhedrosis Virus (CPV) Genome RNA

Kin-ichiro MIURA, Masahiro SUGIURA and Kumiko WATANABE

In order to clear the 5′-terminal structure of CPV–RNA, the method for 5′-terminal labeling by ^{32}P using polynucleotidekinase was examined. Satisfactory labeling was achieved after removal of two or three nucleotides from the complementary 3′-terminal. It was confirmed that the 5′-terminal of every segment RNA were equally labeled without any degradation of a main chain of RNA molecule.

Alkaline digestion and ribonuclease T_2 digestion of the 5′-terminal-labeled CPV–RNA gave ^{32}pGp and ^{32}pXpYp in an equal amount each other. As the ribonuclease T_1 digestion gave the same nucleotides, ^{32}pXpYp should be ^{32}pXpGp. Since the X nucleoside resist to these kinds of digestion, the 2′-position of ribose would be blocked. The mononucleotide ^{32}pX was obtained from the 5′-terminal labeled CPV–RNA by a nuclease from *Penicillium*, which was supplied by Dr. A. Kuninaka, or by the successive treatment of the nuclease S_1 from *Aspergillus*, which was supplied by Dr. T. Ando, and venom phosphodiesterase. From the behavior in chromatography ^{32}pX was considered as a derivative of 5′-adenylic acid. Hereafter this will be written as pA*. This is the first time to show the presence of a strange nucleoside in a viral RNA.

Pancreatic ribonuclease A digestion of the 5′-^{32}P-CPV–RNA gave two trinucleotides. These are identified as ^{32}pGpGpCp and ^{32}pA*pGpUp by chromatography.

Ten segments of the 5′-terminal-^{32}P-CPV–RNA were separated by gel electrophoresis. Each segment was extracted with alkaline treatment and digested by a nuclease from *Penicillium*. Two labeled mononucleotides, pG and ^{32}pA*, were detected equivalently for every segment. Thus, it is

concluded that every one of ten segments of CPV genome RNA has the same terminal structure shown as follows:

Terminal Structure of Single-Stranded RNA Transcribed by the Cytoplasmic Polyhedrosis (CP) Virus

Kunitada SHIMOTOHNO, Yasuhiro FURUICHI and Kin-ichiro MIURA

CP virus contains segmented double-stranded RNA as its genome, and RNA polymerase, which synthesizes single-stranded RNAs carrying length of each genome segment.

In order to clear which strand of the double-stranded RNA is transcribed to the single-stranded RNA by the viral RNA polymerase, the 5′-terminal structure of the single-stranded RNA was analysed by using the specifically labeled nucleoside triphosphate as a precursor for the 5′-terminal nucleotide. Nucleoside triphosphates labeled by ^{32}P at β (or γ) position were prepared for this purpose. Any β- (or γ-) ^{32}P-labeled GTP or γ-^{32}P ATP was not incorporated into the single-stranded RNA product. On the other hand β-^{32}P-labeled ATP was incorporated. Upon ultracentrifuging in a glycerol density gradient, the incorporation of β-^{32}P-labeled ATP into the product RNA was confirmed.

The terminal nucleotide of the single-stranded RNA product was then analysed. Aspergillus nuclease S_1, which cleaves nucleic acid into 5′-nucleotides (Ando, T.: Biochim. Biophys. Acta 114 (1966) 158), gave only β-^{32}P-labeled ADP (^{32}ppA). Ribonuclease T_1 yielded ^{32}ppApGp. Therefore, the 5′-terminal sequence of the single-stranded RNA transcribed by CP virus is identified as ppApGp ------. The RNA carries diphosphate at the 5′-terminal, although the substrate was triphosphate.

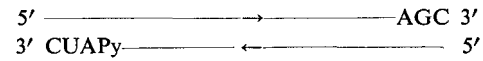
The 3′-terminal of this RNA was identified as C by the separate experiments (Annual Meeting of Japanese Biochem. Soc., Nov. 1972). Thus the single-stranded RNA produced by CP virus has the following structure:

It is concluded that the RNA chain carrying U at the 3′-terminal in the double-stranded RNA genome segment was used as a template on transcription, and it was completely read out. The fact that the counterpart of U is a nucleoside derivative of A is noteworthy, since it may participate for the recognition of the template strand.

The 3′-Terminal Structure of Avian Reovirus RNA

Yasuhiro FURUICHI and Kin-ichiro MIURA

Avian reovirus RNA was oxidized by periodate and reduced by ^{3}H-sodium borohydride to label the 3′-terminal nucleoside. Since only the ^{3}H-trialcohol of cytosine appeared on alkaline hydrolysis, it is concluded that both the 3′-termini of double-stranded RNA is cytosine. Considering the results of ribonuclease digestion of the ^{3}H-labeled RNA, the nucleotide sequences near 3′-termini of every segment RNA are written as follows. The arrangements of three nucleotides from the 3′-termini are common for all the genome segments as shown in RNA of CPV.



Nucleotide Ratio of the Genome Segments in a Cytoplasmic Polyhedrosis Virus from Silkworm

Yasuhiro FURUICHI, Umeko RAI, Kin-ichiro MIURA

CPV–RNA is fractionated into ten bands in a polyacrylamide gel electrophoresis. Each band obtained from the all ^{32}P-labeled CPV–RNA preparation was extracted with 0.3 N KOH. The nucleotide composition of every RNA segment was analysed by paper chromotography and paper electrophoresis. As shown in Table 1, there are only little differences in the composition among the segments. The complementarity between purines and pyrimidines is maintained in all the segments.

Using the ^{32}P-labeled CPV–RNA, the cluster of adenylic acid residues was examined by digestion with pancreatic ribonuclease AI and Takadiastase ribonuclease T_1 which cleave respectively at pyrimidine nucleotides and guanylic acids in RNA. Any longer adenylic acid cluster than hexanucleotide was not detected in the oligonucleotide chromatography in the pre-

sence of urea. Polyadenylic acid sequence found in a messenger RNA and a gene of eukaryote cells or some viruses would not exist in CPV–RNA.

Table 1. Nucleotide composition of RNA segment in CPV

Segment	Hydrolysis & Analysis	Nucleotide composition (%)				Purine / Pyrimidine	GC content (%)
		Ap	Up	Cp	Gp		
I	A	30.5	31.1	19.0	19.4	0.99	38.4
II	A	28.1	27.6	22.6	21.7	0.99	44.3
III	A	27.9	27.5	21.9	22.7	1.02	44.6
IV	A	27.7	29.2	21.3	21.7	0.98	43.3
V	A	28.4	27.9	21.4	22.3	1.02	43.7
VI	A	27.4	29.2	20.5	22.9	1.01	43.4
VII	A	30.0	30.8	19.5	19.7	0.98	39.2
VIII	A	28.6	27.4	22.5	21.5	1.00	44.0
IX	A	25.5	31.1	19.7	23.7	0.96	43.4
X	A	27.2	29.4	21.0	22.4	0.98	43.4
Total	B	29.1	28.4	21.2	21.3	1.02	42.5
CPV–RNA	C	27.8	29.8	21.1	20.3	0.93	41.4

A: 0.5 M KOH 37°C 24 hr, Paper electrophoresis

B: 0.3 M KOH 37°C 18 hr, Two dimensional paper chromatography (U. V. Absorbance)

C: RNase T_2 37°C 18 hr, Two dimensional paper chromatography (U. V. Absorbance)

II. MICROBIAL GENETICS

Absence of Messenger Ribonucleic Acid Specific for Flagellin in *fla*⁻ Mutants

Hideho SUZUKI and Tetsuo IINO[1]

A group of genes called *fla* is known to control flagellar formation in *Salmonella*. It includes as many as twelve (or more) cistrons recognized by complementation tests. Most of the complementation groups are located in clusters close to an H_1 gene. The participation of a *fla* gene in flagella formation is characterized by the lack of specificity for a flagellar phase in contrast to regulator genes adjacent to an *H*-gene. Hence a mutation of *fla* to *fla*⁻ results in the loss of ability to produce flagella both in phase 1 and in phase 2.

It interested us to see if *fla*⁻ mutation accompanies the cessation of transcription of *H*-genes. We utilized the cell-free system for protein synthesis to examine the presence or absence of mRNA specific for flagellin in a *fla*⁻ cell. From the *fla*⁻ strains belonging to one of the following complementation groups, *fla-A*, *-B*, *-C*, *-D*, *-E*, *-F*, *-L*, *-N*, *-P*, and *-Q*, the representative one was chosen, and the salt-precipitable RNA was prepared from the *fla*⁻ cells at a middle log phase of growth. The messenger activity of the RNA for flagellin synthesis was measured by introducing it into *in vitro* system for protein synthesis.

None of the RNAs was found to direct the *in vitro* synthesis of flagellin, although sufficient cell-free synthesis of general protein was observed with these RNAs and a parallel experiment with the RNA of the corresponding *fla*⁺ strain showed the *in vitro* synthesis of flagellin in a considerable amount. To examine the complementability among the RNAs in the cell-free system, the RNA was combined in the following combinations, (*A–F*), (*B–C*), (*B–E*) (*C–D*), (*E–F*), (*F–L*), (*N–P*) and introduced to the *in vitro* system for protein synthesis. With any of these combinations, the synthesis of flagellin was not observed. The mRNA for flagellin has been lost completely in the cells of the *fla*⁻ strains.

[1] Laboratory of Genetics, Faculty of Science, University of Tokyo, Hongo, Tokyo.

It has been assumed that the site of flagellin synthesis is associated with the basal structure of a flagellum and at least some of the *fla*-genes function to produce the components of the basal structure. Any defective component produced by *fla*$^-$ could give rise to a defective basal structure, which then would result in the loss of the function to uncover the transcriptional initiation site of an *H*-gene so that RNA-polymerase may commence synthesis of flagellin mRNA.

III. BIOCHEMICAL GENETICS

Improved Method for Separation and Identification of Rat Serum Transferrins: Thin Layer Acrylamide-gel Electrophoresis with Acrinol Pretreatment

Kazuo MORIWAKI

An improvement in electrophoretic method which will give us better separation and easier identification of rat serum transferrin bands was developed in the present study. A combination of acrinol precipitation and thin layer acrylamid-gel electrophoresis gives satisfactory results.

For the preparation of the gel, acrylamide was dissolved at the final concentration of 3.8% in Tris-citrate buffer (pH 7.4) containing 0.064 M Tris-aminomethane and 0.011 M citric acid. 0.3 M borate buffer (pH 8.6) was used in the electrode vessels. By this discontinuous buffer system the fast-moving transferrins of *Rattus rattus* could be separated well, but the slow-moving ones were occasionally overlapped by the slow α_1-macroglobulin band. Acrinol pretreatment of the serum, which precipitated many kinds of protein, except transferrin and gamma-globulin, was very useful

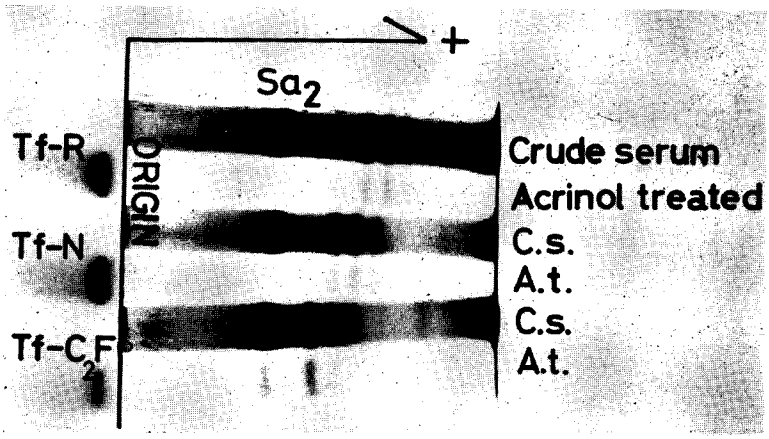


Fig. 1. Separation and identification of serum transferrins by thin layer acrylamide-gel electrophoresis accompanied with acrinol pretreatment.

to separate and identify transferrins in the serum. This is demonstrated in Fig. 1.

As a routine procedure, 100 μl serum was mixed with 1 μl of 0.6 mM $FeCl_3$ solution and diluted 4 time with 300 μl of 5 mM Tris buffer (pH 8.8). To that mixture was added 400 μl of 0.6% acrinol prepared just before use. After 30 minutes, coagulated proteins were removed by centrifugation at 3000 rpm for 10 minutes at 0°C. An equal volume of cold (−10°C) ethanol was added to the supernatant. After standing the mixture in an ice bath for 30 minutes, the protein fraction was spun down at 3000 rpm for 10 minutes at 0°C, after which it was dissolved in 100 μl of 5 mM Tris buffer and examined by the acrylamide-gel electrophoresis method mentioned above.

Using this method one can clearly separate and identify 12 transferrin types (R, R_1, R_2, N_1, N, C_1, C_2, C, D, E, F and G) in *Rattus rattus*. That these bands are transferrin has been confirmed by Fe^{59}-labelling and autoradiography.

The Second Scientific Expedition to Southeast, Southwest and Central Asia for the Study of Rodents. IV. Electrophoretic Survey of Polymorphic Transferrin Type in the Black Rat, Rattus rattus

Kazuo MORIWAKI, Kimiyuki TSUCHIYA, Hatao KATO,
Toshihide YOSIDA and Tamiko SADAIE

Serum transferrin polymorphism in black rats, *Rattus rattus*, collected from Southeast, Southwest and Central Asia was surveyed using the method of acrinol precipitation followed by thin layer acrylamide-gel electrophoresis that was newly developed in our laboratory (For technical details, see Moriwaki, This Annual Report,). Sera of 270 rats obtained from these areas were analyzed and 12 transferrin bands were identified as schematically demonstrated in Fig. 1. Among them, R and N are the same as those bands already found by us using starch-gel electrophoresis in Japanese populations (Moriwaki *et al.* Genetics 63: 193, 1969) and C, D, E and F are the same as those previously reported, also using starch-gel, in Pacific Island population (Malecha and Tamalin, Amer. Nat. 103: 664, 1969). An additional 5 transferrins, R_1, R_2, N_1, C_1 and C_2, have been found for the first time by the present expedition. The numbers in the various localities

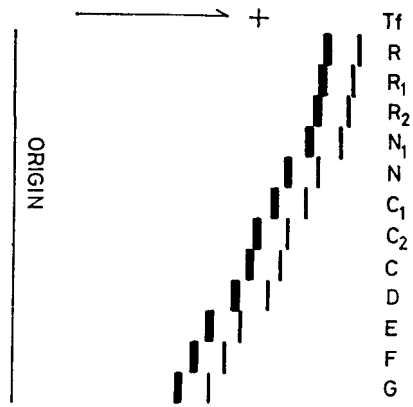


Fig. 1. Schematic demonstration of 12 transferrin bands in *Rattus rattus* separated by acrinol precipitation and acrylamide gel electrophoresis.

of each transferrin phenotype observed are summarized in Table 1 and the frequencies of the transferrin alleles in each locality were computed.

According to our previous data obtained from Southeast Asia and Oceania (Moriwaki *et al.* Annual Report of this Institute No. 20: 44, 1969 and No. 21: 36, 1970), the Tf-C, -D, -E and -F alleles were observed only in the Oceanian type *R. rattus* ($2n=38$). It is interesting to note that *R. rattus* in the Northern parts of India and Pakistan had the Tf-C, -D, -E and -F alleles, though their chromosomes were of the Asia type, $2n=42$. Furthermore, a Ceylon population having 40 chromosomes also had the Oceanian type transferrins. Another transferrin allele, Tf^{C_1}, has been observed in the following 3 populations having different karyotypes: Kalyani ($2n=42$), Ceylon ($2n=40$) and West Ghats Mountaines ($2n=38$).

These findings suggest that the possibility that such polymorphic changes in transferrin genes as shown above had occurred in the evolutionary history of *R. rattus* prior to the karyotypeic differentiation that produced the Asian, Ceylon and Oceanian types.

Table 1. Distribution of transferrin phenotypes and allelic frequencies in *Rattus rattus* collected from various localities in Southeast, Southwest and Central Asia

Locality of collection	Number of samples	Number of individuals with each transferrin phenotype	Allelic frequencies of transferrins												Remarks
			R	R_1	R_2	N_1	N	C_1	C_2	C	D	E	F	G	
Asian type ($2n=42$)															
Central Luzon, Philippine*	37	R_1(23), R_1N(9), N(5)		.743			.257								
Makassar, Malaysia*	59	R_2(59)			.100										*R. rattus argentiventer*
Kuala Lumpur, Malaysia	10	R(3), RN(2), RD(1), N(1), ND(1), NE(1)	.450				.300				.100	.050			*R. rattus diardi*
Kuala Lumpur, Malaysia	15	R(3), RN(8), RE(1) N(3)	.500				.467						.033		*R. rattus jalorensis*
Kanpur, India	16	C_2F(1), C(9), CD(1), CF(5)							.031	.750	.031		.188		
Kalyani, India	8	RC_1(2), N_1(1), C_1(5)	.125			.125		.750							
New Delhi, India	2	C(2)								1.00					
W. Ghats Mts. India	8	C_1(8)						1.00							Forest
Lahore, Pakistan	30	C(4), CE(12), E(14)								.333		.667			
Islamabad, Pakistan	14	C(3), CE(7), E(4)								.464		.536			
Ceylon type ($2n=40$)															
Kandy, Sri Lanka	16	C_1(4), C_1C(4), C_1D(1), C_1G(1), CG(1), D(2), DE(3)						.438		.156	.250	.094		.063	
Oceanian type ($2n=38$)															
Madras, India	10	C(1), CD(1), D(5), DE(2), DF(1)								.150	.700	.100	.050		

Arsikere,** India	12	ND(1), C(5), CD(1), CF(2), D(3)	.042	.542	.333		.083	*R. rattus rufescence*
Kurnool,**	1	CF(1)		.500			.500	
Nagpur,** India	4	C(3), CD(1)		.875	.125			
Hyderabad,** India	3	CE(3), DE(1), DF(1)		.167	.333	.333	.167	
Poona, India	20	C(10), CD(6), D(4)		.650	.350			
Karachi, Pakistan	3	CE(3)		.500		.500		
Chālus Iran	2	C(2)		.100				

* These amimals were obtained by the First Expedition to Southeast Asia and Oceania in 1968. The frozen sera were analyzed by the new method.

** Located in Deccan Plateau

Serum Transferrin Polymorphism in the Red-backed Vole, Clethrionomys rufocanus befdordiae

Kazuo MORIWAKI and Tsutomu KUWAHATA*

Transferrin polymorphism in the red-backed vole, *Clethrionomys rufocanus bedfordiae*, was surveyed by starch gel electrophoresis (For technical details, see Moriwaki *et al.*, Genetics **63**: 193, 1969). The serum of inbred Long Evans strain rats, *Rattus norvegicus*, was used as a standard sample in the electrophoresis. Serum specimens tested were taken from 148 individuals collected from 9 populations in Hokkaido: Nopporo-1 (32), Nopporo-2 (8), Nopporo-3 (18), Teshio (19), Ebetsu (12), Naganuma (8), Daikoku Island (23), Lake Touya (11), and the laboratory stock obtained from the Nopporo-1 population (17). The sizes of the samples are indicated in parentheses. Electrophoretic variants of transferrin were observed only in the laboratory stock. Out of 17 animals, 2 exhibited a fast moving band (Tf-A), 13 a slow moving band (Tf-B), and 2 both of them (Tf-AB), as demonstrated in Fig. 1. These bands were identified as transferrin by Fe^{59} labelling and autoradiography. The remaining populations all showed the Tf-B band. The Tf-A allele in the laboratory stock seems to have been present only in a very small number of the original Nopporo-1 population.

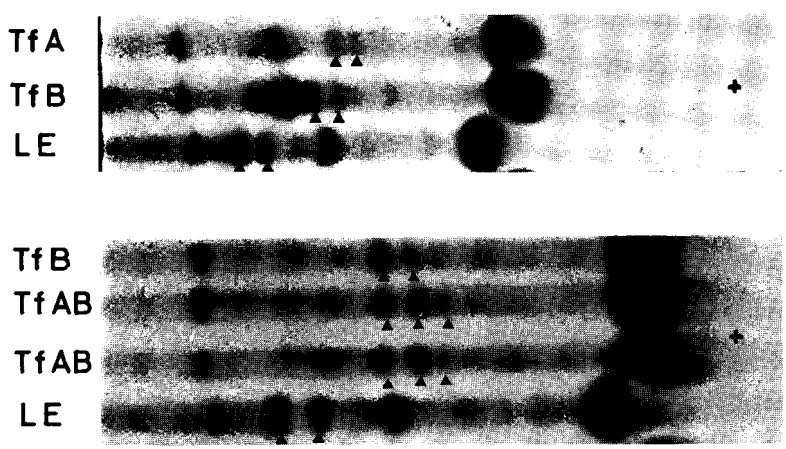


Fig. 1. Three transferring phenotypes, Tf-A, Tf-AB and Tf-B, in the red-backed vole demonstrated by starch gel electrophoresis. Solid triangles indicate the protein bands labelled with Fe^{59}. LE: Serum of inbred Long Evans strain ra as a standard.

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Zymograms of Serum Esterases in the Red-backed Vole (Clethrionomys rufocanus bedfordiae) Separated by Starch-gel Electrophoresis

Tsutomu KUWAHATA* and Kazuo MORIWAKI

Serum esterases of the red-backed vole (*Clethrionomys rufocanus bedfordiae*) were separated by starch-gel electrophoresis (For technical details, see

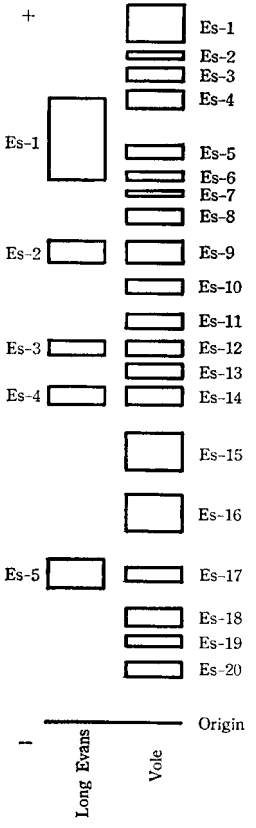

Fig. 1. Schematic representation of the esterase zymograms separated by Starch-gel electrophoresis using the sera of the vole and Long Evans rat.

* Forest Experimental Station, The Ministry of Agriculture, Hokkaido.

Moriwaki *et al.*, Genetics **63**: 193, 1969) and isozyme bands were demonstrated by a histochemical method employing α-naphthylacetate as a substrate.

Sera used in the present study were taken from 148 voles collected from 9 populations which differed in habitat. The esterase zymograms showed different electrophoretic patterns among the individuals; these are summarized schematically in Fig. 1. Throughout this experiment, the serum of inbred Long Evans rats (*Rattus norvegicus*) has been used as a standard.

When the esterease bands were numbered in turn from the fastest, 20 bands in the vole and 5 in the rat were identified on the anodal side (Fig. 1). No esterase bands were observed on the cathodal side. The 20 esterases in the vole can be divided into 2 groups; one consists of 11 bands, Es-2, Es-3, Es-5, Es-7, Es-8, Es-10, Es-11, Es-13, Es-17, Es-19 and Es-20, which are rather variable among the individuals and the populations, and the other consists of 9 bands, Es-1, Es-4, Es-6, Es-9, Es-12, Es-14, Es-15, Es-16 and Es-18, which are considered to be indispensable to the vole, because the esterese patterns in this animal seem always to be constructed on the basis of these 9 bands.

A comparison of the esterase patterns of the 9 populations from Hokkaido showed that the Dikoku Island and Teshio populations had exactly the same patterns, but that the rest of them had obviously different patterns.

When 10^{-5} M eserin, 10^{-3} M PCMB (p-chloromercuribenzoic acid) and 10^{-3} M EDTA (ethylendiamine tetraacetic acid) were used as inhibitors to facilitate the grouping of the esterases, eserin clearly inhibited specific bands, whereas PCMB and EDTA showed no effect. Estereases inhibited by eserin were Es-5 in the Long Evans rat and Es-9 in the vole; these are both considered to be choline esterase.

A Consideration on the Intensity Ratio of Allelic Dimer Isozymes in Heterozygotes

Toru ENDO

Allelic dimer isozyme (or dimer allozyme, Prakish *et al.* 1968, Genetics **61**, 841) generally produces in heterozygotes two parental and one hybrid bands, the latter being intermediate mobility between the parental ones. These three bands are denoted by FF, FS and SS. Their intensity ratio is

expected to be:

$$1FF : (1+a)\,FS : aSS,$$

when specific activity ratio of FF/SS is 1/a. The hybrid band then has the highest intensity of the three.

If FF and SS have the same specific activity but are produced in different amounts (1 : m), the intensity ratio is expected to be:

$$1FF : 2mFS : m^2SS.$$

This case is not very rare in the inheritance of dimeric peroxidase in rice organs (Endo 1971, Japan. J. Genet. **46**, 1). Efron (1970, Science **170**, 751) also reported the same case in alcohol dehydrogenase of corn.

It is expected that when the ratios in specific activity and amounts are 1 : a and 1 : m, respectively, the intensity ratio will be:

$$1FF : m(1+a)\,FS : m^2aSS.$$

The Genic System Controlling Acid Phosphatase Isozymes in *Oryza sativa* and *O. perennis*

Chiang PAI and Toru ENDO

Since 1967, we have continued analysis of the genic system controlling acid phosphatase isozymes in *sativa* cultivars and their wild progenitor, Asian *perennis*. The Indica and Japonica types of *sativa* cultivars similarly show a zymogram composed of three major and three minor bands, but differ in the mobility of the band group. Five different mobility types of the same band group have so far been found among *perennis* strains, two of which represent the Indica and Japonica zymograms. These isozymes are commonly observed in the leaf blade, leaf sheath, lemma and palea. Observations were then concentrated on the leaves. This year, three new mobility types were found in *perennis* populations of W169 (Thailand), W573 (Malaya) and W1183 (British Guiana). Accordingly, eight mobility types of the band group have been identified. Segregation experiments so far made show that a particular mobility of the band group is controlled by a single genetic unit.

However, a new zymogram characterized by the absence of the band group was found in two *sativa* cultivars (1707 and 1708; morphologically con-

sidered as Indicas and having small seeds). These strains showed in leaf sheath, a major band with a higher mobility than the above-mentioned band group, which was absent in the leaf blade. It is noted that the band is commonly seen in leaf blade and leaf sheath of other Indicas. Also the two strains showed a minor band located nearby the start line.

In addition, all *sativa* and *perennis* strains have two blur bands with much higher mobility than the above-mentioned ones. The highest one was found to be beta-naphthylphosphate-specific. The second one as well as all other bands were alpha-naphthylphosphate-specific.

Furthermore, so far as examined, one major and one minor cathodal bands were found to be specific to *perennis* subsp. *barthii* (African *perennis*). All Asian, American and Oceanian *perennis* strains lack those bands. Thus the genic system controlling acid phosphatase isozymes in rice seems to involve at least six gene loci.

Amino Acid Sequence of Human κ Chain

Tomotaka SHINODA

The primary structure of the variable region of the human type K Bence-Jones Protein Ni with an Inv (3) genetic marker was determined by sequence analyses of the tryptic and chymotryptic peptides: the primary structure of the constant region was deduced by partial sequence analyses of the peptides and by their homologies. Twenty-five major tryptic peptides were isolated by chromatography on Dowex 1– ×2 and AG 50– ×4 using volatile buffer systems of increasing pH and ionic strength. The complete sequences of 18 of these peptides were determined. These comprised the entire variable NH_2-terminal half and 7 peptides from the COOH-terminal half of the protein. Thirty-three chymotryptic peptides, covering all but two of the total of 218 residues, were isolated from the S-carboxymethylated protein. Complete or partial sequence analyses of the peptides covering all but two residues of the variable region of the molecule were made to obtain overlaps for the tryptic peptides recovered from this region. For the remaining peptides covering the COOH-terminal half only the partial sequence or the amino acid compositions and the end groups were determined.

From these results all the tryptic peptides could be arranged in order. Only one of the tryptic peptides in the NH_2-terminal half of the specimen

had the same sequence as that reported for the corresponding region of other human κ chains, whereas all the tryptic peptides covering the sequence from residues 108 (in Ag numbering system) to the COOH-terminus corresponded exactly to the sequences reported for the constant region of human κ chains of Inv (3) allotype. For details, see J. Biochem., **73**: 417–431 and ibid., **73**: 433–446.

Functional Heterogeneity of the Variable and Constant Regions of Human Bence-Jones Proteins

Tomotaka SHINODA and Yasuharu TSUZUKIDA*

One of the key problems in investigation of the molecular basis of the mechanism of the antigen-antibody reaction is the mutual arrangements of the prosthetic groups which are located on the surface of antibody molecules. As an approach to the problems we determined the relative rate of trinitrophenylation of amino groups in the molecule of human type K Bence-Jones proteins of known primary structure, on the basis that more reactive ones are at the surface of the molecules. Lysine-169 of the constant region of the Bence-Jones Protein Ni for which the complete sequence analysis has been done in our laboratory, appears to be on the surface of the molecule since it is the most reactive lysine residue toward the trinitrophenylation reaction under a mild condition. This surface position for lysine-169 seems to be the case in general for type K Bence-Jones protein since it is also demonstrated to be highly reactive in another type K Bence-Jones protein (Ka). Lysine-103 of the variable region is less reactive to the reagent thus appears to be less exposed. Lysine-41/42 and lysine-183 are much less reactive than any of the above mentioned lysine residues. The NH_2-terminal aspartic acid residue appears to be the least exposed since it is the least reactive among the rapidly reactive amino groups of type K Bence-Jones protein. The relative reactivity among these amino groups of the specimen Ni appears in the order lysine-169>lysine-103>lysine-41/42$\approx$ lysine-183>aspartic acid-1. The above order of the reactivity is also the case for the specimen Ka. Table 1 summarizes the results of the sequence analyses of peptides containing these reactive amino groups. Details are in the press in J. Biochem.

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Table 1. Summary of amino acid sequences of peptides containing the reactive amino groups

Specimen	Peptide		Sequence
Ni	TNPCnl		1 4 (TNP-)Asp-Ile-Gln-Met
			36 46
	TNPCn8	(a)	Tyr-Gln-Gln-Lys-Pro-(TNP-)Lys-Lys-Ala-Pro-Lys-Leu
		(b)	Tyr-Gln-Gln-Lys-Pro-Lys-(TNP-)Lys-Ala-Pro-Lys-Leu
			99 116
	TNPCn19		Gly-Val-Gly-Ser-(TNP-)Lys-Val-Glu-Ser-Lys-Arg-Thr-Val-Ala-Ala-Pro-Ser-Val-Phe
			155 173
	TNPCn26		Glp-Ser-Gly-Asn-Ser-Gln-Glu-Ser-Val-Thr-Glx-Glx-Asp-Ser-(TNP-)Lys-Asp-Ser-Thr-Tyr
			180 186
	TNPCn29		Thr-Leu-Ser-(TNP-)Lys-Ala-Asp-Tyr
Ka	TNPCk1		1 4 (TNP-)Asp-Ile-Gln-Met
			36 46
	TNPCk8	(a)	Tyr-Gln-Gln-(TNP-)Lys-Pro-Gly-Lys-Ala-Pro-Lys-Leu
		(b)	Tyr-Gln-Gln-Lys-Pro-Gly-(TNP-)Lys-Ala-Pro-Lys-Leu
			99 106
	TNPCk19		Gly-Gln-Gly-Thr-(TNP)Lys-Val-Asp-Leu
			155 173
	TNPCk26		Glp-Ser-Gly-Asn-Ser-Gln-Glu-Ser-Val-Thr-Glx-Glx-Asp-Ser-(TNP-)Lys-Asp-Ser-Thr-Tyr
			180 186
	TNPCk29		Thr-Leu-Ser-(TNP-)Lys-Ala-Asp-Tyr

IV. DEVELOPMENTAL GENETICS

Time-Lapse Cinemicrographic Analyses of Embryonic Cell Behaviors of *Drosophila melanogaster* in Culture

Yukiaki KURODA

Behavior, locomotion, cell division and changes in shape and size, of embryonic cells of *Drosophila melanogaster* were analyzed cinemicrographically under *in vitro* culture conditions. Tissue fragments from dechorionated eggs at the stage of sac-like midgut were explanted on cover slips inserted in T-5 flasks as was described earlier by the author. The culture flasks were placed in a plastic incubator box in which the termperature was controlled at 28°C, on the stage of a Nikon inverted phase microscope.

The tissue fragments were observed every day and cells coming out from the explants were recorded by 16 mm cinefilms. Time-lapse cinemicrography was carried out with a Nikon automatic equipment for interval and exposure and a Bolex camera.

Epithelial-like cells and spindle-shaped cells which were found around the explanted tissues one day after cultivation showed an active migration and cell divisions. On the contrary muscle cells which became noticeable after 7 days of cultivation were not exceedingly motile, although they showed a regular pulsation and pronounced movement of many granules in the cytoplasm. Most of the conspicuously motile cells were macrophage-like, they appeared a few days after cultivation and migrated very rapidly at a speed of one cell length an hour (about 30 μ per hour).

After 8 days of cultivation large cell spheres were prominent.Time-lapse cinemicrographic studies indicated that the sheath of the spheres which consisted of monolayer cells expanded and contracted continuously and many cells inside of the spheres were suspended freely in some fluid and active migration and cell divisions were observed through their transparent sheath. Some cell spheres were separated into several small compartments by boundary membranes which were similar in appearance to the sheath of spheres. This suggests that some spheres may fuse into larger spheres. The cell spheres increased in size in further cultivation and retained a healthy appearance for several weeks under *in vitro* conditions employed. As de-

scribed by earlier worker (Schneider, I. (1972) J. Embryol. Exp. Morphol. **27**: 353), the maintenance of these cell spheres may be the first step of the establishment of cell lines from *Drosophila* embryonic cells.

Inhibition by Cyclic AMP and Dibutyryl Cyclic AMP of Aggregation of Embryonic Quail Liver Cells in Rotation Culture

Yukiaki KURODA

Recently it has become known that cyclic 3′:5′-adenosine monophosphate (c-AMP) and dibutyryl cyclic 3′:5′-adenosine monophosphate (DB c-AMP) were effective in the inhibition of DNA synthesis and cell growth and also in inducing morphological changes and enzyme production in a variety of cultured cells of higher animals. In the present experiment the effects of c-AMP and DB c-AMP on aggregation of embryonic quail liver cells were examined in rotation culture.

Dissociated single cells were obtained from 7-day embryonic quail livers by the standard procedure previously described. Three ml of cell suspensions each containing 2×10^6 cells in a 25 ml Erlenmeyer flask were rotated on a gyratory shaker with a constant speed of 70 rpm at 38°C for 24 or 48 hours. C-AMP and DB c-AMP were added to the culture medium at the concentrations of 0, 0.01, 0.03, 0.1, 0.3 and 0.6 mg/ml. Twenty aggregates harvested from each flask were photographed, measured for their diameters, and calculated for mean diameters with standard deviations. The effects of c-AMP on aggregation of embryonic quail liver cells are shown in Table 1.

Table 1. Effects of c-AMP on aggregation of 7-day embryonic quail liver cells

Concentration (mg/ml)	Diameter of aggregates (Mean±S.D. μ)	
	24 hours	48 hours
0	202.5±39.7	269.0±47.2
0.01	166.0±26.2	259.0±41.2
0.03	143.5±26.3	238.5±33.9
0.1	122.5±21.9	243.5±36.2
0.3	117.5±24.7	157.5±27.7
0.6	101.5±23.3	131.0±17.3

C-AMP had concentration-dependent inhibitory effects on embryonic liver cells. At the concentration of 0.6 mg/ml aggregates formed after 24 and 48 hours of rotation culture had half the diameter of those obtained in respective control cultures.

Aggregates formed after 24 hours in media containing c-AMP at various concentrations were transferred to normal medium in which they were rotated for additional 24 hours. Aggregates which were depressed in size in the previous cultivation with c-AMP recovered partially their cohesiveness in further cultivation in normal medium, although they did not yet reach the size of control aggregates.

When DB c-AMP was added to the medium, the sizes of aggregates obtained after 24 or 48 hours of rotation culture are shown in Table 2.

Table 2. Effects of DB c-AMP on aggregation of 7-day embryonic quail liver cells

Concentration (mg/ml)	Diameter of aggregates (Mean±S.D. μ)	
	24 hours	48 hours
0	231.0±45.8	333.5±67.1
0.01	174.5±37.9	198.0±34.6
0.03	114.5±21.8	110.0±17.6
0.1	89.5±17.2	97.0±15.5
0.3	92.5±25.7	94.0±13.9
0.6	85.0± 9.2	87.5±14.8

DB c-AMP had stronger effects than c-AMP at the corresponding same concentrations. At the concentrations of more than 0.03 mg/ml no more increase was found in size of aggregates for the additional 24 hours of cultivation. When aggregates obtained after 24 hours in the presence of more than 0.03 mg/ml of DB c-AMP were transferred to normal medium, again no recovered increase of aggregate size was found.

Effects of Dextran Sulfates on Aggregation of Embryonic Quail Liver Cells in Rotation Culture

Yukiaki KURODA

Dextran sulfate is one of polyanionic compounds which bind with cell

membrane and induce the increase in negativity of cell membrane, resulting in a disturbance of cohesion among cells. This agent inhibited the metastasis of cancer cells and also decreased the saturation density of tumor cells in monolayer culture by modifying the surface properties of cell membrane. In the present experiment the effects of dextran sulfates of various molecular weights on aggregation of embryonic quail liver cells were examined in rotation culture.

Three ml of cell suspensions containing 2×10^6 cells dissociated from 7-day embryonic quail livers were rotated by the standard procedure at 38°C for 24 or 48 hours. Dextran sulfates of molecular weights of 7,500, 45,000 and 60,000 were added to culture medium at the concentrations of 0, 1, 3, 10 and 30 μg/ml. Twenty aggregates harvested from each flask were photographed, measured for their diameters and calculated for mean diameters with standard deviations. The effects of dextran sulfates of various molecular weights on 48-hour aggregation of embryonic quail liver cells are shown in Table 1.

Table 1. Effects of dextran sulfates on 48-hour aggregation of 7-day embryonic quail liver cells

Concentration (μg/ml)	Diameter of aggregates (Mean±S.D. μ)		
	Dextran sulfate MW=7,500	Dextran sulfate MW=45,000	Dextran sulfate MW=60,000
0	308.5±30.2	270.0±26.6	354.5±39.4
1	220.5±35.7	264.0±30.4	262.5±34.9
3	145.0±36.9	276.0±23.7	175.5±37.3
10	117.5±33.1	277.5±24.5	99.5±14.3
30	95.5±14.0	275.5±29.7	83.0±11.9

Dextran sulfates of molecular weights of 60,000 and 7,500 had pronounced inhibitory effects on aggregation of embryonic liver cells, whereas that of molecular weight of 45,000 had no effect. These results indicate that dextran sulfates added to culture medium may modify the surface properties of cell membrane. The mechanisms by which dextran sulfates induce the depression of cohesiveness of embryonic liver cells are now under investigation.

Differential Inhibition by Fucoses and Concanavalin A of Aggregation of Normal Liver and Hepatoma Cells in Rotation Culture

Yukiaki KURODA

In a series of experiments in which effects of carbohydrate-containing substances on the mutual cohesiveness of normal and malignant cells were examined, it had been previously found that hexosamines and their acetyl derivatives had differential inhibitory effects on normal liver and hepatoma cells. In the present experiments, effects of D- and L-fucoses and concanavalin A on aggregation of embryonic normal liver cells and DAB-induced rat hepatoma cells were investigated in rotation culture.

Single cell suspensions were prepared by trypsinization from 7-day embryonic quail livers and monolayer cultures of DAB-induced rat hepatoma cell lines, dRLN-61, dRLa-74 and dRLh-84, and were rotated for 24 or 48 hours under standard conditions. Hapatoma cell lines, dRLN-61, dRLa-74 and dRLh-84, had a low, moderate and high tumor-producing activity, respectively. D-Fucose and L-fucose were added to culture medium at concentrations of 0, 1, 3, 10 and 30 mM. Concanavalin A (NBC) was tested at concentrations of 0, 0.001, 0.003, 0.01, 0.03 and 0.1%. The effects of fucoses on 24-hour aggregation of normal liver and hepatoma cells are shown in Table 1.

Table 1. Effects of fucoses on 24-hour aggregation of normal and hepatoma cells

Fucose	Concentration (mM)	Inhibition of aggregation of		
		Normal liver cells	Hepatoma dRLa-74 cells	Hepatoma dRLh-84 cells
D-Fucose	0	−	−	−
	1	−	−	−
	3	−	−	+
	10	−	−	+
	30	−	−	+
L-Fucose	0	−	−	−
	1	−	−	+
	3	−	+	+
	10	−	+	+
	30	−	+	++

−: 0~30% inhibition, +: 30~70% inhibition, ++: 70~100% inhibition.

D-Fucose had differential inhibitory effects on normal liver cells and hepatoma cells. At concentrations of more than 3 mM D-fucose affected the aggregation only of hepatoma dRLa-84, whereas no effects were found on the aggregation of normal liver cells and hepatoma dRLa-74 cells.

L-Fucose had stronger inhibitory effects than D-fucose. The effects of L-fucose on aggregation of normal liver cells and hepatoma cells were correlated to tumor-producing activities of cells: no effects on normal liver cells, moderate effects on dRLa-74 cells and strong effects on dRLh-84 cells.

The effects of concanavalin A on aggregation of hepatoma cells having different tumor-producing activities are shown in Table 2.

Table 2. Effects of cancanavalin A on 24-hour aggregation of rat hepatoma cells

Concentration (%)	Inhibition of aggregation of		
	Hepatoma dRLN-61 cells	Hepatoma dRLa-74 cells	Hepatoma dRLh-84 cells
0	−	−	−
0.001	−	−	+
0.003	−	+	+
0.01	−	+	++
0.03	*	*	*
0.1	*	*	*

For symbols see the footnotes in Table 1. * indicates flocculation.

Concanavalin A had stronger inhibitory effects on the aggregation of hepatoma cells, which had higher tumor-producing activities at concentrations up to 0.01%. At the concentrations of more than 0.03% of concanavalin A hepatoma cells did not aggregate, but they flocculated.

Colony-Forming Activity of Embryonic Human Diploid Cells in Culture

Yukiaki KURODA

To elucidate the mechanisms of somatic mutations, phenotypic expression in cultured mammalian cells, and also to establish screening procedures for detecting potential mutagens present in human environment, the culture

conditions were examined under which the best colony formation of embryonic human diploid cells was obtained.

Lung cells derived from a 5-month human embryo were cultured in monolayer in Eagle's BM. Karyotype analysis indicated that more than 86% of cells had normal diploid female chromosomes. The effects of various inoculation numbers of cells and culture media on colony-forming activity of cells were tested. Cells dissociated from monolayer cultures at sixth to tenth transfer generations were counted and 10^3, 3×10^3, 10^4, 3×10^4 or 10^5 cells were inoculated in 60 mm plastic petri dishes in 4 m of Eagle's BM, Eagle's MEM, Puck's N16 or Ham's F12 medium each supplemented with 10% calf serum or in Puck's N16CF medium.

After 10 to 16 days of cultivation at 37°C in the atmosphere of 5% CO_2 and 95% air, cells in petri dishes were fixed in methyl alcohol and stained in May-Grünwald-Giemsa solution. The numbers of colonies consisting of more than 50 cells were counted under a binocular microscope and the colony-forming activity (plating efficiency) was calculated as follows:

$$\text{Colony-forming activity} = \frac{\text{Average number of colonies}}{\text{Number of cells inoculated}} \times 100\%$$

The results obtained are shown in Table 1.

Table 1. Comparison of colony-forming activity of embryonic human diploid cells in various media

Number of cells inoculated	Colony-forming activity (%)				
	Eagel's BM +10% CS	Eagle's MEM +10% CS	Puck's N16CF	Puck's N16 +10% CF	Ham's F12 +10% CS
10^3	0.83	1.25	0.43	0.37	10.70
3×10^3	3.70	2.66	1.09	0.77	11.24
10^4	2.68	1.77	0.55	1.33	6.24
3×10^4	1.20	1.01	0.61	0.88	*
10^5	>0.52	*	>0.48	>0.43	*

* The number of colonies was not counted because a confluent cell sheet was formed.

Ham's F12 medium supplemented with 10% calf serum was the best of all media tested, followed by Eagle's BM, Eagle's MEM, Puck's N16CF and Puck's N16. In Ham's F12 medium with 10% calf serum the best colony-forming activity of cells was obtained when 3×10^3 cells were inoculated on one petri dish.

Effects of 8-Azaguanine, EMS and MNNG on Colony-Forming Activity of Embryonic Human Diploid Cells in Culture

Yukiaki KURODA

In the previous investigation it was found that embryonic human diploid cells displayed the best colony-forming activity when cultured in Ham's F12 medium supplemented with 10% calf serum. In the present experiments the effects of some drugs on colony-forming activity of cells were examined. Drugs employed were an antimetabolite, 8-azaguanine (8AG), a mutagenic substance, ethyl methan sulfonate (EMS) and a carcinogenic agent, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG).

10^4 cells were inoculated in 60 mm petri dishes in media containing various concentrations of 8AG, EMS or MNNG and were incubated for 14 days at 37°C in the atmosphere of 5% CO_2 and 95% air. The cell colonies in harvested cultures were fixed, stained and counted and the colony-forming activity (plating efficiency) was calculated. The results obtained are shown in Table 1.

Table 1. Effects of 8AG, EMS and MNNG on colony-forming activity of embryonic human diploid cells

Substance	Concentration	Average number of colonies	Colony-forming activity	Surviving fraction
8AG	0 μg/ml	559.5	5.60	1.00
	1	234.7	2.35	0.42
	3	16.7	0.17	0.03
	10	0	0	0
	30	0	0	0
EMS	0 M	397.0	3.97	1.00
	10^{-3}	307.0	3.07	0.77
	3×10^{-3}	44.0	0.44	0.11
	10^{-2}	1.0	0.01	0.002
	3×10^{-2}	0	0	0
MNNG	0 M	407.0	4.07	1.00
	10^{-6}	311.5	3.12	0.77
	3×10^{-6}	309.0	3.09	0.76
	10^{-5}	212.0	2.12	0.52
	3×10^{-5}	33.3	0.33	0.08
	10^{-4}	0.3	0.003	0.0007

From concentration-survival curve for each drug, D_0 value was calculated to be 1.3 μg/ml for 8AG, 2.2×10^{-3} M for EMS and 1.7×10^{-5} M for MNNG. If cells treated with EMS or MNNG were cultured in selection medium containing 8AG, the effects of EMS or MNNG on mutation frequency from 8AG sensitivity to resistancy may be determined by calculating the colony-forming activity of cells.

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

Kiyoshi MINATO

To elucidate the effects of high molecular fractions of calf serum on the growth of mammalian cells, further analyses of some fractions which could sustain cell growth were carried out with HeLa S3 cells in monolayer culture.

In Eagle's MEM supplemented with dialyzed calf serum, cells grew almost at the same rate as that in medium supplemented with whole serum. In the medium supplemented with calf serum which was salted-out with saturated ammonium sulfate, the growth rate of cells decreased to 70~80% of control culture with whole serum. The serum fraction which was salted-out with 50~100% saturated ammonium sulfate was effective in providing 50~60% of the growth rate of cells in control culture.

With a fraction which was precipitated with 50% saturated ammonium sulfate, the initiation of cell growth was delayed for 3 or 4 days and about one half of cells in culture died. After a lag period, the growth rate of cells was 50~60% of control culture. With only one fraction precipitated either with 0~50% or 50~100% saturated ammonium sulfate, no growth rate of cells shown in control culture was obtained.

Some attempts were made to substitute the osmotic pressure of serum protein by that of other substances. A non-metabolizable disaccharide, D-cellobiose, had no effects as in culture with no supplementation. A synthetic macromolecule, polyvinyl pyrrolidone (PVP) k-90, was effective a little at the concentration of 0.1% in sustaining survival and growth of cells. At lower or higher concentrations than 0.1% PVP was less effective. Linoleic acid and putrescine which were used by Ham (1963, 1964) as substitutes

of albumin and α-globulin of serum were tested with single or combined supplementation. These substances were not effective in promoting cell growth.

Genetic Analysis of Developmental Mechanisms in Hydra

Tsutomu SUGIYAMA

A new project has been started to study the genetics of fresh water hydra. The aim of the project is to identify and to analyze the function of genes which are involved in various developmental processes such as budding, regeneration, transplantation, cell migration and cell reassociation.

Representative species of fresh water hydra native to Japan have been collected (*Hydra parva, H. magnipapillata, H. japonica* and *Pelmatohydra robusta*) and laboratory conditions for their culture by asexual and sexual reproduction are being examined.

One temperature sensitive strain of *H. magnipapillata* was obtained. This strain shows normal morphology at 18°C but shows twisted filamentous stem at 23°C. The morphology of the wild type is almost identical at both temperatures.

Other morphological and conditionally lethal mutants are being sought.

V. CYTOGENETICS

The Second Scientific Expedition for the Study of Rodents to Southeast, Southwest and Central Asia, I. Members, Aims and Schedule of the Expedition

Tosihide H. YOSIDA

With support of a grant-in-aid for scientific research from the Ministry of Education we organized the second expedition for the study of rodents to Southeast, Southwest and Central Asia from September 27 to November 22, 1972. The members of the expedition were as follows:

Tosihide H. Yosida (Leader)
Kazuo Moriwaki
Hatao Kato
Kimiyuki Tsuchiya
} Department of Cytogenetics, National Institute of Genetics

The expedition was undertaken with the following aims.

1) Localization of a border line dividing the habitats of Asian- and Oceanian-type black rats: The black rat, *Rattus rattus*, is a peculiar animal in that, as we found previously, it shows a local variation within the same species in the number and the shape of chromosomes despite the fact that there are no differences in the external features. This phenomenon is referred to as a "chromosome polymorphism or geographical variation". The black rats found in East and Southeast Asia possess 42 chromosomes, while those in Oceania (Australia, New Zealand and New Guinea) have only 38 chromosomes, whose morphology also differs markedly from those of the former. It has been confirmed by several researchers that the Oceanian-type black rats are also distributed widely throughout North and South America, Europe, and Africa. There is general agreement that the black rat first appeared in the Indo-Chinese area, from where it migrated to Europe through Southwest (India and Pakistan) and Central Asia and from Europe they migrated to Oceania, American and Africa, probably accompanying man. It is reasonable to assume that the transition in chromosome type from the Asian ($2n=42$) to the Oceanian ($2n=38$) might have occurred during the migration period between Asia and Europe, and that the border-line dividing the distribution of these two types would be

located somewhere in Southwest Asia. One of the aims of the expedition was to pinpoint such a border region.

2) A search for a transient type of black rat with 40 chromosomes: In the previous paper we predicted that the karyotype of the black rat evolved through the following sequence: $2n=42 \rightarrow 2n=40 \rightarrow 2n=38$, and, though a black rat with $2n=40$ has not yet been found in a natural population, it probably occurs somewhere in the world as a transient type between the Asian and Oceanian types. We suspected that this type might be found somewhere in Southwest Asia.

3) Elucidation of the relationship between the chromosomal changes and speciation in the genus *Rattus*: We have examined the chromosomes of 13 species of rats collected in Southeast Asia and Oceania during the previous expedition, and obtained cytogenetical data implying that all the rats belonging to the genus *Rattus* have been derived from the black rat through chromosomal rearrangements, and that the polymorphism seen in the black rat reflects a transitory phase in speciation within the genus *Rattus*. Since there have been inadequate karyological studies of rodent species in these areas, the present expedition aimed to collect and examine as many rats belonging to the genus *Rattus* as possible.

4) Comparative survey of the electrophoretic patterns of serum transferrin in the genus *Rattus*: Diversiforms of electrophoretic patterns of serum transferrin have been previously demonstrated in the genus *Rattus* collected from Japan, Southeast Asia and Oceania by Moriwaki *et al.* Recently they have succeeded in comparing these transferrins at the molecular level, that is, in their amino acid compositions. The fourth aim of the expedition was to pursue a study within the genus *Rattus* of the possible parallelism between karyotypic variation mentioned above and molecular variation in the serum transferrins.

5) Development of a new experimental animal: Recently we have succeeded in breeding several species of wild rodents collected during the first expedition in the laboratory. Some of them are now proliferating. We planned to bring back to our laboratory various rodents collected during the second expedition in order to establish new experimental animals.

The schedule of the expedition was as follows:

Tokyo (left Sept. 27, 1972)→Hong Kong→Kuala Lumpur (Malaysia)→ Colombo, Kandy (Sri Lanka)→Madras (India)→Calcutta, Nadia (India)→ Bangalore, Mysore (India)→Poona (India)→New Delhi, Kanpur (India)

Karachi (Pakistan)→Islamabad (Pakistan)→Lahore (Pakistan)→Teheran (Iran)→Ankara (Turkey)→Istanbul (Turkey)→Europe→Tokyo (arrival Nov. 22, 1972)

Acknowledgements: The members of the expedition wish to express their gratitude to the following researchers for their kind help in the collection of animals.

Mr. G. W. Chau: Urban Services Dept. Hong Kong.

Mr. B. L. Lim: Institute of Medical Redical Research, Kuala Lumpur, Malaysia.

Dr. H. F. Fernando: Central Agricultural Research Inst., Peradeniya, Sri Lanka.

Dr. P. J. Sanzeeva Raj: Department of Zoology, Christian College, Madras, India.

Dr. G. K. Manna: Department of Zoology, University of Kalyani, Kalyani, India.

Mr. T. Tachibana: Kasturba Gandhi National Memorial Trust, Asrikere, Mysore, India.

Dr. G. B. Deodika, Dr. M. D. Gadgil: Maharashtra Association for the Cultivation of Science, Poona, India.

Dr. S. S. Rajan: Indian Agricultural Research Institute, New Delhi, India.

Dr. A. S. Srivastava: U. P. Institute of Agricultural Sciences, Kanpur, India.

Dr. Z. Khan: National Health Laboratory, Islamabad, Pakistan.

Dr. I. Khan: Pakistan Medical Research Center, Lahore, Pakistan.

Dr. C. M. Tahir: Rice Research Institute, Lahore, Pakistan.

Dr. D. W. Walton: University of Karachi, Karachi, Pakistan.

Dr. P. Mostashfi: Department of Genetics, University of Teheran, Teheran, Iran.

The Second Scientific Expedition for the Study of Rodents to Southeast, Southwest and Central Asia, II. Animal Species Collected from Southeast, Southwest and Central Asia, and Their Chromosome Numbers

Tosihide H. YOSIDA, Kazuo MORIWAKI, Hatao KATO,
Kimiyuki TSUCHIYA and Tomoko SAGAI

Species of rodents and insectivores collected from Southeast, Southwest and Central Asia by the second expedition are listed in the following table (Table 1).

Table 1. Species and chromosome numbers of rodents and other small mammals collected in Southeast, Southwest and Central Asia by the second expedition

Localities collected	Species	No. of individuals collected	Chromosome numbers
Hong Kong	*Rattus huang*	2	46
	R. rattus flavipectus	10	42
Kuala Lumpur	*R. rattus diardii*	5	42
(Malaysia)	*R. muelleri*	6	42
	R. jalorensis	7	42
	R. annandalei	7	42
	R. argentiventer	4	42
	R. sabanus	8	42
	R. whiteheadi	1	36
	R. rajah	1	36
	R. exulans	1	42
Kandy	*R. rattus*	19	40
(Sri Lanka)	*Bandicota bengalensis*	2	42
	Mus legata	1	40
Kalyani	*R. rattus*	2	42
(India)	*Mus musculus*	1	40
	Funambulus palmarum	2	?
	Suncus murinus	6	40
Madras	*R. rattus*	23	38
(India)	*Mus musculus*	3	40
Mysore	*R. cutchicus*	2	36
(India)	*R. rattus*	20	38, 42

(Continued)

Table 1. (continued)

Poona (India)	*R. rattus*	20	38
Kanpur (India)	*R. rattus*	17	42
	Bandicota bengalensis	2	42
	Mus booduga	1	40
	Tatera indica	2	66
Delhi (India)	*R. rattus*	2	42
	Suncus murinus	3	40
	Bandicota bengalensis	1	42
Rawalpindi (Pakistan)	*R. rattus*	14	42
	Bandicota bengalensis	3	42
	Suncus murinus	1	40
Lahore (Pakistan)	*R. rattus*	29	42
	Bandicota bengalensis	5	42
	Mus booduga	1	40
	Tatera indica	1	66
	Millardia meltada	1	50
Karachi (Pakistan)	*R. rattus*	4	39
	Funambulus pennanti	1	?
	Parachinus micropus	1	?
Teheran (Iran)	*Meriones libycus*	3	?
Near Caspian See (Iran)	*R. rattus*	7	38

The Second Scientific Expedition for the Study of Rodents to Southeast, Southwest and Central Asia, III. Karyotype Analysis of the Black Rat, *Rattus rattus*, Collected in Southwest and Central Asia

Tosihide H. YOSIDA, Hatao KATO, Kimiyuki TSUCHIYA, Kazuo MORIWAKI and Tomoko SAGAI

Black rats collected in Kalyani, Delhi and Kanpur, located in Northern India, had 42 chromosomes, that is, the Asian karyotype, but those collected in Madras and Poona had the Oceanian karyotype ($2n=38$). Among 12 rats collected in Poona, however, only one rat had Asian type chromosomes ($2n=42$). Fifteen black rats were collected in Mysore, India. Among

them 8 rats had the Oceanian karyotype ($2n=38$), but the other 7 rats were Asian ($2n=42$). The black rats collected in Rawalpindi and Lahore, which are located in northern Pakistan, had Asian type chromosomes. Among 13 rats collected in Lahore, only one had 41 chromosomes as the result of loss of one acrocentric chromosome. The karyotypes of 3 rats collected in Karachi, Pakistan, were remarkable in having 39 chromosomes, suggesting the hybrid between the Asian and Oceanian types. All of three had same karyotype, namely one pair of the large metacentric chromosome (M_1), but only one of the M_2-metacentrics was included in their karyotypes. All 7 black rats collected near the Caspian Sea in Iran were of the Oceanian type ($2n=38$). All 10 black rats collected in Kandy, Sri Lanka (Ceylon) had $2n=40$ chromosomes. This karyotype is characterized by having only one pair of M_2-metacentric chromosomes and it is suggested that it developed by Robertsonian fusion of pairs No. 11 and 12 in the karyotype of the Asian rat (Fig. 1). This seemed to be a transient type between Asian ($2n=42$) and Oceanian types. Through these analyses of the karyotypes of black rats from India, Pakistan, Iran and Ceylon, we could confirm that

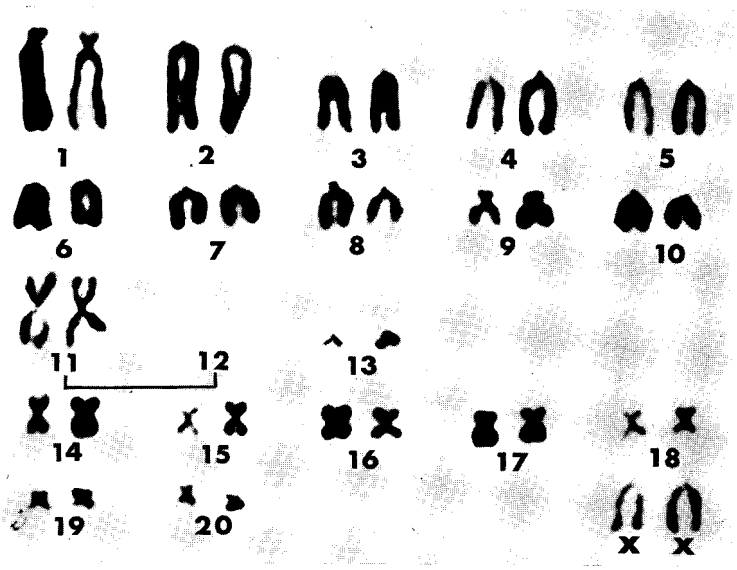

Fig. 1. Karyotype of the black rat, *Rattus rattus*, collected in Kandy, Sri Lanka (Ceylon).

the Asian type developed first in Southeast Asia, and from there migrated to Europe through Southwest and Central Asia. The first Robertsonian fusion of pairs No. 11 and 12 in the Asian type occurred somewhere in Southwest Asia, and thus gave rise to the Ceylon type ($2n=40$). Thereafter the Oceanian type ($2n=38$) could have arisen from the Ceylon type by a second fusion of pairs No. 4 and 7. The latter type remained isolated on Ceylon island, while the former became distributed from there to Central Asia and Europe.

A study of the Ceylon type of the black rat has been published in Jap. Jour. Genetics **47**: 451–454 (1972).

Giemsa Banding Patterns of Chromosomes in Seven Rattus Species

Tosihide H. YOSIDA and Tomoko SAGAI

The Giemsa banding patterns of the metaphase chromosomes were compared in seven *Rattus* species. Four species (*R. rattus tanezumi*, *R. norvegicus*, *R. exulans* and *R. muelleri*) all had $2n=42$ and their karyotypes and banding patterns were similar, slight differences were observed. Another subspecies (*R. rattus rattus*) and two other species (*R. fuscipes* and *R. conatus*) had lower chromosome numbers than those above. They had large biarmed chromosomes developed probably by Robertsonian fusion of the acrocentric chromosomes found in the first four species. The origin of the arms of the biarmed chromosomes was recognized by their characteristic banding patterns. The remaining one species, *R. sabanus*, had $2n=42$ chromosomes, but its karyotype differed markedly from the other species described above in having two small metacentrics, although those are seven in the others species. The banding patterns of the chromosomes in this species, however, were also very similar to those of the others, and therefore the seven small metacentrics, which characterize the karyotypes of many *Rattus* species, seem to have originated by pericentric inversion within small acrocentrics, such as those found in the karyotype of *R. sabanus*. This study was published in Chromosoma (Berl.), 41: 93–101.

Stable Telocentric Chromosomes Produced by Centric Fission in Chinese Hamster Cells in vitro

Hatao KATO, Tomoko SAGAI and Tosihide H. YOSIDA

Metaphase examination of pseudodiploid Chinese hamster cells revealed that spontaneous breaks or fissions occurred rather frequently (2.9%) at the centromeric regions of subtelo- or metacentric chromosomes, resulting in the production of telocentric chromosomes. The centromeric fissions appeared to occur in every member of the chromosome complement. An attempt was made to isolate cells possessing such telocentrics from the cell population and two clonal lines were established which had one and two telocentric chromosomes, respectively. The banding and labeling patterns of these chromosomes both indicated unequivocally their X chromosome origin. They were transmitted successively to the daughter cells during a 3-month culture period, showing no tendency to fuse to produce a metacentric chromosome. (Chromosoma **40**: 183–192).

Non-Random Localization of the Centromere in Mammalian Chromosomes

Hirotami T. IMAI

Since Levan *et al.* (Hereditas **52**: 201, 1964) proposed a standard nomenclature for chromosomes in terms of arm ratio, it has been adopted by many mammalian cytogeneticists in the original form and by several workers after further modification. Nobody, however, has paid attention to the statistical distribution of the centromere along the chromosome as a means of obtaining basic information about the nature of chromosome rearrangements. If the position of the centromere is random, morphologic classification of chromosomes can only be done by using the arbitrary criteria proposed by Levan *et al.* But if the pattern of distribution is non-random, this biological character can be used for the classification of chromosomes. In order to solve this problem, the present paper examines statistically the frequency distribution of centromere localization in 16,817 chromosomes observed in 723 mammals.

The position of the centromere of metaphase chromosomes is expressed either by the arm ratio (r_w) or by the size of the short arm (S_w) using weight,

according to the photo cut-out technique of Wurster *et al.* (Cytogenetics **10**: 153, 1971). The mean weight of the short arms of a homologus pair of chromosomes expressed as a percentage of the total weight of the X-containing haploid set is designated as S_w, in the same way that of long arms as L_w, and that of the whole chromosome as C_w, where $C_w = S_w + L_w$ and $r_w = L_w/S_w = (C_w - S_w)/S_w$. The relative weight of the *Y* chromosome is calculated from its percentage of the total weight.

The frequency distribution of the value of r_w is shown in Fig. 1. The

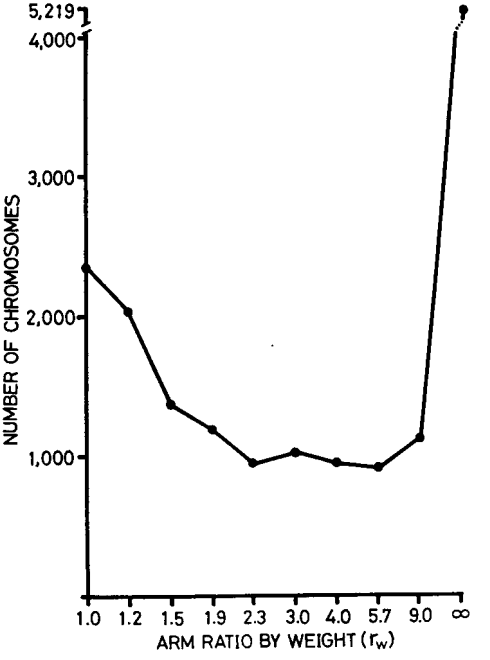

Fig. 1. Frequency distribution of the arm ratio by weight (r_w).

figure exibits a U-shaped curve, suggesting a non-random localization of the centromere. In order to know whether the non-random distribution of r_w can be observed in every size of chromosome, the relationship between frequency distribution of r_w and C_w was investigated. As shown in Fig. 2, a V-shaped distribution was observed, which demonstrates the non-random localization of the centromere more clearly than does the U-shaped pattern seen in Fig. 1.

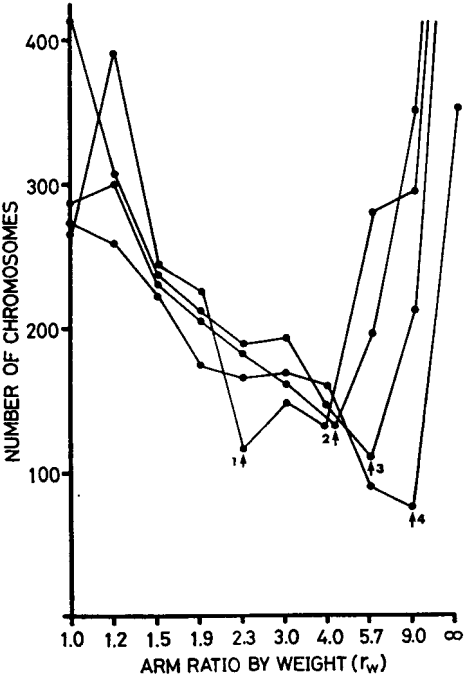

Fig. 2. Relationship between the frequency distribution pattern of the arm ratio (r_w) and the size of chromosomes (C_w). The arrows attached to the numerical orders indicate the antimode when $2.0 \leq C_w < 3.0$, $3.0 \leq C_w < 4.0$, $4.0 \leq C_w < 5.0$ and $5.0 \leq C_w < 6.0$, respectively.

It is interesting to note that the value of r_w at the antimode tends to increase as value of C_w increases, *i.e.*; when (1) $C_w < 3.0$, (2) $3.0 \leq C_w < 4.0$, (3) $4.0 \leq C_w < 5.0$, and (4) $5.0 \leq C_w$, $r_w = 2.3$, 4.0, 5.7, and 9.0, respectively (Fig. 2). This evidence is best interpreted by assuming that the antimode is fixed for all sizes of chromosomes at a point located distance "k" per cent from the end, where k is a constant fraction of the total weight of the haploid set. According to this assumption, the antimode is expressed by the arm ratio as $r_w = (C_w - k)/k$. k is, then, computed as $k = C_w/(r_w + 1)$. When $C_w = 2.0$ and $r_w = 2.3$, $k = 0.6$. The same k values, 0.6, were obtained in the cases where $C_w = 3.0$ and $r_w = 4.0$, $C_w = 4.0$ and $r_w = 5.7$, and $C_w = 6.0$ and $r_w = 9.0$. These data suggests that the antimode is fixed at a point lying about 0.6 per cent distance from the end

of the chromosome, that is, the point where $S_w = 0.6$.

The frequency distribution of S_w when $C_w = 1.9$, 2.5, 3.4, 4.3, 5.1, and

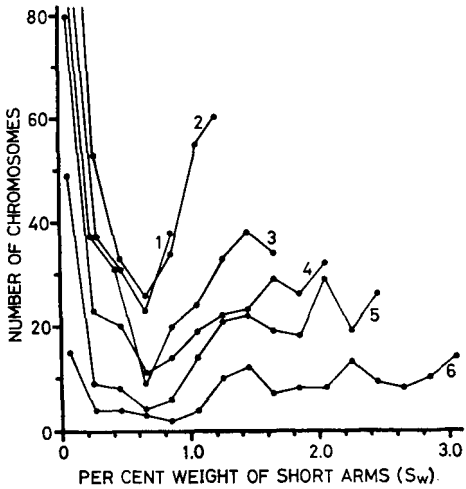

Fig. 3. Frequency distribution of the size of short arms (S_w). The numerical orders show the distribution pattern of S_w when $C_w = 1.9$, 2.5, 3.4, 4.3, 5.1, and 6.4, respectively.

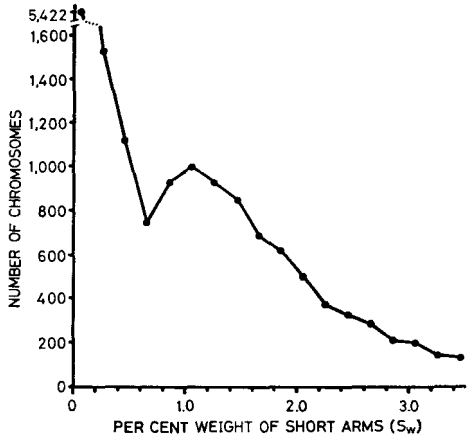

Fig. 4. Frequency distribution of the size of short arms (S_w).

6.4 is shown in Fig. 3. The figure shows an obvious V-shape having an antimode lying at about $S_w = 0.6$, which strongly supports the previous conclusion that the antimode is fixed at a point lying at about 0.6 per cent of the total length from the end. As the same V-shaped pattern was found for all sizes of chromosomes (Fig. 4), the antimode lying at $S_w = 0.6$ seems to be an intrinsic characteristic of mammalian chromosomes.

Proposal of a New Criterion for the Classification of Mammalian Chromosomes

Hirotami T. IMAI

The present author found that the frequency distribution of the size of short arms (S_w) is V-shaped with an antimode at $S_w = 0.6$, which means in other words that the position of the centromere is non-random in mammalian chromosomes (see this Ann. Rep. 46). Although the biological meaning of this non-random distribution is unknown, the antimode at $S_w = 0.6$ seems to be a clear and convenient landmark for classifying the chromosomes of mammals. Based on this intrinsic character, it is possible to group mammalian chromosomes into two distinct categories, $S_w \leq 0.6$ and $S_w > 0.6$.

In this context, it is interesting to note that 97.4% of the "acro- or telocentrics" identified by Hsu and Benirschke (Springer-Verlag, 1967, 1968, 1969) in their Chromosome Atlases have $S_w \leq 0.6$ and 94.4% of their "meta-, submeta- and/or subtelocentrics" $S_w > 0.6$ (see Imai, Ann. Rept. **22**: 48, 1972). The same phenomenon is found in the various nomenclature systems proposed by Baker (1967), Nadler and Lay (1967), Nadler (1968), Thaeler (1968), Wurster and Benirschke (1968a, b), and Yong (1969) (Table 1). This evidence suggests that most mammalian cytogeneticists have *a priori* discerned this discontinuity in the frequency distribution of short arm size. The present author proposes to designate the chromosomes with $S_w \leq 0.6$ "acro- and telocentrics" (A & T) and those with $S_w > 0.6$ as "meta-, submeta- and subtelocentrics" (M, SM & ST).

This quantitative classification has certain advantages for analysing karyotype evolution. There is some evidence that chromosome morphologies have often changed from "A & T" to "M, SM & ST" or *vice versa* by centric fusion or fission, or by pericentric inversion all three of which must

have played an important role in the karyotype evolution of mammals (*e.g.*, Hsu and Arrighi, Cytogenetics **7**: 417, 1968; Wurster and Benirschke, Chromosoma **25**: 152, 1968).

As to the chromosomes with $S_w > 0.6$, it may be convenient to classify meta-, submeta- and subtelocentric according to the standardized criterion proposed by Levan *et al.* (Hereditas **52**: 201, 1964). The antimode at $S_w = 0.6$ is expressed by the arm ratio in weight (r_w) as $r_w = (C_w - 0.6)/0.6$, where C_w is the whole size of a chromosome. Then chromosomes can be defined by means of the arm ratio in weight within the range of $r_w < (C_w - 0.6)/0.6$ as follows; metacentric (median *sensu stricto* and median region) as $1.0 \leq r_w < 1.7$, submetacentric (submedian) as $1.7 \leq r_w < 3.0$, subtelocentric (subterminal) as $3.0 \leq r_w$ (the terms recommended by Levan *et al.* were shown in brackets). In the same way, acrocentric (terminal region) is defined as $(C_w - 0.6)/0.6 \leq r_w$ and telocentric (terminal *sensu stricto*) as $r_w = \infty$. All the chromosomes with $C_w = 1.2$ are grouped here as acrocentrics, since their short arms are $S_w < 0.6$. It is interesting to note that most of the minute chromosomes identified by Hsu and Benirschke (1967, 1968, 1969) have $C_w < 1.2$. The present nomenclature is summarized in Fig. 1.

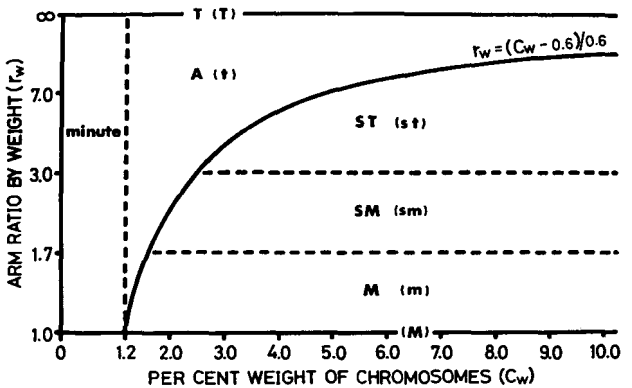

Fig. 1. Nomenclature of mammalian chromosomes. Solid line; $r_w = (C_w - 0.6)/0.6$. Broken lines; limit lines of arbitrary classification. M; metacentric. SM; submetacentric. ST; subtelocentric. A; acrocentric. T; telocentric. (M); median *sensu stricto*. (m); median region. (sm); submedian. (st); subterminal (t); terminal region. (T); terminal *sensu stricto*. The terms recommended by Levan *et al.* (1964) are shown in brackets. Minute; chromosomes with $C_w < 1.2$, which are a category of acrocentrics, since they have short arms less than 0.6 ($S_w < 0.6$).

Table 1. Correlation between various nomenclature systems and the new criterion by arm size

Authors	Chromosome designation by authors	New criterion by the size of short arms (S_w) $S_w \leq 0.6$	$S_w > 0.6$	Materials	References
Baker	A	51 (98.1%)	1 (1.9%)	15 species of	Southwestern
	(M, SM & ST)	6 (3.1)	190 (96.1)	bats	Naturalist **12** (1967)
Nadler & Lay	A	58 (96.7)	2 (3.3)	5 species of	Z. f. Säugetierkunde
and Nadler	(M & S)	1 (0.7)	140 (99.2)	*Meriones* and	**32** (1967)
				4 species of	Cytogenetics 7 (1968)
				Spermophilus	
Thaeler	A	68 (88.3)	9 (11.7)	8 species of	Chromosoma **25** (1968)
	(M, SM & ST)	14 (10.6)	118 (89.4)	*Thomomys*	
Wurster	(A & SA)	294 (84.2)	55 (15.8)	5 species of Bovoidea and	Chromosoma **24** (1968)
& Benirschke	(M & SM)	7 (1.4)	485 (98.6)	32 species of carnivores	Chromosoma **25** (1968)
Young	A	167 (96.0)	7 (4.0)	11 species of	Chromosoma **27** (1969)
	(S & M)	10 (7.0)	132 (93.0)	rats	

A=acrocentric, M=metacentric, S or SM=submetacentric, ST=subtelocentric, SA=subacrocentric.

Comparison between the Length Method and the Weight Method for Formulating the Centromeric Position of Mammalian Chromosomes

Hirotami T. IMAI

The location of the centromere has been considered to be the most useful landmark for the morphological classification of metaphase chromosomes. Nevertheless, if we want to understand chromosome rearrangements quantitatively, it is desirable to observe the position of the centromere in interphase chromosomes, since the chromosome rearrangements which have played an important role in karyotype evolution would have occurred on the stretched DNA strands during interphase. However, the position of the centromere of interphase chromosomes can only be computed indirectly from two-dimensional images of metaphase chromosomes.

Assuming a uniform thickness and a uniform width for long and short arms, the per cent length of each arm against total length of the haploid set (S_1 and L_1) is correlated with the per cent volume of each arm against total volume of the haploid set. If the DNA strands are uniformly folded in the metaphase chromosomes, the S_1 and L_1 values should roughly correspond to the DNA content. Recent electron micrographs of the metaphase chromosomes seem to support this assumption (Comings and Okada, Cytogenetics **9**: 436, 1970; Stubblefield and Wray, Chromosoma **32**: 262, 1971). Furthermore, a linear relationship between the DNA content of metaphase chromosomes and their length has been demonstrated by Radley (Exp. Cell Res. **41**: 217, 1966).

However, the assumption that the widths of the short and long arms are approximately equal in every size of chromosomes has seldom been examined. To study this, the relationship between the length of the short arms (S_1) and the ratio of arm widths (mean width of short arm/mean width of long arm) was examined using the chromosomes of Folios no. 1–25 obtained from the Chromosome Atlas by Hsu and Benirschke (Springer-Verlag, 1967). It was discovered that the ratio of arm widths was about 1.0 in chromosomes with $S_1 > 1.0$, but that it was reduced remarkably in those with $S_1 \leq 1.0$ (Fig. 1). The use of the length method for the determination of the short arm size with $S_1 \leq 1.0$ seems to result in overestimation and error. The sizes of minute short arms can be determined more accurately by the weight

method than the length method. Assuming a uniform thickness of the metaphase chromosomes on a glass slide as well as of the photographic paper used, the S_w values correspond to DNA content in the short arm (see this Ann. Rep. 46).

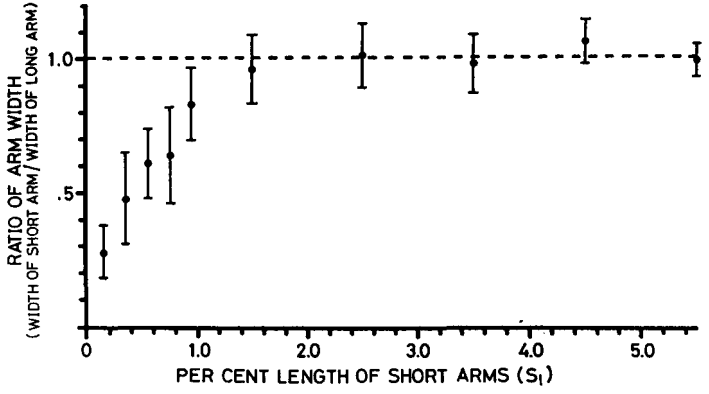

Fig. 1. Comparison of widths of short and long arms. Broken line; expected line assuming that the widths of both arms of a chromosome are qual. Solid circle; the observed ratio of arm widths.

Studies on Chromosomes of the Silkworm, *Bombyx mori*

Akio MURAKAMI and Hirotami T. IMAI

It has been established that the diploid and haploid numbers of silkworm chromosomes are, 56 and 28, respectively. To reach this final figure, however, it has taked about four decades after the first observation by Henking (1892). This would be due to a technical difficulty to estimate the exact chromosome number by the paraffine-sectioning method. During the last two decades, techniques of chromosome preparation have developed remarkably in mammals and others. Recently, Imai (Chromosoma, **37** (1972): 193–200), one of the present authors, demonstrated exact chromosome numbers and clear cut metaphase figures in ants by employing the improved squash method.

This preliminary communication will report on the result of observations on the silkworm chromosomes according to the colchicine hypotonic squash method developed by Imai (1972).

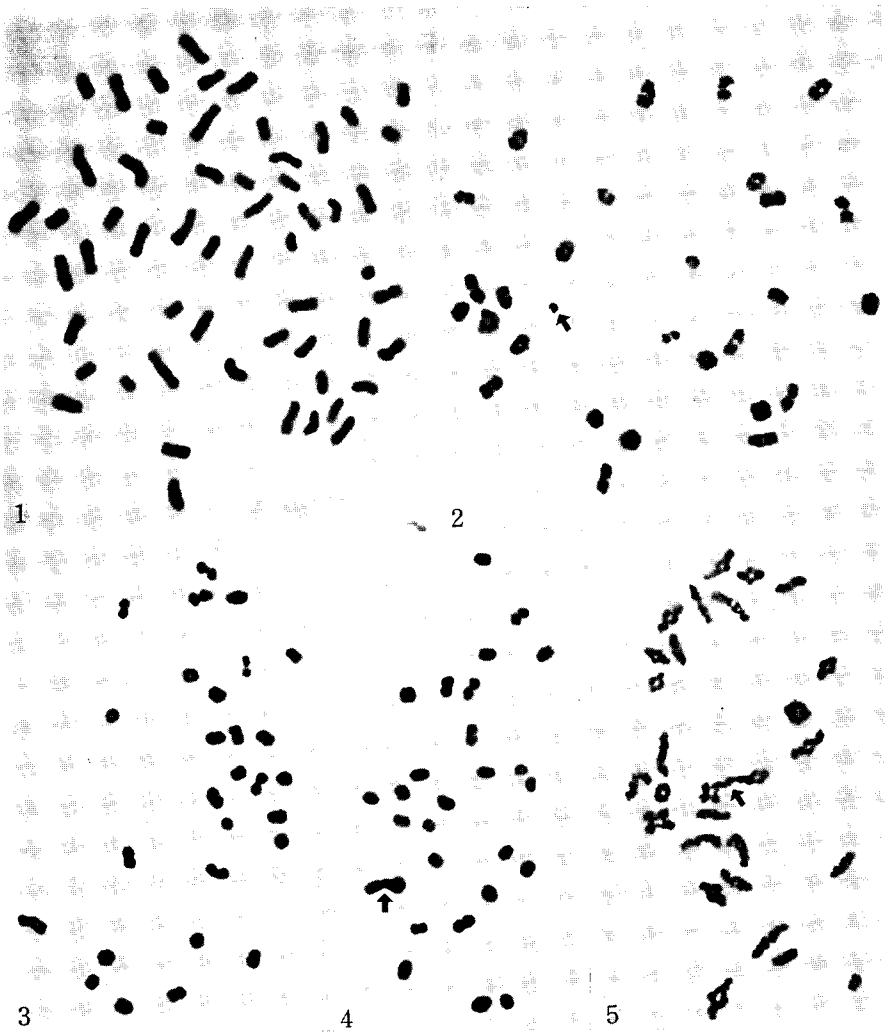

Fig. 1.

FIGURE LEGENDS

1) Mitotic metaphase of spermatogonial cells ($2n=56$).
2) First meiotic metaphase having one minute fragment (arrow).
3) First meiotic metaphase ($n=28$).
4) & 5) First meiotic metaphase and diakinesis with one chain quadrivalent originated from reciprocal translocation (arror).

Testes obtained from 3rd or 5th instar larvae were dipped in a colchicine-hypotonic solution (0.01% colchicine in 0.45% sodium citrate solution) for 30–45 minutes at room temperature. After this treatment, the testis was transfered on a slide slightly tipped to remove the hypotonic solution completely. The testis teared into pieces in a drop of 60% acetic-ethanol (1: 3) fixative was squashed gently by finger pressure. The squashed preparation was frozen on a block of dry ice for a couple of minutes to take off the cover glass using a razor. After the ice was melted by blowing air over it gently with a hair-dryer, the slide was immediately dipped in absolute glacial acetic acid for 30 seconds to reduce cytoplasmic staining, and was dried again with the hair-dryer. The preparation was stained for about 10 minutes in Giemsa solution (Merck) diluted 30 times in Sörensen's phosphate buffer (pH 6.8).

Typical mitotic metaphase figure of spermatogonial cells ($2n=56$) and the haploid chromosome ($n=28$) at meiotic metaphase of spermatocytes are shown in Figs. 1 and 3, respectively. These observations coincide with the standard number of chromosomes of the silkworm reported by authors in the past. It is worthwhile to note that the colchicine-hypotonic squash method is a simple procedure for providing exact and reproducible preparations. Thus, it seems to be certain that the method is useful for detecting cytological abnormalities of the silkworm chromosome. In fact, some observations were carried out on minute fragment chromosomes (Fig. 2) and a translocated chromosome (Figs. 4 and 5), which have been drived from X-rayed silkworms.

However, it can be learned from these figures that silkworm chromosomes could not be characterized each other by the location of centromers. Results obtained in the present experiment support the observations by Friedlander and Wahrman (J. Cell Sci., 7 (1970): 65–89) who indicated that the silkworm chromosome was one of diffused centromer type or holokinetics as often observed in other *Lepidoptera*.

The Sixth Cell Population Change to Occur During Serial Passages of the MSPC-1 Mouse Myeloma

Kazuo MORIWAKI and Tamiko SADAIE

The mouse myeloma MSPC–1 has exhibited cell population changes at

least 5 times during serial passages since 1966. These were detected by single non-disjunction, the appearance of marker chromosomes (A, B and C) and loss of the ability to produce gamma-globulin. In the middle of 1972, a sixth cell population change was detected by the appearance of the D-marker chromosome. This new cell population has grown rapidly, and after 9 generations the former subline having 39 chromosomes and the A, B and C markers had changed completely to the new one having 38 chromosomes and the A, B, C and D markers (see Fig. 1).

Fig. 1. Banded karyotype of a NP-38-ABCD subline cell in MSPC-1 mouse myeloma. D-marker chromosome is translocated on C-marker.

The cell population shift that is described above could be repeated by using frozen tumor cells of subline NP-39-ABC which were stored at −80°C 5 generations before the D-marker was observed. In this case the cell population shift was very fast. For instance, generation 114 consisted of 100% NP-39-ABC cells, generation 117 75% NP-39-ABC and 25% NP-38-ABCD and generation 119 100% NP-38-ABCD.

The rate of cell population shift seems to be affected by the inoculum size of the tumor cells. If a sample of 10^5 cells containing equal numbers of NP-39-ABC cells and NP-38-ABCD cells was transplanted subcutaneously, the resulting tumors contained almost equal numbers of both subline

cells. But if 10^3 cells were tested in the same manner, the NP-38-ABCD cells tended to proliferate more rapidly than the NP-39-ABC cells.

Ecological Genetic Studies on the Differentiation of *Chrysanthemum* Species

Shuzo NAGAMI[1)]

About 40 wild *Chrysanthemum* species are reported in Japan (Kitamura, 1940). In 1972, 22 species among them were sampled from their respective natural populations, and were transplanted in the garden of National Institute of Genetics in Misima for studies on the pattern of species differentiation. The results of pollen investigations are summarized as follows:

1. Ultramicro-structures of pollen grains were observed by using a scanning electron microscope. The pollen grains of all these species were spherical and had many small spines on the surface. In proportion to the increase of *Chrysanthemum* polyploidy, gradual increase was observed in the size of pollen grains, but no increase was found in the number of pollen spines. Clear differences among the 22 species were frequently seen in the shape of pollen spines.

2. *Chrysanthemum* pollen grains did not germinate well on culture media so far devised. But two suitable media were found recently, whose components were as follows: (A) sucrose 25% and agar 1.5%, and (B) sucrose 25%, agar 1.5%, boric acid 250 ppm and malonic acid 10 ppm. In general, the latter gave better pollen germination than the former. Germination rate varied from 0% to 35%. Pollen longevity of *Chr. pacificum* ($2n=90$) was about 6 hours after emission at room temperatures, but it was extended to 72 hours when stored at $-15°C$ with silica gel (Nagami, Akizawa and Iwanami, 1972).

3. Isozymes in the pollen grains of *Chr. Shiwogiku* var. *kinokuniense* ($2n=90$) were examined by starch gel electrophoresis. The results were as follows: (1) bands of acid phosphatase, esterase, asparate aminotransferase, leucine aminopeptidase, alcohol dehydrogenase and malate dehydrogenase (+ nicotinamide-adeninedinucleotidephosphate) were clearly identified, (2) weak bands were observed for malate dehydrogenase (+ nicotinamideadeninedinucleotide) and isocitrate dehydrogenase (+ nicotinami-

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deadeninedinucleotide), but (3) no isozyme band was observed for peroxidase, succinate dehydrogenase, glutamate dehydrogenase (+ nicotinamide-adeninedinucleotide) and α-glycerophosphatedehydrogenase).

VI. MUTATION AND MUTAGENESIS IN ANIMALS

A New Sensitive Test System for the Detection of Weak Chemical Mutagens Using Silkworm Oöcytes

Yataro TAZIMA and Kimiharu ONIMARU

With a purpose of exploiting a sensitive system for the detection of weak chemical mutagens, researches have been carried out with silkworm in two directions: one was screening of sensitive strains and the other was inquiring into the most sensitive cell stages for the chemical treatment. Seven representative strains for differential radiosensitivity have been compared in respect to their sensitivity to chemical mutagens by injecting a same dose of physiological saline solution of mitomycin-C to mature male larvae. The difference thus observed among strains was far less than anticipated, showing only four fold difference between the most and the least sensitive strains. Furthermore, induced mutation frequencies fluctuated from experiment to experiment. In contrast, differences in mutability among different cell stages were far larger than those between strains in both sexes.

It has been known in the silkworm that oöcytes were extraordinarily sensitive to the mutagenic action of radiation around the stage of meiotic metaphase I and II. We, therefore, focussed our study to the treatment of cells at those stages with chemical mutagens. Since meiotic metaphase takes place immediately after oviposition in this insect, we first tried to administer mitomycin-C to ova in the ovariole by injecting it into the body cavity of female pupae one day before emergence. The chemical were injected into wild type female pupae in several doses, and mutation frequencies were measered by specific loci method. Linear relation was obtained when experimental data were plotted on logarithmic scale between administered doses and mutation frequencies, indicating that the injected chemical penetrated the chorion and induced the mutations. However, the mutation frequencies were far lower than expected.

Next, injection was performed at earlier pupal stages: i.e., 3 or 5 days before emergence. Injected dose was 5 μg/head. The results are partly shown in Table 1.

The results clearly indicated that injection three days before emergence

Table 1. Changes in mutation frequency according to the stage of injection into female pupae. (Treated ++♀ × *pe re*♂, 723 rb)*

Injected stage	No. of eggs observed	Mutation frequency (10^{-5})						**Induced Mut. freq.
		pe	*pe* mos	*re*	*re* mos	others	total	
Cont.	26682	11.2	15.0	3.7	3.7		33.6	—
1 day before	19509	20.5	0	15.4	15.4		51.3	1.8
3 days before	14122	226.6	191.2	177.0	49.6		644.4	61.1
5 days before	5910	1895.1	1844.3	1336.7	930.6	50.7	6006.7	597.3

* Injection: MMC 5 μg in 0.025 ml physiological saline per head.
** Induced mutation frequency: $\times 10^{-5}/\mu$g/locus.

was 10 times and that five days before was more than hundred times as effective as the injection one day before. The effectiveness seemed to be inversely correlated with the formation of a chorion, which is completed one day before the emergence. The amount of chemicals to be incorporated into the egg plasm might be very limited after the completion of the chorion but it might be fairly large before the completion of the chorion.

These findings seemed to permit us to develop a very sensitive test system for the detection of weakly mutagenic chemicals. This was confirmed by the examination of the mutagenicity of a weak mutagen, Panfuran. This chemical is a derivative of nitrofuran and widely used in Japan as clinically useful topical anticeptics. The weak mutagenicity of this compound to the silkworm has already been reported by us (Tazima and Fukase, 1966). The effect was so indistinct that we could hardly conclude the positive effect. In the present experiment, the chemical was injected into female pupae four days before emergence in doses 4.5 and 17.5 μg/head. The results were diffnitely positive.

Thus, the method was concluded to be a very sensitive test system which could be utlized for the detection of weakly mutagenic chemicals.

Test for Mutagenicity of Mercuric and Cadmium Compounds Using Oöcyte System of the Silkworm

Yataro TAZIMA and Yosoji FUKASE

Ever since the causality of organic mercuries and cadmium compounds was recognized with regard to serious human injuries, a suspicion has been aroused upon the mutagenic action of those compounds. In order to get an answer to this question we carried out experiments with use of silkworm oöcyte system soon after we noticed its high sensitivity. The compounds we tested were Methylmercury chloride, Mercuric phenylacetate, Cadmium carbonate and Cadmium hydroxide. Those compounds, disolved in a physiological saline solution, were injected into wild type female pupae four days before emergence, whereby enough quantity of chemicals could have been incorporated into egg plasm. Doses given were 0, 0.1, 1, 10 and 100 μg per head in cases of mercuric compounds and saturated concentration and its 1/4 in cases of cadmium compounds. Injection volume was 0.025 ml/head. Mutations were measured by specific loci method using *pe* and *re* marker genes. Results for Methylmercury chloride are shown in Table 1.

Table 1. Results of mutagenicity test for Methylmercury chloride. (Treated $++\,♀ \times$ *pe re* ♂, 723 C108)

Dose μg/head	No. of eggs observed	Mutation frequency (10^{-5})				
		pe	*pe* mos	*re*	*re* mos	total
0	30365	9.9	23.1	13.2	6.6	52.8
0.1	39992	20.0	45.0	10.0	10.0	85.0
1	33275	27.0	57.1	15.0	6.0	105.1
10	31158	35.3	22.5	9.6	12.8	80.2
100	3947	25.3	50.6	0	0	75.9

As seen from the table, the compound seemed to exert toxic effects to growing oöcytes at higher doses but almost no mutagenic effects upon the silkworm germ plasm. Likewise, Mercuric phnylacetate, Cadmium carbonate and Cadmium hydroxide failed to be detected of their mutagenicity by this test system.

Since the method used in this experiment is very sensitive one, it can be concluded that those chemicals are not mutagenic in the silkworm.

Consequences of the Selection for Mutation Frequencies Presumably Due to Replication Instability in the Descendants of Mitomycin-C Treated Silkworm

Kimihraru ONIMARU and Yataro TAZIMA

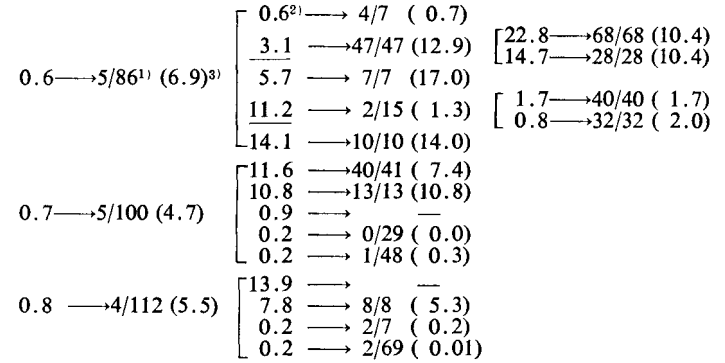
We reported earlier (Onimaru and Tazima, 1969, this Report No. 19) that in the descendants of EMS and/or Mitomycin-C treated silkworm, a mutation from + to *re* had continued to occur generation after generation. In that experiment wild type males were treated and crossed to *pe re* females. In the F_1 (hereinafter represented by C_1) several mutants, whole types and mosaics for *pe* and *re*, appeared in addition to expected wild type. Those wild type females were back crossed to *pe re* males and C_2 were obtained. The expected phenotypes in this generation were 1 +: 1 *pe*, because *pe* is epistatic to *re* and recombination does not occur in the female. It was observed in C_2 that, in addition to those expected phenotypes, *re* mutants appeared in some batches (29 betches among 418 tested) and the frequency of *re* within a batch was 4.8% on an average. Wild type females from those *re* producing batches were back crossed again to *pe re* males and thus the strains were maintained (C_3, C_4 ...). The occurrence of the *re* mutation continued to later generations.

The frequency of *re* producing batches to total observed was 29/418 in C_2, 77/221 in C_3 and reached 100% in C_4. The frequency of the appearance of *re* within a batch varied remarkably among batches and in high *re* producing batches the ratio of + to *pe* became lower than unity. Wild type females from non-*re* producing batches in C_3 were tested in a similar way, by sampling 50 eggs from each of 20 batches randomly, but no *re* producing batches were discovered in C_4 (0/198). Those observation strongly suggested that the observed case could be regarded as due to the replication instability as already reported for *Schizosaccharomyces* and *Drosophila*.

Since the above experiment was carried out on mixed batch basis, next experiment was undertaken on separate batch basis, so as to investigate more exactly the change in the frequency of *re* producing batches. The experiment started from three batches of C_1. The frequency of *re* producing batches in C_2 were 5/86, 5/100 and 4/113, in which the numerator denotes a number of *re* producing batches and the denominator a total number of observed batches. In C_3 the frequency reached 100% in some families but was fairly low in others, showing large variation among families. In C_4

all batches became *re* producing. However, the effect of selection was not clear with regard to the frequency of *re* eggs within a batch, which is shown in parenthesis in the Figure 1.

Fig. 1. Procedure and consequences of the selection of mutation frequency in the descendants of mitomycin-C treated individuals.



Note:

1) 5/86 denotes 5 *re* producing egg batches among 86 tested.
2) Per cent of *re* eggs within each *re* producing egg batches.
3) Average percentage of the *re* eggs within *re* producing egg batches.

It should be noted that the occurrence of *re* mutation in all batches was attained as early as in C_3 in this experiment, one generation earlier than the former experiment which was carried out on mixed batch basis. When mutation frequency was reasonably high, the appearance of *re* producing batches could be expected to reach 100% in C_3. This was actually observed for about half number of families. These results suggested that in addition to a instable primer at least a co-factor is necessary for the production of mutation in this strain.

Further Studies on the Instable p^M Allele of the Silkworm I. Evidence for the Occurrence of Recombination Between Translocated and Free Second Chromosomes in the Female

Yataro TAZIMA and Akio OHNUMA

In the previous report (this Annual Report No. 22) we described an

instable p^M allele locating on the second chromosome segment of a W.II translocation. Although very seldom mottled females with regard to p^M marking appear and when crossed to p males, they produce daughters of several phenotypes, belonging to the same multiple allelic group: p^B, p^M, p^{Sa}, $+^p$, and p. In contrast, their sons comprised only one phenotype, p. The newly arisen phenotypes were transmitted to their female offspring as if each of them were a complete mutation that had occurred at p^M locus on the translocated segment. For the interpretation of the appearance of several phenotypes belonging to the same allelic group, an assumption was necessary that mutation had occurred several times at the same locus during very early cleavage stages in the p^M mottled female. Such instability of p^M locus can be interpreted by assuming, as was the case for variegated position effects in *Drosophila*, that an euchromatic region involving p^M locus and a heterochromatic region of W chromosome were brought in a close proximity due to the translocation. It is only hypothetical and does not explain why existence of two kinds of chromosome region in close proximity cause instability of p^M locus.

In order to see if the two free second chromosomes behave freely from the translocated segment of the second chromosome, $W \cdot p^M/Z, pY/py$ females were crossed to py/py males. The results were surprising. It was revealed that recombination could occur between translocated and a free chromosome even in the female.

Among seven families tested six behaved just as expected. Whereas, one family showed quite abnormal segregation; i.e., $p^M Y$♀ 61, $p^M y$♀ 63, $p^{Sa} Y$♀ 100, py♀ 1, pY♂ 68, and py♂ 179.

The newly arisen $p^{Sa} Y$ females were transmitted *per se* to the next generation, when they were back crossed to py males. These results could only be interpreted by assuming the occurrence of recombination between translocated and a free second chromosome during a very early cleavage stage, giving rise to a mosaic comprising two kinds of genetic constitution; W.p^M/Z, py/pY and W.p^{Sa}Y/Z, py/py. The former genetic constitution accounts for the appearance of $p^M Y$♀ 61, $p^M y$♀ 63, pY♂ 68, py♂ 68, and the latter may account for the appearance of $p^{Sa} Y$♀ 100 and py♂ 111.

These evidences suggest that the observed instability at p^M locus is not only due to mutations occurring at p^M locus but also due to an unknown mechanism of chromosome recombination.

X-ray-Induced Crossing-Over in Oogenesis of the Silkworm

Akio MURAKAMI

Physical and chemical agents have been known to induce or enhance the genetic recombinations or corssing-overs in both meiotic and mitotic cells. Recently, the repair mechanism in recombination for radiation-induced DNA lisions has attracted special attention.

Tanaka (1913) found that in the silkworm, crossing-overs do not normally occur in oogenesis. Later, many investigators have attempted to produce artificial induction of crossing-overs by means of X-irradiation to oogenesis of the silkworm. They found a number of exceptionals which might have resulted from the induced crossing-overs. Most of the experiments, however, was lacking in sufficient progeny tests except for a few cases as criticized by Tanaka (1953) and dealt with the middle or late growth stage oocytes in pupae although the most likely cell stage for induction of crossing-overs may be an early growth stage oocytes. In the present study an investigation was carried out by using egg-color (*pe* and *re*) markers to know whether X-rays could induce crossing-overs in oogenesis of the silkworm.

Early stage oocytes in fourth instar larvae carrying the heterozygous genotype $\left(\frac{+\ re}{pe\ +}\right)$ were irradiated with 180 kVP X-rays (1000 and 2000 R at a dose-rate of 300 R/min) and they were then crossed to male moths having the homozygous genotype for *pe* and *re* genes. Spontaneous crossing-overs were also checked in unirradiated control. By this procedure, any crossing-overs could be detected by finding exceptional black and yellowish-white eggs among normal yellowish-white and red eggs. Since exceptional yellowish-white eggs could not be distinguished from normal ones, a progeny test has been done for black exceptional eggs. In view of the mating procedure, however, the crossing-over type black eggs also could not be distinguished from the polyploid type black eggs. To make clear this confusion, the moths developed from the exceptional black eggs were back crossed again to moths carrying the homozygous for *pe* and *re* genes. Since it was well-known that tetraploid ($4n$) on triploid ($3n$) type eggs were generally much larger or smaller in size than crossing-over $2n$ type eggs, difference in egg size was useful for distinguishing each other. In addition, by comparing egg-color segregation ratios in their hybrids, it was pos-

sible to classify them into crossing-over and polyploid types: female moths which produce yellowish-white and black eggs in the ratio of 1 to 1 and male moths which produce yellowish-white, red and black eggs in various ratios could be regarded as the crossing-over type. While, female moths which produce yellowish-white, red and black eggs in various ratios could be regarded as the incidence of polyploid type. A lack of this class male moth would be interpreted to be due to retardation of XO type male cells in the process of gametogenesis.

The results indicated that the frequency of exceptional black eggs increased with doses of X-rays. One crossing-over type moth was found out of five exceptionals examined in the control experiment. Thus, the spontaneous frequency of the crossing-over in silkworm females was estimated to be 3×10^{-5}. This suggests that the crossing-over in oogenesis of the silkworm does not completely lacked, but is very strongly inhibited. Three females (17×10^{-5}) and two males (11×10^{-5}) crossing-over types were found out of ten exceptionals in the 1000 R series and one female (12×10^{-5}) out of twelve exceptionals in the 2000 R series. Although this experimental scale was not enough, one can be said that there were less frequently induce crossing-overs in the 2000 R series than in the 1000 R series in contrast to polyploid. This suggests that the polyploid, which may be due to inhibition of cell-division, would be much more frequently induced with the high doses than with the low doses. No crossing-over was observed in pupal oocytes. In any case, it may be given a conclusion that X-rays could enhance the crossing-over in early growth stage oocytes of the silkworm.

A Comparison of Relative Mortalities by 2 MeV Neutrons and X-rays of Young and Aged Dormant Silkworm Embryos

Akio MURAKAMI and Tamiko IWASAKI*

To establish a relationship between the radiosensitivity and the aging is one of important and fascinating subjects in radiobiology. It has generally been thought that there are various biological and technical limitations in insects as compared to mammals to investigate such a biological event. The silkworm has a biological advantage that the duration of dormant em-

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bryonic stages can be regulated by the temperature with a slight change in the physiological activity of embryos. The present experiment carried out to make clear a relationship between the radiosensitivity and the age of embryos was investigated by comparing the sensitivity of embryos at two different stages which were exposed to radiations having two different ion densities.

Embryos of the C108 silkworm strain at two different dormant stages, prehibernated (3 month-old) and post-hibernated (10 month-old) stages, were exposed to either 2 MeV neutrons (at a dose-rate of 100 rads/min) or 180 kVp X-rays (at a dose-rate of 300 R/min) and embryonic mortalities were determined by the reduction of hatchability. The neutron beam was obtained from a Van de Graaff accelerator in the National Institute of Radiological Sciences, Chiba and gamma contamination was less than 10 per cent.

It was clearly observed that there was a larger shoulder in the dose-response curve for young dormant embryos than in that for aged ones regardless of the radiation quality. This was more pronounced with neutrons than with X-rays. Practically, no shoulder was observed in the curve for aged dormant embryos exposed to 2 MeV neutrons. LD_{50} of X-rays and 2 MeV neutrons for young emrbyos was about 1400 and 350 rads, respectively, while that for aged embryos was about 600 and 100 rads, respectively. This finding indicates that aged embryos were much more sensitive in embryonic mortality than young ones. Comparing the LD_{50} value of 2 MeV neutrons with that of X-rays, a relative effectiveness (or RBE) of 2 MeV neutrons for the radiation-induced embryonic mortality can be estimated. Thus, RBE values for young and aged dormant embryos were about 4 and 6, respectively, suggesting that the RBE would be increased with the age of embryos. A possible reason for changes in radiosensitivity and RBE in the process of embryonic development may be due to either the increase in radiosensitivity or the decrease in repair mechanism in accordance with the process of senescence of embryos.

In comparing the RBE values for the embryonic mortality in the silkworm of 2 MeV neutrons with that of 14 MeV neutrons in the previous report (Murakami and Kondo, this Report, No. 14 (1964): 106–107), the former is 1.2 to 1.8 times more effective than the latter, indicating that the RBE for the embryonic mortality clearly depends on the LET. This observation would be interpreted as due to the chromosomal damage as revealed cytolo-

gically in *Artemia* which was exposed to 2 MeV neutrons (Iwasaki *et al.*, Radiation Res., **45** (1971): 288–298).

Mutagenesis of Acridine Orange in Cleavage Nuclei of the Silkworm

Akio MURAKAMI

In the previous paper (Murakami, 1972), it was shown that acridine orange (AO) was mutagenic for germ-cells in female silkworm pupae, but not for male pupae. The explanation for these findings was that AO, which had taken into ooplasm of oocytes (or eggs), would affect metabolic and non-crystallized DNA in either meiotic oocytes or cleavage nuclei by intercalating with DNA bases. In yeast cells, however, it was observed that 5-aminoacridine was mutagenic for meiotic cells, but antimutagenic for mitotic cells (Magni *et al.*, 1964). The present study was carried out to check whether AO is mutagenic for mitotic cleavage nuclei in the silkworm.

Mutation frequencies were estimated by the specific-locus method using egg-color genes, *pe* and *re*. For selectively testing the mutagenic action of AO for mitotic cleavage nuclei, AO was injected into seven day-old female pupae with double markers. After emergence they were then mated to C108 wild type male moths. The germ-cells of female pupae at the stage of injection were prophase I oocytes. Oviposited F_1 eggs were scored on the visible phenotype with complete or mosaic mutations.

A positive mutagenic effect of AO was clearly observed on chromosomes originated from the wild type male. The majority of mutants recovered was mosaics which had small mutated areas on the egg, while complete mutations detected was remained on a level of the control. It is of interest to note that the mutant area in mosaics produced by treated pupae with double markers was less than that in mosaics produced by treated wild type pupae which were mated to marker male moths. If mutational events occurred in oocytes, mutated areas may become a large size, on the contrary, mutational events occurred in somatic cells at the stage of cleavage divisions, the areas may become small size in the process of egg development. Thus, it may be concluded that most of mutants detected in the present experiment would be originated from mutational events in the stage of mitotic cleavage divisions.

Although in a small scale experiment, it was observed that AO was mutagenic for meiotic spermatocytes and that the ratio of complete to mosaic mutations was an equal. It was also observed that the mutants detected in the treated wild type female pupae (Murakami, 1972) would be originated from mutational events in both mitotic cleavage nuclei and meiotic oocytes. These findings in the silkworm seem to be incompatible with observations in yeast cells (Magni *et al.*, 1964) and in *E. coli* (Streisinger *et al.*, 1966). One of possible interpretations for this contradiction among different biological materials would be the difference in biophysical conformation of their DNA molecules.

Mutagenesis of Quinacrine Compounds in the Silkworm

Akio MURAKAMI

The acridine compounds are commonly used in medicine as antibacterial, carcinostatic and antimalarial drugs. They are also used as dyestuffs in the textile industry. Quinacrine hydrochloride (QH) or atebrine, and quinacrine mustard (QM) are acridine derivatives that have been used as antimalarial and carcinostatic agents, respectively.

In the silkowrm, acridine orange, acridine yellow and proflavine are highly mutagenic for female germ-cells (or prophase I oocytes) in pupae after injection regardless of the lighting condition (Murakami, 1972). In contrast, these acridines are not mutagenic for mature sperm. Therefore, female germ-cells in silkworm pupae seem to be a simple and relevant mutational assay system for acridines and related compounds.

This communication reports a positive mutagenic effect of QH and QM on silkworm oocytes as well as on cleavage nuclei, but not on mature sperm.

QH (0.25 and 0.50 mg/capita) and QM (0.04 mg/capita) in saline were injected into the ventral abdomen of eight day-old wild type C108 silkworm pupae. The germ-cells at this stage pupa are in prophase I oocytes and mature sperm in female and male, respectively. They were then mated after emergence to marker strain moths carrying double recessive egg-color genes, *pe* and *re*. F_1 eggs were examined for their egg color to determine the occurrence of mutations.

The results indicated that mutagenicity of QH was low for pupal oocytes

treated, while that of QM was markedly high and about ten times more effective than QH. No significant mutagenic effect of both compounds was observed on mature sperm. This sex difference in mutagenic activity of the quinacrine compounds for pupal silkworms may be caused by the differential DNA conformation in germ-cells, either female meiotic and mitotic cells or mature sperm.

In comparing the mutagenicity of other acridine compounds, acridine orange, acridine yellow and proflavine, for pupal silkworm oocytes, QH was the least effective among them. It is known that QH did not bind less strongly to DNA than did proflavine (Drummond *et al.*, 1965). Hence it can be said that intercalation of acridines and related compounds between DNA bases would be a necessary condition to induce mutation by these compounds, but not a sufficient one.

The majority of mutants recovered in the present experiment was mosaic types as observed in the experiments with acridine orange. This may be interpreted as being due to preferential insertion of quinacrine compounds into one of double-stranded DNA molecules. If so, a possible mechanism how complete mutants appear in meiotic oocytes would be an induction of recombination-like events between paired homologous DNA molecules if one of them (temporarily) contains an intercalated compound.

VII. RADIATION GENETICS AND CHEMICAL MUTAGENESIS IN MICROORGANISMS AND PLANTS

Participation of DNA Polymerase I in the Repair of γ-ray Damaged DNA in Permeable *Bacillus subtilis* Cells

Takehiko NOGUTI and Tsuneo KADA

Gamma-ray irradiation reduces biological activity and molecular size of cellular DNA. We have recently shown that the DNA damage was repaired in *B. subtilis* cells made permeable by toluene treatment when they are supplied with substrates and cofactors for DNA polymerase and ligase (Noguti, T. and Kada, T., J. Mol. Biol. **67**: 507,1972). Mutant cells lacking DNA polymerase I failed to repair the DNA damage in the above system (Table 1). Gamma-ray irradiation and toluene-treatment of wild cells showed remarkable high level of DNA synthesis compared to the non-irradiated control. On the other hand, the *pol*$^-$ cells showed a very reduced level of DNA synthesis upon γ-ray irradiation. This γ-ray stimulated DNA synthesis seemed to be an essential process of the repair.

Table 1. Repair of γ-ray damaged DNA in toluene-treated *pol*$^+$ and *pol*$^-$ cells

Strain	γ-ray dose (kR)	Incubation medium	Incubation period (min.)	% transformants
HA 101 (*pol*$^+$)	0	—	0	100
	0	buffer	10	59
	0	complete	10	41
	30	—	0	4.8
	30	buffer	10	3.2
	30	complete	10	33
HA 101 (59) F (*pol*$^-$)	0	—	0	100
	0	buffer	10	73
	0	complete	10	39
	30	—	0	8.1
	30	buffer	10	4.1
	30	complete	10	5.5

HPUra(6-(p-hydroxyphenylazo)-uracil) has been reported to be a specific inhibitor for semiconservative DNA replication of *B. subtilis* cells (Brown, N. C., J. Mol. Biol. **59**: 1, 1971), and more recently to inhibit action of pol III (Mackenzie, J. M. *et al.*, Proc. Nat. Acad. Sci. U. S., **70**: 512, 1973). The γ-ray stimulated DNA synthesis was refractory to this inhibitor with a dose sufficient enough to inhibit ATP dependent DNA synthesis to less than one tenth.

These results led to conclude that the type I DNA polymerase is actually involved in the above repair.

Purification of an Enzyme Specifically Involved in Repair of Gamma-ray Irradiated DNA

Takehiko NOGUTI and Tsuneo KADA

The involvement of a third enzyme in addition to DNA polymerase I and DNA ligase relevant to the γ-ray irradiated DNA was shown last year and described in the previous annual report (Noguti, T. and Kada, T., No. **22**: 68, 1972). Gamma-ray irradiation *in vitro* usually reduces priming activity of DNA for DNA polymerase I. However, the priming activity of DNA extracted from irradiated cells was rather more enhanced than that without irradiation. It was further shown that treatment of irradiated DNA with cellular extract brought about remarkable enhancement of the priming activity. If the cellular extract was heated at 100°C or treated with pronase, the above effect was disappeared. The SDS or phenol treatment also caused inactivation of the activity. Because such an effect was incubation dependent, we assume that our extract contains an specific enzyme. This cellular enzyme was expected to play a role in the repair by converting damaged DNA into a better primer for DNA polymerase I.

We made efforts to purify this cellular enzyme. The assay method of its activity consists of measuring the amount of radioactive precursors incorporated into the primer DNA which had been irradiated with γ-rays, treated with the enzyme and purified again. In the present study, *Micrococcus lysodeikticus* DNA polymerase (type I) was prepared and used.

The cellular extract (F–I) containing less than 2% of cellular DNA was prepared from *B. subtilis* cells grown in C^+ medium by treating with Brij-58 and lysozyme at low temperature (at 7–8°C). Subsequently, the extracted

protein was condenced by ammonium sulfate precipitation (F–II). Specificity of this enzyme fraction toward γ-ray damaged DNA is shown in Table 1. After dialysis, the F–II was adsorbed on DEAE cellulose column and eluted by a linear gradient with NaCl. Most of active fractions were collected and condensed by dialysis against solid sucrose (F–III). After dialysis, the F–III was adsorbed on phosphocellulose column and eluted by a linear gradient with $(NH_4)_2SO_4$. Two peaks appeared between 0.05 M and 0.2 M $(NH_4)_2SO_4$ concentrations. The mode of action of these active fractions is now under investigation.

Table 1. Specific enhancement of priming activity of gamma-ray irradiated DNA after treatment with F–II fraction

Gamm-irradiation on T7 DNA (kR)	Incubation (min.)	Priming activity (cpm)
0	0	430
	60	466
4	0	188
	60	1750
8	0	125
	60	2060

Mapping of *rec* Genes in *Bacillus subtilis*

Yoshito SADAIE and Tsuneo KADA

Past isolation and characterization studies on bacterial mutants defective in genetic recombination have been carried out mainly in *E. coli*, revealing the roles of different *rec* genes in processes of recombination, repair and mutation as well as in other DNA metabolisms. Recombination-deficient strains of *Bacillus subtilis* were also isolated. Three of *rec* mutations are actually mapped (Hoch, J. A. and Anagnostopoulos, C., J. Bacterol. **103**: 295, 1970; Sinha, R. P. and Iyer, V. N., J. Mol. Biol. **72**: 711, 1972). Studies on the recombination in *B. subtilis* have unique features; chase of transforming DNA in the course of integration into host chromosome and biochemical studies of DNA molecules in mutants with various genetic defects in the recombination capacity.

Through isolations of γ-ray sensitive strains of *B. subtilis*, we charac-

terized several recombination-deficient strains. Some of our studies are already described (Kada, T., Tutikawa, K. and Sadaie, Y., Mutation Res. **16**: 165, 1972; Sadaie, Y. and Kada, T., Mutation Res. **17**: 138, 1973). The chromosomal location of the *rec* mutations was first studied in a strain named M45 which showed very reduced frequencies both in genetic transformation and PBS1 transduction. One class of revertants of M45 having intermediate sensitivities to radiations and mitomycin between the M45 and wild strains possessed a mutation in the *recA* gene. This mutation was linked to *ura-1* and causes reduction in the frequency of transformation but not that of PBS1 transduction and therefore designated as *recA45*. PBS1 phages grown on the M45 or on their revertants, did not give any Rec^+ transductant among Ura^+ transductants in recipient cells of GSY 1037 (*ura-1 recA1*). When DNA isolated from the revertants of M45 was used for transformation of the M45, no Rec^+ transformant was obtained and most transformatns were of *recA45* type. DNA from the wild strain could transform the M45 to Rec^+ and a few *recA45* type transformants were detectable. These results with another observation indicate that the strain M45 carries a *recA45* mutation and an additional *rec* mutation linked closely to the *recA*. We tentatively name this new *rec* gene *rec*45.

Efficient Procedures for Detection of Frameshift Mutagens

Tsuneo KADA and Yoshito SADAIE

There are increasing numbers of reports indicating that many carcinogens have mutagenic capacity after metabolisation in mammals (Maher, V. M., Miller, E. C., Miller, J. A. and Szybalski, W., 1968, Mol. Pharmacol. **4**: 411; Fahmy, O. G. and Fahmy, M. J., 1972, Int. J. Cancer **9**: 284; Cookson, M. J., Sims, P. and Grover, P. L., 1971, Nature, New Biology **234**: 186; Huberman, E., *et al.*, 1971, Proc. Nat. Acad. Sci. U. S. **68**: 3195). Important carcinogens such as polycyclic aromatic hydrocarbons or acetylaminofluorens are really frameshift mutagens *in vivo* (Ames, B. N., Sims, P. and Grover, P. L., 1972, Science **176**: 47; Ames, B. N., Gurney, E. G., Miller, J. A. and Bartsch, H., 1972, Proc. Nat. Acad. Sci. U.S. **69**: 3129). Because the frameshift mutagenesis may involve some specific interactions between mutagens and DNA, it is usually required to exame a sample with plural

testing strains having different sites for mutation detections.

We deviced a simple method named "rec-assay" for screening new chemical mutagens by comparing cellular sensitivities of wild and recombinationless strains against different chemicals (Kada, T., Tutikawa, K. and Sadaie, Y., 1972, Mutation Res. **16**: 165). Because diverse chemical damages in DNA are repaired through cellular recombination functions in wild cells but not in recombination-deficient cells, most agents showing increased lethal activity on *rec*$^-$ over *rec*$^+$ cells may have damaged cellular DNA. It is highly probable that such DNA-damage-provoking agents are mutagenic.

We have isolated a number of *rec*$^-$ strains on which genetic and biochemical studies were carried out (Sadaie, Y. and Kada, T., 1973, Mutation Res. **17**: 138). It was recently found that certain *rec*$^-$ strains were remarkably sensitive to typical frameshift mutagens compared to wild strains. Among other repair-deficient mutant strains studied, three *pol*$^-$ strains as well as five *hcr*$^-$ strains showed cellular sensitivities which are similar to those of wild strains against typical frameshift mutagens. Strains carrying either *recB* or *rec43* (this carried by the strain L43 isolated by us) showed remarkably increased sensitivities to frameshift mutagens compared to wild cells. Furthermore, the strain M45 carrying a *recA* gene (*recA45*) and a *rec45* gene (tentatively named) showed the highest sensitivity. The above findings indicated that comparative studies on cellular sensitivities of wild and M45 strains may be efficiently applied to the detection of frameshift mutagens.

Actually we tested, in collaboration with Dr. Y. Shirasu, a number of pesticides and found at least two potent frameshift mutagens: 2,4-Dinitrophenylthiocyanate and Sodium p-dimethylaminobenzenediazosulfonate.

Radiation Effect upon Sex Expression in Cucumber

Taro FUJII

In a monoecious plant like cucumber, the sex expression could be changed with environmental conditions, such as temperature, day length or hormones like ethylene or kinin etc. in spite of genetic background controlling the sex factors. It is well known that ionizing radiations modify certain enzymatic or hormonal system in addition to the genetic effect. Present experiments were conducted to examine the effect of γ-rays on the sex expression in cucumber.

Dry seeds of commercial variety "Kaga-Fushinari" were exposed to 10 and 20 kR of ^{137}Cs γ-rays at a dose rate of 40 kR/h. Twenty seeds were used in each lot and the seeds irradiated in Feb. 28, 1972 were sown in soil inside the greenhouse. The development of female flowers were examined during the successive internodal growth of the plants. In most cases, the first female flower was noticed on the 6th node but in some instances on the 7th or 8th node. Two or more female flowers clustering on the same node were also observed and there was no male flower on such nodes. Most of the plants continued their growth exceeding 60 nodes or leaves but the present examinations were terminated at the 50th node, since some plants did not survive beyond this stage. Data on the effect of γ-rays on the sex expression is presented in Table 1. The average number of nodes that developed female flowers decreased from 9.1 in the non irradiated control to 7.5 or 6.5 with 10 or 20 kR treatments. The total number of female flowers in the control was 221, but it decreased considerably after the radiation exposure. A reduction over than 50% of female flowers was noticed by a 20 kR treatment. We conclude that the γ-ray exposure to cucumber seeds alters the sex expression by reducing the number of female flowers. Though mechanisms involved are not clear, one possible explanation may be a change in the rate of auxin synthesis, since this hormone is reported to be radiosensitive (Brown, G. N. and F. G. Tayler Jr., Rad. Bot. 7: 67, 1967). Differential radiosensitivities of growth hormones could perhaps account for alterations in the normal balance of sex expression and development. Examination of hereditary alterations, if any, is now under way.

Table 1. Average number of female flowers developed in γ-ray irradiated cucumber

Dose of γ-rays	Node number					Average number of nodes developed female flowers	Total number of female flowers
(kR)	1–10	11–20	21–30	31–40	41–50		
0	1.2	3.5	2.7	3.8	5.5	9.1	221
10	1.6	1.3	1.6	3.0	3.8	7.5	145
20	0.6	0.6	1.2	1.7	2.2	6.5	101

Further Studies on the Fractionation Dose Effects with γ-rays in Maize Pollen

Taro FUJII

Recovery of premutational damage or decrease of mutation frequency in maize pollen grains by fractionation dose treatments with γ-rays has already been reported (Fujii, T., Japan. J. Genet. **46**: 243, 1971). To obtain further information about the extent of recovery, maize pollen grains having a *Bz* gene were irradiated with 2 or 3 kR γ-rays as well as with these doses splitted into two equal parts by administering them in two fractionations with 2 and 3 hours intervals. Subsequently, the irradiated pollen grains were used to pollinate the recessive stock. Mutation from *Bz* to *bz* can be observed in the F_1 seeds which have bronzy aleuron color.

A single dose of 2 or 3 kR showed a mutation frequency of 6.76 or 9.11% (Table 1). In the fractionation treatments, there was a decrease in mutation frequency but the frequency did not vary much between the two interval periods. The frequency of whole type mutations was markedly lower in the fractionation lots than in the single irradiation lots, while the frequency of partial type mutations did not show any significant difference between the single and fractionated treatments. Although the nature of mutational course or origin in these two mutant types are not clear, the present results confirm the earlier findings that up to 2 hours interval period there enhanced

Table 1. Mutation from *Bz* to *bz* by single and fractionated irradiations with γ-rays in maize

Treatment with γ-rays (kR)	Number of seeds	Mutation frequency (%)		
		Whole (a)	Partial (b)	(a)+(b)
Single treatment				
2	5534	4.37	2.39	6.76
3	5575	6.13	2.98	9.11
Fractionated (2 hours interval)				
1 +1	5937	2.53	1.58	4.11
1.5+1.5	4881	4.13	2.29	6.45
Fractionated (3 hours interval)				
1 +1	5612	2.51	2.07	4.58
1.5+1.5	5257	4.20	2.21	6.41
Not irradiated	7646	0.04	0.09	0.13

recovery, and no further recovery was observed beyond this interval. In the present material, the 2 hours interval period appears to bring about the maximum recovery and the rate in recovery of premutational damage induced by γ-rays seems to be approximately 30 per cent.

Modification of Radiation Damage in Rice

Sujit BAGCHI and Taro FUJII

Ten cultivated varieties of rice were subjected to 10–40 kR of γ-rays with a dose rate of 10 kR/hr from a ^{137}Cs source. Considering the germiability at 40 kR as a criteria for selection, four varieties were taken into account in the present experiments. Among these four varieties two (Nos. 451, 647) showed higher germiability and were presumed to be radiation resistant and the other two (Nos. 545, 564) showed considerably low germiabilities and assumed to be radiation sentitive. In the next part of these experiments, the four varieties were subjected to irradiation under different conditions, such as dry-seed irradiation, dry-seed irradiation combined with pre- or post-treatment of temperature at 60°C for 30 min. as well as wet-seed (24 hr soaked in water) irradiation. These experiments were conducted primarily to observe differential radio-tolerant capacities in the above four vatieties which were selected as radio-resistant and sensitive ones. Two quantitative characters such as germiability and seedling height were taken into account. It was found that the radiation tolerance capacity of germination is not always reflected by the seedling height, thus showing non-dependency with each other.

In the next part of these experiments all the four varieties were subjected to fractionated dose treatments both in dry as well as in wet-seed conditions, i.e., each treatmental dose was divided into two equals leaving a gap of time in between. In the dry-seed fractionated dose treatment, the time intervals were 24–96 hrs for applied doses of 10–50 kR. In the case of wet-seed treatments, the time intervals were 6–24 hrs for applied doses of 2.5–20 kR. It was found that, in the dry-seed treatment at 50 kR dose with 96 hr interval, the germiability and seedling height were very low when compared with the value at 50 kR dose with 48 hr intervals. This lag in the germiability and in the seedling height has been thought to be due to lack of metabolic-repair (Traut H., Proc. IAEA, 67–78, 1966). In the wet-seed treatment, the ger-

miability did not show any marked decrease except in one sub-treatment, however when the mean seedling height was considered, a pronounced decrement could be observed except in three sub-treatments. The detrimental effects of irradiation in seedling height of wet-seed treatments is presumed to be due to the damage occurred at the metabolic-pathway, thus hindering the metabolism proper. Though any definite conclusion could be drawn from the above experiments, this sort of damage was more pronounced in the treatment using wet-seeds than dry-seeds.

Copper Dependency of an Induced Dwarf Rice Mutant

H. K. SHAMA RAO, Taro FUJII and Tsuneo KADA

Seeds of eleven dwarf rice mutants and parent Norin-8 obtained from Dr. T. Kawai were cultured in the modified Murashige and Skoog's medium containing copper, cobalt and molybdenum or in the modified White's lacking them, in order to ascertain the physiological or biochemical differences, if any. Interestingly one of the mutants, MGS–95, was found to develop into normal seedlings in the modified Murashige and Skoog's medium but not in the White one. This observation made us to conceive the Murashige and Skoog's medium to contain an element (or elements) which may be a specific requirement for this mutant.

Since the modified White's medium lacked Cu, Co or Mo, it was supplemented individually with these minor inorganic elements for culturing MGS–95 and Norin-8. Observations made at 5, 10, 20 and 30 days after culturing revealed, in the presence of Cu, 100 per cent callus initiation around 10 days in the mutant, while the parent required 30 days to attain this stage. In the absence of Cu, 80% callus initiation occurred around 20 days in Norin-8 and only 25% in MGS–95. The presence of molybdenum did not have any effect on early callus initiation in both the mutant and parent. On the other hand about 50 to 60% calluses were observed around 10 days in the presence of cobalt.

Optimum Cu requirement in MGS–95 was ascertained by culturing in the modified white's medium supplemented with 0, 0.025, 0.05, 0.1 and 1.0 mg/l of Cu, respectively. Besides early callus initiation in the mutant, there was also a shift from shoot to root callus formation which was maximum (76%) at 0.05 mg/l Cu concentration. Still higher concentrations inhibited

root callus development.

The differences in the concentration of 2,4-D in the White's (20 mg/l) and the Murashige and Skoog's media (2.2 mg/l) does not appear to have influenced the nature and frequency of callus development. It was confirmed that even at 20 mg/l concentration of 2,4-D early callus initiation as well as the shift from shoot to root callus development appeared only in the presence of various Cu concnetrations in the supplemented White's medium.

These results have clearly demonstrated an induced differential response for copper requirement of a mutant. Since the strain MGS–95 was produced as a result of mutation, it is probable that a genetic change is exercising on the level of a protein to exhibit the copper dependent character involved in the regulation of callus formation. It is required in future to elucidate what type of gene expression is involved in the copper effect.

Radiation and Giberellin Effects in the Induced Dwarf Rice Mutants

H. K. SHAMA RAO and Tsuneo KADA

Seeds of eight dwarf rice mutants and parent Norin-8, kindly supplied by Dr. T. Kawai, National Institute of Agricultural Sciences, were used in this study. Of these 8 mutants, one was derived from γ-rays (MGS–28), 3 from X-rays (MGS–46, 50 & 95), 2 from pile neutrons (MGS–54 & 55) and 2 from ethylene imine (MGS–130 & 131) treatments.

Moisture stabilized (12.5%) dry seeds were exposed to 0, 9.5, 28.5 and 47.5 Krads of ^{137}Cs γ-rays and 0, 1.5, 2.6 and 4.4 krads of 14.1 MeV fast neutrons. There were 4 replications. The neutron doses administered were determined by the seed elements composition analysis and sulfur activation. Seedlings were raised under constant temperature ($\pm 28°C$) and continuous illumination (2800 lux) inside a phytotron. The extent of radiation damage was measured based on height recorded on 14 days old seeldings.

To study the GA_3 effect on the induced dwarf rice mutants and parent, well germinated seeds were first sterilized in equal volume of ethyl alcohol and 30% vol. H_2O_2 for 10 min., and subsequently transplanted aseptically on 0.5% agar media inside 500 ml. beakers. After the establishment of roots, 4 ml of 20 μg/l GA_3 was applied inside each beaker. Growth differentiation was recorded after 14 days growth of these seedlings raised under

constant temperature (±28°C) and continuous illumination inside the growth room.

MGS–46, 55 and 95 were radiosensitive with significantly pronounced effect at higher doses of both γ-rays and 14.1 MeV fast neutrons. For e.g. with 28.5 krads of γ-rays, the percentage seedling height were 38.3, 31.4 and 37.5 respectively compared to 58.1 in the parent Norin-8. With 2.6 krads of fast neutrons, the seedling height percentages were 65.3, 55.9, 43.9 and 84.6 for MGS–46, 55, 95 and Norin-8 respectively.

On the other hand in MGS–130 and 131, the seedling height percentages were significantly higher than in Norin-8, i.e. 63.1 and 61.4% with 28.5 krads of γ-rays and 87.2 and 88.2% with 2.6 krads of fast neutrons and thus revealed radioresistance. The other 3 mutants MGS–28, 50 and 54 were like the parent Norin-8. RBE's amongst the mutants and parent however varied only between 7.3 and 8.8. Interestingly only the mutants derived after ethylene imine treatment were radioresistant; of the 3 radiosensitive mutants, one had been induced with pile neutrons and the other 3 with X-rays.

Growth promotion with GA_3 was observed to a similar extent in the parent and the radiation induced mutants (125 to 150% over control) except for the absence of such an effect in MGS–95 (93.7% over control) which was found to be copper dependent for callus differentiation.

In MGS–130 and 131 the growth was rather inhibited i.e. 84.8 and 61.9% of control, respectively. Since these 2 mutants are now relatively less sensitive to radiations, a certain target site or function responsible for radiation sensitivity might have been modified as a result of mutation. Further, the absence of GA_3 effect may be the consequence of genetic alteration in the above presumed biological site or function. In the case of MGS–95, it may be interesting to examine in future if the GA-dependence of growth is also related to copper concentrations.

Biological Effects of Neutrons after Post-irradiation Storage or with Gamma-ray and Neutron Combination Treatments in Rice

H. K. SHAMA RAO

The present experiments were conducted to find out the influence of post-irradiation storage as well as pre/post gamma-ray and neutron combination

treatments on the biological effects in rice. Moisture stabilized dry seeds of commercial variety Norin-8 were used. The seedlings were raised under constant temperature condition. Seedling heights were recorded 14 days after sowing and the extent of radiation damage was measured in relation to percentage seedling heights over respective controls.

In the storage experiment, the 14.1 MeV fast neutron doses administered were 1.5 and 2.6 krads and the ^{137}Cs gamma-ray doses were 19.0 and 38.0 krads, respectively. Post-irradiation storage treatment consisted of storing for 10, 20 and 30 days at 30°C. It may be noted that enhanced radiation damage occurred up to 20 days storage with some recovery at 30 days, in both radiation treatments. For, e.g., with 2.6 krads of neutrons, the seedling height percentages were around 94, 84 and 89% at 10, 20 and 30 days storage periods, respectively. The damage was much greater with 38 krads gamma-rays, the seedling height percentages being around 67, 47 and 69% for similar periods of storage, respectively. At 20 days storage where the damage was maximum, it was nearly 50% more with gamma-rays compared to neutrons. Even the fast neutrons were found to enhance the radiation damage by nearly 16% with 20 days post-irradiation storage as compared to about 50% damage with gamma-rays.

In the pre/post neutron and gamma-ray combination experiment, the doses administered were 0.7 and 9.5 krads of neutrons, and 9.5 and 19.0 krads of gamma-rays. Although both the treatments showed dose dependence for seedling heights reduction, the extent of radiation damage differed in the combination procedures. For e.g., neutrons when administered immediately after the gamma-ray treatment (9.5 krad +1.3 krad) revealed seedling height percentage to be 64%, whereas for a similar total dose when gamma-rays were used as a post-treatment the seedling height was around 79% of control. Similar trends were observed even with lower doses of neutrons (0.7 krad), the gamma-ray dose being constant (9.5 krad). The seedling height recovery as a result to post-irradiation gamma-ray treatment could be explained on the repair processes known to occur with such low LET treatments. On the other hand, the densely ionizing fast neutrons when administered as a post-treatment after gamma-rays could block the recovery processes. The extent of recovery could therefore be much less than when gamma-rays are used as a post treatment.

Genetic Fine Structure Analysis of *wx* Locus in Maize

Etsuo AMANO

Since the *wx* character of starch is expressed in pollen grains, infrequent intracistronic recombination are detectable relatively easily for a higher plant. When independently induced *wx* mutants were crossed with standard *wx* testers and if any non-*wx* (*Wx*) recombinant pollen were produced, they could be scored as blue black grain among light-brown colored pollen of parental character by appropriate iodine staining. The frequency of blue black grains might reflect the map distance between the tester *wx* site and the tested *wx*. Preliminary mapping data have been reported (Ann. Rep. Nat. Inst. Genetics, **20**: 85, 1969).

Further experiments using other *wx* testers such as wx^{R} (presumably intracistronic deletion) indicated different distribution of the *wx* sites from the detailed work of Nelson (Genetics **60**: 507, 1968). According to orthodox linkage experiments, he mapped marker loci and *wx* sites as bz–wx^{H21}–wx^{C}–wx^{90}–v. However, his pollen analysis data themselves suggested a different order which would also give a good interpretation for the present experiments. Using the same standard *wx* stocks to determine the EMS induced *wx* sites, a preliminary map which was constructed refering to two *wx* stocks, wx^{C} and wx^{H21} required only a little modification in the present experiments. If the wx^{H21} site locates between wx^{C} and wx^{90} as suggested in the present experiments and Nelson's pollen data, the distribution of the *wx* mutant sites will be relatively even, i.e. two mutant sites locating outside wx^{C}, three between wx^{C} and wx^{H21}, seven between wx^{H21} and wx^{90}, and eight outside wx^{90}. If the wx^{H21} must be placed outside wx^{C}–wx^{90} region, distribution of *wx* mutant sites may become very uneven, since most of the mutants induced by EMS should be placed outside wx^{H21}. The order of the standard *wx* stocks should be determined by orthodox linkage test with larger population and compared with the pollen data, while further diallele cross experiments with more *wx* mutants are required.

Mixed Pollination for Obtaining Inbred Mutant Line in Maize

Etsuo AMANO

In maize, a number of conveninent marker stocks have been available

for mutation studies. Mutagen treated dominant homozygous plants can be pollinated with an endosperm marker tester (*C* sh_1 *bz wx*, *y*) to detect recessive mutations in these loci on the cob. However, since the induced mutant genes could only be detected as heteroallelic hybrids with the tester marker, there are some difficulties in obtaining established mutant lines. Genetic back-ground may interfere with the results of analytical studies such as genetic fine structure mapping in *wx* locus, and progenies of hybrids in maize often show unstable growth. Moreover, identification of segregated newly induced mutant genes would be possible only for special cases like leaky *wx* mutants, or after laborious tests of recombination with the tester. To avoid these difficulties, pollination of the mutagen-treated plant was made with mixed pollen of untreated parental line and the tester line. Pollen of the two lines were collected from tassels cut in the field on a previous day and kept separately in vases. Roughly equal volumes of pollen of a stock 6311R (untreated C^{I} Sh_1 *Bz Wx*, *Y*) and a stock 639 (*C* sh_1 *bz wx*, *y*) were mixed in a Petri dish. Pollinations were made using small brushes. Test pollination to 639 resulted in good fertility and expected segregation ratio, suggesting absence of disturbance like certation. When the mixed pollen were pollinated to 6311R, inbred kernels could be separated from hybrid ones. This classification can be tested further, since the latter will show hybrid vigor when planted in the field. If a mutant could be detected after seed treatment with a mutagen on a cob as hybrid kernels, presumably half of the 6311R kernels on the same cob may carry a newly induced mutant gene. Inbred homozygous mutant will be segregated in the next generation from some of the inbred kernels. A few mutant cobs have been obtained in this way by preliminary experiments.

Some Experiments with Cultured Haplopappus Cell Lines

Etsuo AMANO

Five callus lines of *Haplopappus gracilis* ($2n=4$) have been induced and maintained in our laboratory since 1971. Origins and morphological characteristics were described previously (Ann. Rep. Nat. Inst. Genetics **22**: 73, 1972). These lines have been kept on agar slant containing inorganic salts (Eriksson, T, Physiol. Plant. **18**: 976, 1965), vitamines, yeast extract (0.3%), 2.4 D (2 ppm) agar (0.8%) and saccharose (2%). Among

the lines, HL (induced from leaf), and HNC (spontaneous callus appeared on the stem surface) seemed to possess better characters for suspension culture than those of HSe (from seed), HR (from root) or HS (from stem). Liquid suspension culture was made in small Erlenmeyer flasks kept on a rotating stand (2 or 6 rpm). Component of the liquid medium used was the same as that of slant medium shown above except agar.

At 28°C, the fresh weight of HL cells in 20 ml of liquid medium increased linearly for a week and resulted in ten-fold increase of weight. The cell volume, measured either by natural sedimentation for 60 min. or by centrifugation for 1 min. at 2000 rpm, continued to increase linearly for 20 days. The results were comparable except that the centrifugation method gave packed cell volumes which were almost half of those obtained by the sedimentation method. The linear growth in initial phase might suggest a low mitotic activity in some of daughter cells. Rough estimate of the mitotic cell cycle was about 35 hours according to the increase of fresh weight.

The chromosome number of the five cell lines was studied on samples kept for more than one year on agar slant. The cell lines were cultured in liquid suspension of 20 ml for a week. After 20 hours of cold treatment, cells were collected by centrifugation (2000 rpm, 1 min.) and fixed in 1:3 acetic alcohol fixative. The chromosome number was determined with acetocarmine squash preparations. Among the five cell lines, HS contained tetraploid cells (10%) and an aneuploid cell (one with three chromosomes in 64 metaphases). One cell in the 88 HR cells examined had only two chromosomes. Other cells and three cell lines (HL, HNC and HSe) showed either normal four metaphase chromosomes or eight anaphase chromosomes. To test effect of the suspension culture, small samples were taken from fresh slant cultures and stained by acetocarmine stain without pretreatments. Only a small number of metaphases could be examined in this way, but the chromosome number was the same as that of the suspension culture.

By a simple sedimentation procedure, a fine cell suspension, free of large cell aggregates, could be obtained from a cell line, HNC. This cell sample could incorporate ^{3}H-TTP into acid-insoluble fraction when incubated for two hours with appropriate precursors and energy sources. Addition of 5% Brij-58 which can increase permeability in yeast cell, was not successful to promote ^{3}H-TTP incorporation in the present material.

VIII. POPULATION GENETICS (THEORETICAL)

Population Genetics, Molecular Biometry, and Evolution

Motoo KIMURA and Tomoko OHTA

Amino acid substitutions in protein evolution and protein polymorphisms in natural populations are not separate phenomena; they represent two aspects of a single phenomenon, i.e., the spreading of neutral or nearly neutral mutations, caused by random genetic drift.

The behavior of the neutral mutants within a finite population was analysed mathematically. In particular, the constancy of the evolutionary rate was explained in terms of constant occurrence of neutral mutations.

The most rapidly evolving molecules known so far are the fibrinopeptides A and B. It has been found that the middle segment of the proinsulin molecule shows nearly the same evolutionary rate as fibrinopeptides A and B. Since these molecules have no known function after they are removed from the rest of the molecules, so selective constraint must be minute for them, and hence, almost all mutations (amino acid replacements) are likely to be selectively neutral. Thus, the rate of amino acid substitutions in evolution is approximately equal to the mutation rate for them.

From the standpoint of the neutral mutation-random drift theory, the average amino acid composition of proteins represents the equilibrium condition under mutational substitutions of nucleotides, as can be predicted from the code table. This prediction is in fact turned out to be in good agreement with the actual observations. For details, see Proc. 6th Berkeley Symp. of Mathematical Statistics and Probability Vol. **5**: 43–68.

The Effective Number of a Population with Overlapping Generations: a Correction and Further Discussion

James F. CROW and Motoo KIMURA

An approximate formula is proposed for the effective number, N_e, of a population such as the human population where generations overlap and reproduction occurs at various ages. When y is the age (e.g., in years), the variance-effective number is given by

$$N_{ev} = \bar{l} \int_0^\infty N_y v_y dy = \bar{l} V,$$

where N_y is the number of age y and v_y is Fisher's reproductive value at age y; V is the total reproductive value of the population, and $\bar{l}$ is the weighted mean probability of surviving into the reproductive period,

$$\bar{l} = \int_0^\infty l_y^2 b_y e^{-my}\, dy\,.$$

The formula can also be written

$$N_{ev} = N_0 \bar{l} \tau\,,$$

where τ is the mean age of reproduction. These are also a good approximation to the inbreeding-effective number.

These formulas all assume that the population has attained a stable age ratio and that the age-specific birth and mortality rates are constant. Modifications when these conditions are not met are discussed. We also retract an earlier, incorrect formula. For details, see Amer. J. Hum. Genet. **24**: 1–10.

Fixation Probability of a Mutant Influenced by Random fluctuation of Selection Intensity

Tomoko OHTA

The probability of fixation of a mutant gene in a finite population was investigated by taking into account the effect of random fluctuation of selection intensity. It was assumed that the change of gene frequency per generation by selection is given by $sx(1 - x)$ where s is the selective advantage and x is the gene frequency. It was shown that not only the product of the effective population number and average selection coefficient ($N_e\bar{s}$) is important, but also the ratio of the mean and the variance of selection coefficient ($\bar{s}/V_s$) has an important influence on fixation probability. In particular, when this ratio is small, a mutant gene, even if selected against, becomes fixed in the population like a selectively neutral mutant. In general, when random fluctuation of selection intensity is not negligible it may be convenient to use 'effective selection coefficient'. The bearing of the present findings on the neutral mutation-random drift theory of evolution and variation at the molecular level was discussed. For details, see Genetical Research **19**: 33–38.

Fixation Time of Overdominant Alleles Influenced by Random Fluctuation of Selection Intensity

Tomoko OHTA and Motoo KIMURA

It was demonstrated that the number of generations until fixation or loss of an overdominant alleles is influenced by random fluctuation of selection coefficients. When $2\bar{s} < V_s$, where $\bar{s}$ is the mean selection coefficient against either homozygote and V_s is the between-generation variance of the selection coefficient, overdominance generally accelerates rather than retards fixation of segregating alleles. This finding should have important bearing on our consideration of the behaviour of polymorphic variants which are nearly neutral but have very slight overdominance. When the population size (N_e) is extremely large, not only $N_e\bar{s}$ but also $\bar{s}/V_s$ have to be considered in discussing the effectiveness of overdominance. For details, see Genetical Research **20**: 1–7.

Simulation Experiments of Multilocus System

Tsuneyuki YAMAZAKI and Motoo KIMURA

In order to obtain information on what type of chromosomes are established by selection when many loci are tightly linked, we performed simulation experiments under the following assumptions: (1) Population size—100 diploid individuals, (2) The number of linked loci on a chromosome—88, (3) Recombination value between adjacent loci—0.002 without interference, and, (4) Individual fitness—determined by the multiplication of fitness values among loci.

The results are summarized as follows: 1) When there are two overdominant alleles at each locus and the selection coefficient against either homozygote is 0.1, almost complete linkage disequilibrium is established among segregating loci after approximately 200 generations. Then, the population consists of two complementary chromosome types, and fixation (or loss) of alleles seldom takes place. 2) When more than two alleles exist at each locus with 10% selection coefficient (against homozygotes) complementary chromosome types are established similar to the two allelic case. The maximum number of complementary chromosomes is equal to the number of alleles per locus. 3) When the selection coefficient (s) of

overdominant loci is small, the rate of fixation or loss of alleles is slower with linkage than without linkage. However, when selection coefficient is larger than a certain value, linkage tends to accelerate fixation rather than retard it. Moreover, the most efficient selection coefficient to maintain polymorphisms in a population is neither a large s nor a small s, but the optimum is an intermediate value of s, though it is a function of recombination fraction. 4) When frequency-dependent selection of minority-advantage type is operating, two complementary chromosome types are also established by selection; namely, one with majority alleles in most loci, and the other with minority alleles.

IX. POPULATION GENETICS (EXPERIMENTAL)

Spectra of Heterozygosities and of Evolutionary Rates among Different Loci

Takeo MARUYAMA and Tsuneyuki YAMAZAKI

Kimura and Ohta suggest that protein polymorphism and protein evolution should be regarded not as separate phenomena, but as two aspects of a single process. They further argue that most of naturally occurring polymorphisms are due to random frequency drift of selectively neutral mutants in finite populations, (Kimura and Ohta, 1971, Nature **229**: 467). On the other hand, Kimura, King and Jukes have argued that most of molecular evolutionary changes are due to fixation in species of the neutral mutants, (Kimura, 1968, Nature **217**: 624; King and Jukes, 1969, Science **164**: 788).

Under the hypotheses of neutrality, Kimura and Crow showed that the "effective number" of allele at a locus is $1 + 4Nu$, where N is the population size and u is mutation rate per gene per generation, and Kimura proved that the evolutionary rate of amino acid substitution is exactly equal to mutation rate per site per year and is independent of other factors. Both of these quantities are observable and are known to vary greatly among loci. A considerable amount of data for heterozygosity is available in published literatures, and Dayhoff's *Atlas of Protein Sequence and Structure* provides the evolutionary rates for a number of proteins. Since both of these quantities are proportional to mutation rate, if they are really two different aspects of a single process, their spectra ought to be similar to each other. Namely, the distributions of the values of $4Nu$ and of the evolutionary substitution rates should have an identical pattern. The purposes of this note are to obtain the spectra from available data and to examine whether or not they appears to be identical.

The spectrum of the values of $4Nu$ among different loci is constructed from data of the *Drosophila* species. From the literatures were collected gene frequency data of some 140 loci and the value of $4Nu$ was calculated for each locus by first computing the reciprocal of the homozygote probability and then substructing one from it. The whole range of the values were divided into ten classes of equal interval. The distribution of the values with

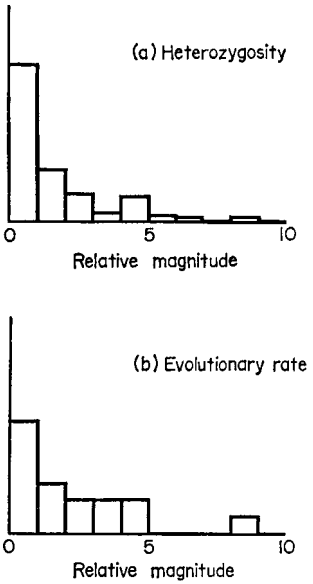

Fig. 1. The spectra of the evolutionary rates (nv) and of the heterozygosities ($4Nu$) among different loci.

respect to the classes is the spectrum and is presented in Fig. 1 (a). On the other hand, the amino acid substitution rate, which is denoted by v in this note, given in Dayhoff's *Atlas* (page 50) was multiplied by the number (n) of amino acid residues of that protein. This value (nv) should be the mutation rate per locus per year because of Kimura's principle that the evolutionary rate is equal to mutation rate. Since the rates in Dayhoff's *Atlas* were calculated from mammalian data and mammals have approximately same length of one generation time, the value of nv should be proportional to mutation rate per gene per generation which is therefore comparable to the value of $4\,Nu$. The values of nv among different proteins were also divided into ten classes of equal interval and the distribution is presented in Fig. 1 (b). The two spectra presented in the Figure appear to be quite similar, and therefore the result is consistent with the hypothesis of Kimura and Ohta and with the neutral hypothesis of molecular evolution.

Evidence that Enzyme Polymorphisms are Selectively Neutral

Tsuneyuki YAMAZAKI and Takeo MARUYAMA

In a recent paper, Johnson, G. B. (Nature New Biol. **237**: 170, 1972) has examined published data with special reference to the neutral hypothesis of protein polymorphisms and concluded that they appear to be contrary to the following theoretical expectation based on the neutral hypothesis. Here we denote by n_e the inverse of the homozygosity (the effective number

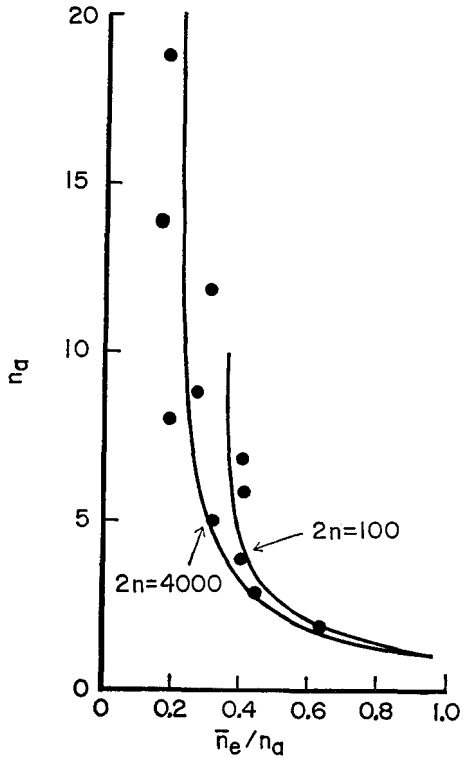

Fig. 1. The relationship of the actual to the effective number of alleles in the combined data, (all the data based on a sample of size greater than 100 genes are included). The curves indicate the theoretical expectations of the relationship. Each dot indicates the average coordinate of the analysed data which belong to a given value of n_a. The number of protein loci used for this figure is 255.

of alleles) and by n_a the actual number of alleles. In these notations, Johnson's prediction is that, if all the alleles at loci in question are selectively neutral, the ratio, n_e/n_a, should decrease as n_a increases. He has presented three different sets of data, all showing the opposite to the above prediction, and has argued that the finding is against the truth of the neutral hypothesis.

In this note, we have extended the same examination as Johnson to more data including a number of different organisms and also provided the exact theoretical expectation of ratio $\bar{n}_e/\bar{n}_a$ as a function of $\bar{n}_e$ and sample size (Fig. 1). (A bar indicates the expectation of the quantity under consideration.) On the contrary to Johnson's claim, we find that the observed data are in good accord with the theoretical expectations, while our theoretical expectation turned out to be the same as his prediction.

Since we could provide the precise theoretical expectation as a function of $\bar{n}_a$ and the sample size, we have included all the alleles reported in the literatures used for the analysis, while Johnson had excluded those whose frequency is less than 0.01. It is possible that the exclusion of less frequent alleles had lead Johnson to the false conclusion. It is certain that quantitatively more precise theoretical expectation, than Johnson's qualitative prediction merely on the direction of the slope, had been necessary to draw any conclusion from the kind of analysis attempted by him. The present paper provides both the exact expectation and the analysis based on more data of some two hundred fifty loci. The latter is particularly important, for the controversy is not concerned with a set of any selected loci, but it is concerned with average fate of all loci.

From Fig. 1 it is clear that the data is consistent with the theoretical expectation based on the neutral hypothesis. We therefore present this result as evidence for the truth of the neutral theory.

Further Evidence for the Neutral Hypothesis of Protein Polymorphism

Tsuneyuki YAMAZAKI and Takeo MARUYAMA

In our previous papers, published polymorphism data were used in conjunction with an invariant principle to conclude that the available data are consistent with the "neutral theory" of Kimura and Ohta, (T. Yamazaki and T. Maruyama, Science **178**: 56–58 and this Report No. 22: 86–87.)

The principle is that when we assess the amount of heterozygosity, given that the gene frequency is specified, the total amount is independent of the population structure. The data then available were some 400 proteins. Since then more data were published and therefore we made the same survey

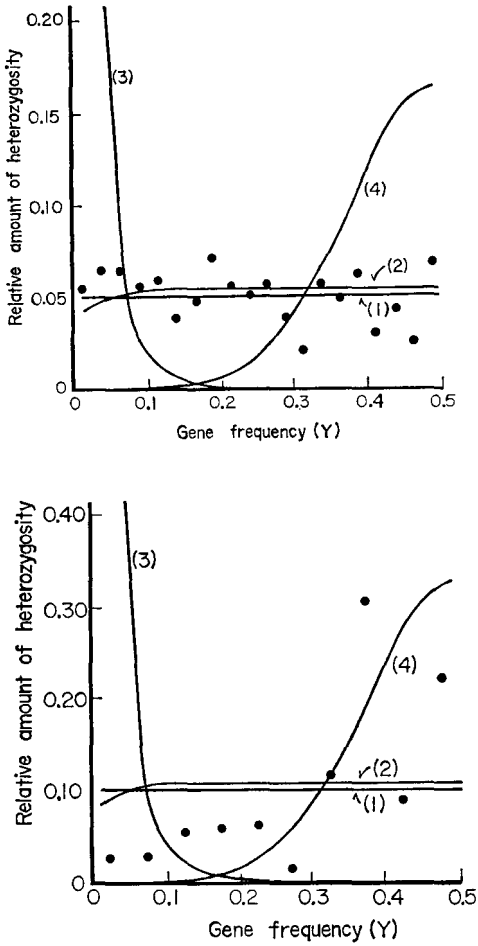

Fig. 1 and 2. Distribution patterns of heterozygosity. The curves indicate the theoretical expectations: (1) neutral; (2) advantageous ($Ns=10$); (3) deleterious ($Ns=-10$); (4) Overdominance ($Ns=10$).

of all available data which amount to 1045 proteins. The present result turned out to confirm the previous ones, and therefore our claim was greatly reinforced. The result is presented in Fig. 1.

We have applied the same analysis to polymorphism data of human blood groups alone. Although the amount of available data is undoubtedly insufficient to draw any strong conclusion, the result indicates that the distribution of heterozygosity deviates from that of neutrality. This is probably due to some sort of balance selection. The result is presented in Fig. 2.

Allelism between Lethal Genes in Japanese and Korean Natural Populations of *Drosophila melanogaster*

Chozo OSHIMA, Takao K. WATANABE and Jong-Kil CHOO[1)]

Many flies of *D. melanogaster* were collected simultaneously in September 1971 at two sites of Anyang, the suburbs of Seoul, Korea and in November at Katsunuma and Shikishima in Yamanashi prefecture, Japan. The frequency of lethal second chromosomes extracted from each male fly was reported in the previous Ann. Rep. No. 22: 90.

The frequencies of allelism between lethal genes concealed in the same population and these different populations were determined by half and full diallel crosses. The results were shown in Table 1.

Although the distances between two sites of Anyang and between two sites of Yamanashi were about 8 km and 20 km respectively, no remarkable difference was found between frequencies of allelism within and between populations. The map distance between Anyang and Yamanashi was measured to be about 1000 km, but the frequency of allelism between lethal genes in both populations was higher than that expected between genetically unrelated populations.

Among many individual lethal genes, 8 lethal genes (a — h), locating at different loci, were confirmed to appear on several chromosomes as shown in Table 2.

Seven lethal genes, except h, were found to be located with another lethal genes on a chromosome and on the other hand, seven lethal genes, except c, were found to be concealed in both Japanese and Korean populations

[1)] Visiting researcher from Department of Biology, Chung-Ang University, Seoul, Korea.

Table 1. Results of allelism between lethal genes

Population	Within population: Number of tested chrom.	Number of crosses	Number of allelic crosses	Frequency of allelism (%)	Between populations: Number of tested chrom.	Number of crosses	Number of allelic crosses	Frequency of allelism (%)
Katsunuma	40	780	12	1.54	40	1600	23	1.44
Shikishima	40	780	11	1.41	40			

Population	Between populations: Number of tested chrom.	Number of crosses	Number of allelic crosses	Frequency of allelism (%)	Population	Between populations: Number of tested chrom.	Number of crosses	Number of allelic crosses	Frequency of allelism (%)
Katsunuma	40	760	3	0.39	Katsunuma	40	960	11	1.15
Anyang 1	19				Anyang 2	24			
Shikishima	40	760	0	0.00	Shikishima	40	960	4	0.42
Anyang 1	19				Anyang 2	24			
Total Japan	80	3440	18	0.52					
Korea	43								

Table 2. Allelismic relationship between lethal genes found commonly in Japanese and Korean populations

Chromosome Symbol	Population	Lethal gene (tentative symbol)							
040 A*	Katsunuma	a	b						
040 B	Katsunuma	a							
013	Anyang 1	a							
024	Anyang 2		b						
061 *	Katsunuma		b		d				
091	Shikishima		b						
045 A*	Katsunuma			c	d				
045 B	Katsunuma			c					
005 A*	Anyang 2				d	e			
005 B	Anyang 2				d				
043 A, B	Katsunuma					e			
071 A, B, C	Shikishima					e			
063 *	Katsunuma						f	g	
023	Anyang 2						f		
035	Anyang 2							g	
037	Anyang 2								h
055	Katsunuma								h

*; double lethal chromosome.

respectively. Such lethal genes would be persisted for a long time in these natural population, but a migration of insect by a seasonal wind from Korea to Japan is unknown.

Allelism between Sterility Genes in Japanese and Korean Natural Populations of *D. melanogaster*

Chozo OSHIMA, Takao K. WATANABE and Jong-Kil CHOO

The frequency of sterility second chromosomes among semilethal and quasinormal chromosomes extracted from Katsunuma, Shikishima and Anyang 1, 2 natural populations was reported in the previous Ann. Rep. No. 22: 91.

The frequencies of allelism between sterility genes concealed in these populations were determined by the fertility test crosses of hybrid flies between different sterility lines. The results are shown in Table 3.

Table 3. Results of allelism between sterility genes

	Population	Within population: Number of tested chrom.	crosses	allelic crosses	Frequency of allelism (%)	Between populations: Number of tested chrom.	crosses	allelic crosses	Frequency of allelism (%)
♀	Katsunuma	17	136	1	0.74	17	204	3	1.47
	Shikishima	12	66	1	1.52	12			
♂	Katsunuma	32	496	15	3.02	32	736	40	5.43
	Shikishima	23	253	32	12.65	23			
♀	Anyang 1	14	91	16	17.58	14	140	10	7.14
	Anyang 2	10	45	4	8.89	10	140	10	7.14
♂	Anyang 1	6	15	0	0.00	6	48	0	0.00
	Anyang 2	8	28	1	3.57	8			

	Population	Between populations: Number of tested chrom.	crosses	allelic crosses	Frequency of allelism (%)		Population	Between populations: Number of tested chrom.	crosses	allelic crosses	Frequency of allelism (%)
♀	Katsunuma	17	238	6	2.52		Katsunuma	17	170	0	0.00
	Anyang 1	14					Anyang 2	10			
♀	Shikishima	12	168	0	0.00		Shikishima	12	120	0	0.00
	Anyang 1	14					Anyang 2	10			
♂	Katsunuma	29	174	0	0.00		Katsunuma	29	232	0	0.00
	Anyang 1	6					Anyang 2	8			
♂	Shikishima	22	132	0	0.00		Shikishima	22	176	0	0.00
	Anyang 1	6					Anyang 2	8			
♀	Total Japan	29	696	6	0.86	♂	Total Japan	51	714	0	0.00
	Korea	24					Korea	14			

It was confirmed by the allelism test that three female sterility genes locating at different loci respectively, existed in both Japanese and Korean populations. One of them, named *fs* 302, was found in one chromosome extracted from Katsunuma population, and it appeared in 4 chromosomes among 14 chromosomes extracted from Anyang 1 population. This fact could be hardly considered only by the occurrence of common mutation at each locus.

Effects of Density of Larvae and Temperature on a Factor of Fitness in *Drosophila melanogaster*

Seido OHNISHI and Chozo OSHIMA

Fitness of organisms is greatly influenced by environmental factors such as temperature, nutritions and population density. Developmental time from egg to adult, one of the component of fitness, was examined under several different temperatures and densities. The fertilized eggs of Oregon-R strain were collected for two hours and placed on the medium in small vials (20×90 mm) and thereafter they were kept under constant (25°C) and fluctuating (20–30°C, one cycle a day) temperatures respectively. Three kinds of densities (20 60 and 180 eggs per vial) and five replications for each were adopted simultaneously under both temperature conditions. All flies emerged were counted every eight hours and then the developmental time and the viability (ratio of numbers of adult to egg) in every vials were obtained as shown in Table 4.

The developmental time was prolonged significantly by the density in-

Table 4. Mean developmental time (hours from egg to adult) and viability (ratio of number dault to egg) in three densities under constant (25°C) and fluctuating (20～30°C) temperatures

Density*	Developmental time		Viability	
	const.	fluct.	const.	fluct.
20	201.25±0.40	217.17±1.09	0.890±0.037	0.930±0.034
60	203.99±1.08	218.70±0.54	0.923±0.026	0.817±0.037
180	210.71±0.73	224.52±1.50	0.823±0.018	0.777±0.010

* Number of eggs per vial.

creased under both temperature conditions and also more prolonged by the fluctuating temperature.

On the other hand, the viability decreased proportionately with the density increased under the fluctuating temperature, while such a result could not be found under the constant temperature. From the results, an optimum density was presumed to be about 60 eggs per vial. It is evident that the density of larvae seems to control the viability, and its pattern is influenced by the condition of temperature. The coefficient of correlation between the developmental time and the viability was estimated to be -0.5797 for the constant and -0.6335 for the fluctuating temperature.

X. EVOLUTIONARY GENETICS

Population Size and Rate of Evolution

Tomoko OHTA

It is suggested that in evolution there is much substitution of nearly neutral mutations, for which the selection intensity varies from time to time or from region to region. Since the variance among the selection coefficients of new mutants decreases when the environment becomes uniform, the probability of a mutant being advantageous to the species as a whole increases in more uniform environment. Therefore the rate of gene substitution increases in smaller populations, as smaller populations are likely to be distributed over less varied environments.

The adequacy of the model was discussed in relation with the following facts or plausible postulates. 1. A large number of amino acid substitutions during a period corresponding to the formation of new species. 2. Rapid evolution at the phenotypic level of populations having a small size. 3. Many extinctions and expansions of the species in the past. For details, see Jour. Molecular Evolution **1**: 305–314.

Evolutionary Rate of Cistrons and DNA Divergence

Tomoko OHTA

The ratio of DNA divergence to cistron divergence was estimated for various comparisons of Drosophila and mammalian species. It was shown that a negative correlation exists between this ratio and the length of one generation. It was proposed that the majority of the amino acid substitutions in evolution are the result of random fixation of nearly neutral mutations for which selection intensity fluctuates considerably but the average selection coefficient is very slightly negative. On the other hand, the evolutionary rate of DNA divergence is negatively correlated with the generation time if the majority of mutations are completely neutral and if mutation rate at the nucleotide level is mildly dependent on the generation time. For details, see Jour. Molecular Evolution **1**: 150–157.

On the Stochastic Model for Estimation of Mutational Distance between Homologous Proteins

Motoo KIMURA and Tomoko OHTA

A set of simple equations is derived which gives the relationship between the observed fraction of amino acid differences per codon and the "evolutionary distance" per codon. Holmquist's stochastic model is used in which the mutation is assumed to occur at random among the four bases (A, T, G, C) in the variable part of the molecule. Let P_d be the observed fraction of amino acid differences per codon and let D_E be the evolutionary distance (in terms of the number of nucleotide substitutions) per codon. Then,

$$P_d = 1 - (1 - \lambda)^2 (1 - \lambda/4)$$

$$D_E = -(9/4) \log_e (1 - 4\lambda/3)$$

where λ represents the fraction of nucleotide sites for which the two cistrons differ from each other ($0 \leq \lambda \leq 3/4$). For details, see Jour. Molecular Evolution 2, 87–90.

XI. HUMAN GENETICS

Effect of Changing Parental Age Patterns on Chromosomal Aberrations and Mutations

Ei MATSUNAGA

Among a number of possible genetic consequences of family planning (Matsunaga, E. (1966) JAMA **198**: 533), the effect of changing parental age patterns upon recurring mutations, both chromosomal and genic, is the one which we can appreciate as of obvious "eugenic" relevance. Here, the argument is based on deduction from two empirical facts. First, demographic data in most industrialized countries show that there has been over several decades, as a result of family planning practiced by more and more people, a gradual concentration of childbearing at younger ages than in former times. Second, there are strong, positive associations of parental ages with the production of certain chromosomal aberrations and gene mutations. Whatever biological mechanisms are involved in such associations, the genetic consequence of younger parenthood is beneficial because it lessens the additional genetic burden that would have arisen if the parents had borne children at advancing ages.

Using demographic data in Japan, it was shown that the incidence of certain trisomies (21-trisomy, XXY and XXX, 13-trisomy and 18-trisomy) must have decreased by 30–40% during the past 20 years since 1948, simply because of the changing parental age patterns caused by family planning. In addition, it was deduced that certain dominant conditions (chondrodystrophy, Apert's syndrome and Marfan's syndrome) must have decreased by about 20% during the same period. Since they include relatively common disorders like Down's syndrome, a reduction in incidence by this amount through family planning would represent a major saving in medical care and in human suffering. (For details, see *Social Biology* **20**: 82, 1973).

Genetic Effect of Selective Abortion Upon the t(21q Dq) Translocation

Ei MATSUNAGA

Among a variety of chromosomal aberrations, a balanced heterozygote carrying the t(21qDq) translocation is probably the most important indication for prenatal diagnosis. The incidence at birth of carriers of this type of translocation has been estimated as $3/21{,}996 = 1.4 \times 10^{-4}$ (U. N. (1972) Ionizing Radiation II), and the risk of having a child with Down's syndrome is 10% for female carriers while it is reduced to about 2% for male carriers (Hamerton, J. L. (1968) Cytogenet. **7**: 260). If prenatal diagnosis followed by selective abortion of unbalanced heterozygotes could be performed in every carrier parent after one affected child is born, the reduction in the incidence at birth of inherited cases of Down's syndrome would be only a few per cent. However, if carriers could be diagnosed by premarital counseling with reference to his or her relatives being affected with translocation Down's syndrome, the reduction would be much greater. On the other hand, if reproductive compensation took place, the prevalence of carriers would increase. This point may be outlined as follows.

Assume that premarital diagnosis of all carriers could be made in order to monitor each of the pregnancies, and full reproductive compensation by the births of two phenotypically normal children were introduced. Then the prevalence of carriers in the nth generation, y_n, would be such that

$$y_n = (1/2 + k)\, y_{n-1} + 2\, m,$$

where k is the relative proportion of balanced heterozygotes among phenotypically normal progeny of male carriers, and m is the mutation rate giving rise to a balanced heterozygote of the t(21qDq) translocation. While the value of m has been estimated as $0.6\text{–}0.7 \times 10^{-5}$ (Polani, P. E. *et al.* (1965) Cytoget. **4**: 193; Kikuchi, Y. *et al.* (1969) Jap. J. Human Genet. **14**: 93), it is still an issue whether or not k is significantly greater than 0.5 (Hamerton, J. L. ibid.; Dutrillaux, B. and Lejeune, J. (1969) Ann. Genet. **12**: 77; Jacobs, P. A. *et al.* (1970) Ann. Human Genet. **34**: 119). With k equal to 1/2, the prevalence of carriers would increase from the present level of 1/7,000 to 1/5,000 in four generations, and to 1/4,000 in eight generations. However, if k is 0.6, as is suggested by the family data given by Hamerton,

the prevalence would increase to 1/3,700 in four generations, and to 1/2,200 in eight generations. It is to be noted that, if carriers were diagnosed only retrospectively, then the rate of increase in their prevalence would become slightly slower.

Needless to say, if, based on prenatal diagnosis, not only fetuses that are unbalanced heterozygotes, but also those that are balanced heterozygotes, could be aborted, the eugenic effect would be as great as in the case of dominant conditions. This raises of course an ethical issue. The above calculation implies that, even if we go without sacrificing fetuses that are balanced heterozygotes, the extent of the possible consequent dysgenic effect need not be of serious concern, at least within several generations.

Evidence for a Second Structural Locus Determining Tetrazolium Oxidase in Man

Tomotaka SHINODA, Ei MATSUNAGA and Jushiro KOSHINAGA*

Upon electrophoresis of tissue extracts in starch gels and stained for tetrazolium oxidase, two different zones of the activity are demonstrated, one of these corresponds to that of hemolyzates and the other to a distinct form absent in hemolyzates. The former, having a greater mobility, is provisionally referred to as soluble or cytoplasmic form (*s*-TOX), and the latter as mitochondrial or bound form (*m*-TOX). The both forms of the enzyme appear to have common substrate specificities and physicochemical characteristics, as far as the data obtained from our preliminary experiments are concered.

An inherited variation in tetrazolium oxidase has recently been reported by Shinoda (Jap. J. Hum. Gent., **15**: 144–152) for a Japanese family which was followed up to three generations. The variant has been referred to as TOX 2–1, heterozygous for TOX^1 and TOX^2 alleles at TOX locus. We now suggest an existence of a second structural locus determining this enzyme in man, based on the results of isozyme typing for various organs obtained by authopsy.

During the course of the survey for multiple molecular forms of enzymes in man, we encountered a rare case in which the enzyme in *m*-TOX fraction exhibited an altered electrophoretic pattern. The electrophoretic pattern

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of *s*-TOX fraction of this subject was found to be identical with that of the other individuals. This unusual pattern was constant in starch gel electrophoresis of extracts obtained from various organ, such as skeletal muscle, lung, heart, brain, pancreas, spleen, and kidney. The specimens taken from the individual were analyzed for other enzymes of over 20 different kinds, and all of them were found to belong to one of the common types or one of the phenotypes when the enzyme systems were polymorphic. These data strongly suggest that the altered form in *m*-TOX fraction of the individual is not the result of secondary modifications, but a true structural alteration resulting from a mutation at the *m*-TOX locus of the individual. Based on these evidences we proposed the second structural locus for tetrazolium oxidase in man.

Isozyme Variations in Man

Tomotaka SHINODA, Ei MATSUNAGA and Jushiro KOSHINAGA*

Isozyme variations in red cells and tissues obtained by autopsy have been investigated for the total of 16 different enzyme systems. As to isozymes in red cells, acid phosphatase adenosine deaminase, phosphogulcomutase, 6-phosphogluconate dehydrogenase and *s*-glutamate-pyruvate transaminase were found to be polymorphic, with the practecally the same frequencies in the previous results. Estimated frequencies for respective enzyme systems were as follows; AP : $P^a = 0.20$, $P^b = 0.80$; ADA : $ADA^1 = 0.97$, $ADA^2 = 0.03$; PGM_1 : $PGM_1{}^1 = 0.76$, $PGM_1{}^2 = 0.24$; PGD : $PGD^A = 0.91$; $PGD^C = 0.09$; s-GPT : $GPT^1 = 0.60$, $GPT^2 = 0.40$. Other variants found in red cells were PGM 7–1 (1/340), PHI 3–1 (1/340), PHI 4–1 (3/340), s-GOT 2–1 (3/340), s-GOT 3–1 (1/340), and s-ICD 2–1 (1/340), respectively. No variants were found in red cell samples with respect to other enzyme systems.

As to tissue specimens, the following enzyme systems were found to be in multiple forms: they were adenosine deaminase (ADA 2–1, 3; ADA 1, 61; total 64), alcohol dehydrogenase (ADH_3 1, 37; ADH_3 2–1, 47; ADH_3 2, 14; total 98), 6-phosphogluconate dehydrogenase (PGD A, 57; PGD AC, 7; total 64), phosphoglucomutase PGM 1, 39; PGM 2–1, 21; PGM 2, 4; total 64), phosphohexose isomerase (PHI 1, 63; PHI 4–1, 1; total 64), m-glutamate-

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oxaloacetate transaminase (GOT 1, 53; GOT 2–1, 11; total 64), and s-glutamate pyruvate transaminase (GPT 1, 20; GPT 2–1, 24; GPT 2, 6; total 64).

In addition to these enzyme systems an isocitrate dehydrogenase was found to be highly heterogeneous, not only in s-fractions but also in m-fraction. There are three types of s-ICD; types 1 and 2 exibit only one major band either the most cathodal in the former or the most anodal in the latter, whereas the third one consists of three major bands, two of these are identical with those of either type 1 or type 2, respectively. The remaining band has an intermediate mobility between the bands 1 and 2. This figure suggests that the third type is likely to be heterozygous form for types 1 and 2. At present we are not very certain whether these phenomena are under genetic control.

Structural Studies on Human Immunoglobulins

Tomotaka SHINODA

Amino acid sequence analyses were carried out using a fragment obtained by specific scission at Asn-Gly bond with a chemical agent from purified human type K IgA myeloma protein (Mu). Peptides were obtained by tryptic and chymotryptic digestions of the fragment following column chromatography on Chromobeads-P in volatil buffer systems. After purification by high voltage paper electrophoresis at pH 3.7 or 6.5, peptides were sequenced by manual Edman-dansyl technique. The fragment contained the first 65 residues of the variable region of the α chain.

When the sequence of the fragment is compared with those reported for other human heavy chains, there are several noteworthy aspects: a) the sequence greatly resembles that reported for VHIII heavy chain subgroup; b) there are three regions in which the sequences appear to be less variable; i.e., positions 2 though 10, 17 through 29, and 36 through 43; c) the half-cystine residue at position 22 and two tryptophan residues at positions 36 and 47 are invariant; d) this region seems to have no class specificity in sequence but is rather subgroup specific. The evidence strongly suggests that the variable region of the α heavy cha n has the characteristic structural feature common to other heavy chains and this will give further support to the genetic hypothesis that each immunoglobulin chain is coded by more than one gene, probably two, one for the variable region and the other

for the constant region. Details are in the press in Biochem. Biophys. Res. Commun.

Prenatal Detection of Genetic Disorders: A program in Shizuoka prefecture. (A preliminary report)

Yasuo NAKAGOME, Kazuso IINUMA and Ei MATSUNAGA

In 1972 a program was set out to monitor "high-risk" pregnancies in the Shizuoka prefecture. It was a joint effort of both the Department of Human Genetics, National Institute of Genetics, Mishima and the Shizuoka branch of the "Nichibo", a nation-wide organization of obstetricians and gynecologists. There were about 330 members in the Shizuoka branch, most of them being in private practice. The total number of births in this area was about 60,000 per year and most, if not all, of them were covered by the branch members. The prefecture has no medical college.

Indications of amniocentesis included pregnancies of chromosomal translocation carriers, women who had had a chromosomally abnormal child, women of 40 years or older, carriers of severe sex-linked diseases and carriers of a few metabolic diseases. Amniocentesis was carried out by the branch members and the amniotic-fluid samples were transferred to our laboratory.

Sixty-three cases have been examined so far. Twenty-six of them were by various reasons other than genetic diseases (e.g., hydramnion, history of irradiation in early pregnacy). Feasibility of various culture techniques, effects of storage prior to the start of cultures and the incidence of polyploid cells in cultures were studied using these samples (Nakagome *et al.*, Lancet **2**: 387, 1972 and unpublished data). Examinations of pregnancies of two different translocation carriers, t(6q+; 18q−) and t(Cp−; Dq+), revealed a balanced carrier in the former and an unbalanced Dq+ foetus in the latter. The banding study on the latter family is in progress. There were 23 pregnancies that were examined because of a previous delivery of a child with Down's syndrome (regular trisomy). In one of them, who had had two miscarriages and was 42 years old, the foetus was 47, XXY. The details will be published elsewhere (Iinuma et al., Human Hered., in press). Pregnancies of two known carriers of haemophilia revealed a female and a male foetus respectively. All other cases, including a pregnancy with the

history of previous delivery of cat-cry syndrome and 8 cases with maternal ages over 40, showed a normal karyotype.

G Group Chromosomes in Satellite Associations. (A second report)

Yasuo NAKAGOME

In the last issue of this organ a slight excess of the involvement of no. 21 chromosomes in satellite associations was described (Nakagome (1972) Ann. Rep. Nat. Inst. Genet. **22**: 97). A total of 152 G group chromosomes in satellite associations were then identified by the technique of G-staining. Eighty-seven of them were no. 21s and 65 were 22s. The difference was not significant.

Additional 385 G chromosomes have been scored. The details of the techniques used are described elsewhere (see next page). The results are shown in the table. Out of a total of 537 (152 plus 385) G group chromosomes, 297 were no. 21s and 240 were 22s. There seems to be an excess entry of no. 21 chromosomes into satellite associations ($x^2 = 6.05$, d.f. $= 1$, $p < 0.02$). The details will be published elsewhere (Nakagome, Cytogenet. Cell Genet., in press).

Table 1. No. of G group chromosomes involved in satellite associations

Individuals	Sex	No. of G's associated				x^2
		Total	#21	#22	Expected	
1	F	60	30	30	30	—
2	M	18	10	8	9	0.22
3	M	134	71	63	67	0.48
4	F	59	32	27	29.5	0.42
5	F	33	18	15	16.5	0.27
6	M	18	13	5	9	3.56
7	F	15	10	5	7.5	1.67
8	F	42	23	19	21	0.38
9	M	158	90	68	79	3.06
Combined		537	297	240	268.5	6.05*

* x^2 for combined data, d.f.$=1$, $0.02>p$. Sum of $x^2s=10.06$, d.f.$=9$, $p>0.25$. Heterogeneity $x^2=4.0$, d.f.$=8$, $p>0.5$.

Banding Studies of Human Chromosomes. 1. Characterization of 3 Translocations Involving C or G Group Chromosomes and of a C Trisomy Mosaic

Yasuo NAKAGOME, Kazuso IINUMA and Ichiro MATSUI

Individual chromosomes in the C or G group cannot be distinguished with certainty either by conventional karyotype analysis or autoradiography. However, the techniques of Giemsa banding and quinacrine fluorescence have made it possible to recognize every individual chromosome pair by its characteristic pattern of bands. In the present report, chromosomes involved in 3 unusual translocations, including two Cs and a G, were identified. Further, the points of exchanges were determined within narrow limits. A C group chromosome in a case of mosaic C trisomy was also identified. The techniques of Giemsa staining and quinacrine fluorescence are described elsewhere (Nakagome *et al.* (1973) J. Med. Genet. **10**: 174).

A family with 2 cases of 18 trisomy had a t(Cq+; Eq−) translocation through 3 generations. In every case examined, translocation was identified as rcp (6; 18) (q2; q1). In a pregnancy of a carrier woman, the embryo was identified to be a translocation carrier by amniocentesis.

In a girl with t(Cq−; Dq+) translocation the chromosomes involved were 11 and 14. The karyotype was identified as 46, XX, rcp (11; 14) (q12 or 13; q32?). Both of her parents showed a normal karyotype. In the third translocation, routine chromosome analysis revealed a 45, XX, +C, −E, −G karyotype. One of the C group chromosome was mediancentric and about the size of a no. 12. The karyotype was identified as 45, XX, −17, −22, + der (17), t(17; 22) (p12 or 13; q11?). An extra chromosome in a case of mosaic C trisomy was identified as no. 10. The correlation between phenotype and these chromosomal changes are being studied.

Fluorescence of the Barr Body in Human Amniotic-fluid Cells

Kazuso IINUMA and Yasuo NAKAGOME

Fluorescent staining technique has been proved to be a reliable tool for prenatal identification of fetal sex, as it ensures the presence of the Y chromosome in interphase nuclei of male cells from human amniotic fluid.

Moreover, the sex-chromatin or Barr body when stained with quinacrine mustard has been observed to fluoresce in certain cell types under certain conditions, such as cultured fibroblasts. However, no recognizable fluorescence of the Barr body has been observed in the nuclei of uncultured human amniotic-fluid cells from female fetuses. Our report describes the fluorescence of the Barr body in freshly obtained amniotic-fluid cells.

Approximately 3 ml of fresh amniotic fluid was centrifuged, and the cell pellet was fixed in acetic-methanol (1: 3) for 15 minutes. After centrifugation, one drop of cell suspension was placed on a glass slide, gently blown, and flame-dried. Our fluorescent staining technique was described elsewhere (Iinuma and Nakagome, (1972) Jap. J. Hum. Genet. **17**: 57).

The fluorescent Barr body could be readily identified by its size, intensity of staining, and its usual position next to the nuclear membrane. Restaining with carbolfuchsin confirmed its correspondence with the sex-chromatin. The incidence of nuclei with fluorescent Barr bodies varied from 2% to 16% in a total of 10 cases with the conception of a female fetus. In one case, both Y-chromatin and fluorescent Barr body were observed in the same nucleus: its karyotype from cultured fibroblasts was 47, XXY. For details, see Lancet **1**: 436 (1973).

Polymorphisms of C and Q bands in Human Chromosomes

Kazuso IINUMA, Ei MATSUNAGA, and Yasuo NAKAGOME

Using the new techniques of staining *C* and *Q* bands of human chromosomes described by Arrighi & Hsu (Cytogenetics, **10**: 81, 1971) and by Caspersson *et al.* (Exptl. Cell Res. **60**: 315, 1970), respectively, we have studied a total of 29 unrelated persons, consisting of 13 males and 16 females, without any detectable abnormality in the karyotype. Polymorphism involving constitutive heterochromatin (*C-band* variants) was found to occur at a high rate in chromosomes Nos. 1, 9 and 16, where the length of densely stained heterochromatic regions of the secondary constriction was used as a marker. The results are summarized in Table 1, in which "*cc*" stands for the homozygous form with two "normal" chromosomes and "*Cc*" for the heterozygous form with one normal and one (*C*) with increased length of densely stained heterochromatin in the region of secondary constriction. In our sample there was no case with a homozygous "*CC*" form. It is

Table 1. Summary of *C-band* variants

Chromosome No.	*CC*	*Cc**	*cc*	Total	χ^{2**}	Frequency of *C*
1	0	9	10	19	1.83	0.24
9	0	10	9	19	2.42	0.26
16	0	1	18	19	0.01	0.03

* *C* indicates the variant with increased length of densely stained heterochromatin in the region of secondary constriction.

** Calculated on the basis of the Hardy-Weinberg's equilibrium.

Table 2. Summary of *Q-band* variants

Chromosome No.	*FF*	*Ff**	*ff*	Total	χ^{2**}	Frequency of *F*
3	5	16	5	26	0.18	0.50
4	0	14	12	26	3.53	0.27
13	5	14	7	26	0.18	0.46
14	0	6	20	26	0.44	0.12
15	0	1	25	26	0.02	0.02
21	0	7	19	26	0.10	0.13
22	0	5	21	26	0.08	0.09

* *F* indicates the variant with strongly fluorescing paracentric region.

** Calculated on the basis of the Hardy-Weinberg's equilibrium.

not certain whether the chromosomes designated either as *C* or *c* are homogeneous among different subjects with respect to the quantity of the heterochromatin but the values of χ^2 calculated on the basis of the Hardy-Weinberg's equilibrium showed a good agreement between the observation and the expectation. The frequencies of the variants are also shown in the table.

Polymorphism involving *Q-bands* was observed to occur in chromosomes Nos. 3, 4, 13, 14, 15, 21 and 22, each of them showing either strongly (*F*) or weakly (*f*) fluorescing paracentric regions or satellites. Again, assuming that there are only two types of chromosomes involved in each of the polymorphisms concerned, the results are summarized in Table 2. The χ^2 gives a larger but insignificant value (3.53 for 1 d. f.) for the polymorphism of chromosome No. 4; it is not clear whether this is due to chance alone or to other causes, although it is sometimes difficult to discriminate the fluorescent marker in this particular chromosome. In addition, the length of intensively fluorescing distal part of the long arm of Y chromosome was considerably variable from person to person. The length of the largest Y chromosome was equal to that of D group chromosomes, while the smallest one corresponded to two thirds of No. 21 chromosome.

Further, metaphases from 6 pairs of identical twins, one pair of fraternal twins, one pair of siblings and their parents were analysed with *C* and *Q* banding techniques. A combination of respective markers was characteristic in each individual, and comparison of these combinations within a family revealed a pattern of regular inheritance from parents to children. Particularly, a complete concordance in the combinations within pair of identical twins, whose zygosity was determined independently by the conventional methods using blood types, serum types, and isozyme patterns of red blood cells, was noticeable, in contrast to the discordance between fraternal twins or siblings.

A part of this study was reported in the 17th annual meeting of the Japan Society of Human Genetics (1972).

XII. BEHAVIORAL GENETICS

Genetic Analysis of Selected Populations of *Drosophila melanogaster* for Positive and Negative Phototaxis.[1)]

Jong-Kil CHOO and Chozo OSHIMA

The results of selection of *D. melanogaster* (Okinawa strain) for both photopositive and photonegative directions for 25 generations were reported in the previous Ann. Rep., No. 22: 104–105. Such selections were repeated for more 10 generations, and mean photoscores and variances of both photo-positive and negative populations for two periods were shown in Table 1. Realized heritability was calculated by average ratio of the selection response to the selection differential (Falconer 1960). The regression slops, plotted against the cumulated selection differential, were calculat-

Table 1. Results of selection: Mean photoscores and variances of original photopositive and negative populations, and of reversely and disruptively selected populations

Generation of selection	Original selection			
	Positive		Negative	
	$\bar{X}$	σ^2	$\bar{X}$	σ^2
26–30	3.21	5.43	7.95	7.86
31–35	3.35	6.52	8.40	7.48
	Reverse selection			
	Posi.→Nega.		Nega.→Posi.	
	$\bar{X}$	σ^2	$\bar{X}$	σ^2
28–30	4.39	8.97	7.01	12.27
31–33	6.59	9.82	6.79	9.93
34–35	7.52	10.40	6.03	12.04
	Disruptive selection			
	Posi. ♀ × Nega. ♂		Nega. ♀ × Posi. ♂	
	$\bar{X}$	σ^2	$\bar{X}$	σ^2
27–29	5.47	9.60	5.88	9.18
30–32	6.41	12.30	6.47	10.62
33–35	6.66	9.86	6.59	10.40

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

ed for early 20, late 15 and total 35 generations. Combined female and male realized heritabilities of both photo-positive and negative populations for total 35 generations were 0.0160 and 0.0245 respectively. The responses to both directions of selection were not symmetrical. The realized heritability of photo-positive population for the late 15 generations was very low.

At 27th generation, reverse selections for both photo-positive and negative populations were started. Fifteen pairs of flies showing the most negative phototaxis in the photopositive population were crossed, and such reversed selection was repeated for the following 8 generations. On the other hand, such reverse selection was performed for the photonegative population. Mean photoscores and variances of both populations in the period of reverse selection were shown in Table 1. The effect of reverse selection from positive to negative was rapid and the photoscore decreased to the neutral level by only four generations, but the opposite reverse selection did not show such rapid effect.

At 26th generation, disruptive selections were started. Fifteen females and males, showing the most positive and the most negative phototaxis from photo-positive and negative populations respectively, were crossed to make a hybrid population. Such procedures were repeated for the following 9 generations. The results were presented in Table 1. Mean photoscores of these populations accepting reciprocal disruptive selections for three periods were similar with each other. However, these values seemed to incline gradually to photonegative side.

Hybridization analysis between photo-positive and negative populations was carried out at 26th, 30th and 34th generation, and the results were shown in Table 2. Photoscores of reciprocal hybrid flies at 26th generation

Table 2. Photoscores and variances of hybrid flies between photo-positive and negative populations at 26th, 30th and 34th generation

Population	Gen. 27		Gen. 31		Gen. 35	
	$\bar{X}$	σ^2	$\bar{X}$	σ^2	$\bar{X}$	σ^2
Photopositive popul.	3.01	4.34	2.78	5.95	3.49	8.30
Photonegative popul.	7.86	8.47	8.07	9.42	8.42	6.86
Average	5.43		5.62		5.95	
Posi. ♀ × Nega. ♂	5.62	9.50	6.95	10.17	7.16	9.94
Nega. ♀ × Posi. ♂	5.88	8.62	6.84	9.51	7.08	10.11

was close to the mean of both parental populations, however, those of 30th and 34th generations were slightly biased to photonegative side. From the results described above, some photonegative polygenes manifesting negative phototaxis would be partially dominant or epistatic over the photopositive polygenes.

Comparison of Some Quantitative Characters between Populations Selected for Phototaxis in *Drosophila virilis*[1)]

Chozo OSHIMA and Jong-Kil CHOO

Selections to make photo-positive and negative populations in *D. virilis* (Tokyo strain) were carried out for 40 generations. The results of the first 30 generations were reported in the previous Ann. Rep., No. 22: 103–104. The experimental method using a maze apparatus was quite the same as that of *D. melanogaster*. In this case, body weight and body length (from the 3rd segment of antenna to the end of abdomen) of flies, sampled from both photo-positive and negative populations at 34th, 36th and 40th generations, were measured by Mettler's physical balance and the magnifying projector. Wing length (from the humeral crossvein to the end of 3rd longitudinal vein) was measured at the same time. Each generation was measured with 30 pairs of flies (about 7 days-old), taken at random from the total number of flies of each population. The results of these measurements were shown in Table 3. The body weight of photopositive female and male was definitly lighter than that of photonegative female and male flies respectively. The same relationship was deservedly noticed in the body length, but no difference was observed in the wing length. The ratio of body weight over body length of photonegative flies was greater than that of photopositive flies.

Walking speed of flies to a light way was measured by a long tube, of which inside diameter was 1.5 cm and length was about 1.3 m. The tube was divided into 8 regions (18 cm) by septum having a hole and these regions were numbered from 5 (light) to −2 (dark). These tubes were connected in a straight. A glow lamp (60W) was placed at 30 cm above the 5 region and the −2 region was covered with a dark box. About 40 female and male flies were introduced separately into the 0 region, and they were allowed to

[1)] This work was supported by a grant (92715) from the Ministry of Education.

Table 3. Body weight, body length and wing length of photo-positive and negative flies

Population	Generation of selection	Body weight		Body length		Wing length		Ratio of body weight/body length	
		Female	Male	Female	Male	Female	Male	Female	Male
Photopositive population	Gen. 34	1.75	1.90	3.29	3.22	2.91	2.75	0.53	0.59
	Gen. 36	2.16	1.95	3.44	3.23	2.70	2.59	0.63	0.60
	Gen. 40	1.76	1.68	3.45	3.16	2.57	2.45	0.51	0.53
	Average	$1.89_{\pm.04}$	$1.84_{\pm.03}$	$3.39_{\pm.03}$	$3.20_{\pm.02}$	$2.73_{\pm.02}$	$2.60_{\pm.02}$	0.56	0.58
Photonegative population	Gen. 34	2.69	2.21	3.78	3.39	2.97	2.81	0.71	0.65
	Gen. 36	2.76	2.19	3.82	3.37	2.69	2.51	0.72	0.64
	Gen. 40	2.80	2.26	3.74	3.43	2.70	2.61	0.75	0.66
	Average	$2.75_{\pm.04}$	$2.22_{\pm.04}$	$3.78_{\pm.02}$	$3.40_{\pm.02}$	$2.79_{\pm.02}$	$2.64_{\pm.02}$	0.73	0.65

Table 4. Walking speed of photo-positive and negative flies in a test tube for 5 minutes

Population	Generation of selection	Percentage of flies distributed in regions of a test tube								Total flies
		5	4	3	2	1	0	−1	−2	
Photopositive	Gen. 38 ♀	61.65	6.77	8.27	4.51	8.27	3.76	5.26	1.50	133
	Gen. 40 ♀	82.96	3.70	2.96	2.96	2.22	3.70	1.40	0	135
	Gen. 38 ♂	60.82	5.26	11.70	8.77	4.09	3.51	5.68	1.17	171
	Gen. 40 ♂	78.51	8.26	4.96	1.65	2.48	3.31	0.83	0	121
Photonegative	Gen. 38 ♀	10.00	6.47	5.88	11.76	14.71	29.41	14.11	7.65	170
	Gen. 40 ♀	6.51	4.73	10.06	13.02	15.98	26.63	14.79	8.28	169
	Gen. 38 ♂	5.75	7.47	7.47	5.17	12.64	36.21	16.67	8.62	174
	Gen. 40 ♂	11.03	5.15	13.97	7.35	11.76	21.32	16.91	12.50	136

walk to either light or dark ways and after 5 minutes, the number of flies distributed at each region was counted. Such experiments were performed four times with different flies. From the results represented in Table 4, the walking speed of both photo-positive and negative flies could be compared with each other. About 80 percent of photopositive flies walked fastly to the light way, but about a quarter of photonegative flies did not move and about 20 percent of them prefered the dark way. The behavior of both photo-positive and negative flies could be recognized by such a simple method.

Effects of Urbanization on the Ecological Habitat and of Noise and Light Environments on the Development of *Drosophila*.[1)]

Chozo OSHIMA and Jong-Kil CHOO

In November 1972, *Drosophila* species were collected by sweeping at two places: National Park for Nature Study in Tokyo city and Tamazawa in the suburbs of Misima city. Names of genera, and numbers of species and specimens, collected at two places, were summarized in Table 5.

The montium species group (*D. auraria, D. rufa* and *D. lutea*) was dominant species in autumn at two places, but about fifty percent of specimens collected at Tamazawa were *D. immigrans*. The similar result was observed in 1971 as reported in Ann. Rep., No. 22: 107–108. Furthermore, many

Table 5. Drosophilid flies collected at two places in November 1972

Genus	Number of species (No. of specimens)	
	National Park for Nature Study (Tokyo city)	Tamazawa (Misima city)
Leucophenga	4 (24)	3 (25)
Mycodrosophila	1 (4)	—
Scaptomyza	1 (19)	3 (33)
Drosophila	10 (285)	16 (730)
Total	16 (332)	22 (788)

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

flies of a new species, *D.* (*S.*) *oshimai* (named by Choo and Nakamura: Kontyū, 1973), were collected at Tamazawa and Shizuoka in autumn and winter seasons.

The effects of noise and light environments on the development (from egg to adult) of *D. melanogaster* (Oregon-R strain) were examined. A sound level of 100 phone, 2000 cycle/sec. was produced by a machine, Audio Signal Generator (SNR–11), which set in an incubator with program controllers of photo and temperature conditions. Many eggs laid by several hundreds of female flies for one hour were collected, and each of 50 eggs was transferred to a small vial containing cornmeal, ebios and sugar medium. Two small sheets of gauze were used as a cap of vial in noise environment, and a sponge plug was used in light environment. A group was composed of ten vials having fifty eggs. One group was kept in constant noise and light (2500 lux) environment, and another group was kept in constant quiet and light environment. The temperature in these environments was kept at 25°C. In other cases, light environment was changed to constant dark (0 lux) environment and also to periodical light and dark (L12; D12) environment. Periodical noise and quiet environment (N12; Q12) was also made. The results were presented in Table 6.

Table 6. Time of development (egg to adult) under noise and quiet, light and dark environments

Environment	Mean time of development	Variance	Eclosion rate (%)
Const. L and Q	207 hr 35 min	6 hr 38 min	88.45
Const. L and N	197 hr 35 min	5 hr 49 min	80.40
Const. D and Q	221 hr 20 min	6 hr 45 min	81.47
Const. D and N	210 hr 49 min	7 hr 14 min	86.00
Peri. L12 : D12, Const. Q	212 hr 18 min	4 hr 45 min	90.80
Peri. N12 : Q12, Const. L	190 hr 40 min	6 hr 15 min	89.60

L, Light environment; D, Dark environment; Q, Quiet environment; N, Noise environment.

The light environment accelerated the development about 5 percent faster than the dark environment, and the noise environment accelerated it about 10 percent faster than the quiet and dark environment. However, no significant differences among the rates of eclosion in these environments was observed.

Selection for Geotaxis in *Drosophila melanogaster*

Takao K. WATANABE and Wyatt W. ANDERSON

Geotactic selection and hybridization studies were carried out with a natural population of *D. melanogaster*. Behavior was measured by using Hirsch classification maze. The initial population was near neutral to gravity, yet it responded to both positive (downward) and to negative (upward) selections with a realized heritability of about 0.14. A stabilizing selection toward neutral gravity was also carried out simultaneously. At generations of 6, 9 and 10 all possible hybrid crosses among the selected populations were examined. Each geotactic value of the hybrid population was not different from the mid-parent value. But, average value of the hybrids was slightly deviated to downward. Frequencies of polymorphic inversions of the initial population generally reduced in every population. During the selection of ten generations, neutral population seemed to maintain more inversions than the negative and positive populations. Negative population eliminated all second chromosome inversions, while positive population decreased the frequencies of the third chromosome inversions. There was no significant depression in productivity (number of progeny) of each selected population during the selection for geotaxis.

XIII. APPLIED GENETICS

Geographical Variation on an Isoperoxidase Level in Populations of *Pinus Thunbegii*

Kan-Ichi SAKAI and Shinya IYAMA

Collection of needle-leaves on an individual tree basis was performed in five districts along the sea coast of Japan proper. Two of them are facing Japan Sea and designated for the sake of convenience JE (Niigata-Ken) and JW (Tottori-Ken), while the remaining three on the side of Pacific Ocean, being designated PE (Miyagi-Ken), PC (Shizuoka-Ken) and PW (Hiroshima-Ken). From each of two to five populations in each district, twenty to seventy trees were sampled for their needle-leaves. The squeezed sap of those needle-leaves was investigated for the isoperoxidase variation by the aid of the starch-gel electrophoresis. It has been found from this study that there was a distinct variation among populations in respect of incidence of several isoperoxidase bands. It is of interest to find that the populations growing in western parts of Japan proper had higher incidences in general than those from eastern parts. For example, incidence of Q band in populations from PW and JW was 88 and 52%, respectively, while it was 14, 20 and 13% in the remaining three districts, i.e. PC, PE and JE. The same tendency was also found for other bands. In addition to this, it was observed that the staining intensity of isoperoxidase bands in samples from central and eastern districts looked apparently weaker than that in western districts. Further investigations are under way.

Effect of Population Size on Selection Efficiency of the Bulk Method of Breeding in Autogamous Plants

Shinya IYAMA

The bulk method of breeding is widely used for selection of quantitative traits in autogamous crop plants. The principle of the method is as follows: After crossing between two parental stocks, the progeny populations are propagated for several generations without selection. During this period, the plants become homozygous and desirable recombinations can take place.

Then, selection is applied to the hybrid population. In practice, the size of population which one can maintain is small as compared with the number of possible gene combinations.

A computer simulation experiment was conducted to find how the size of population in each generation influences on the probability of recovery of desirable recombinants. After crossing *AAbb*×*aaBB*, the probability of *AABB* genotype in the F_{11} generation was estimated under various propagation schemes (Table 1). The probability of occurrence of *AABB* on the average for 1,000 runs did not differ among the different schemes if the two loci had the same strength of linkage. However, the frequency of runs in which *AABB* was ultimately lost distinctly differed according to the schemes.

It was concluded from the results that a small population size in early generations (F_2 and F_3) definitely resulted in the loss of desired genotypes. An example of simulation is presented in Table 1.

Table 1. Frequency of runs losing *AABB* before F_{11} under different propagation schemes. (No. of runs=1,000)

	Propagation scheme					Receombination value between *A* and *B*					
	n_1*	n_2	n_3	n_4	n_5 ... n_{10}	0.1	0.2	0.25	0.3	0.4	0.5
(1)	4	4	4	4	1....1	121	86	63	57	58	79
(2)	8	4	4	2	1....1	14	10	3	11	7	5
(3)	8	8	2	2	1....1	18	3	5	4	3	5
(4)	16	4	2	2	1....1	4	2	0	0	0	0
(5)	6	4	3	2	1....1	75	45	32	29	13	18
(6)	6	6	2	2	1....1	52	28	19	16	22	14
(7)	12	3	2	2	1....1	18	2	1	1	0	0
(8)	24	3	2	1	1....1	4	0	0	0	0	0

*) n_i=number of descending plants from each parental plant at *i*-th generation. Population size at F_5 was 256 for (1) to (4) and 144 for (5) to (8).

Gamma-ray Induced Genetic Variation in *Arabidopsis thaliana*

Sujit BAGCHI and Shinya IYAMA

In order to look into the pattern of induced genetic variations in quantitative characters, a pure line, Landberg, of *Arabidopsis thaliana* was irradiated with gamma-rays. The experiment started with treating the seeds of the plants with doses of 20 and 80 kR. The subsequent propagation was

conducted according to the "one plant one offspring" scheme (Sakai and Suzuki, 1964) with population size of about 1,000 in each treatment. At the M_3 generation 20 plants were selected at random from each of the 20 kR and 80 kR bulk populations in addition to the 14 plants from the control population. In the M_4 generation, lines each consisting of approximately 20 plants were grown from the selected plants. Measurements were taken on days to first flowering, plant height and number of leaves on a single plant basis.

The data were subjected to analysis of variance, and genetic and environmental variances were estimated based on the components of between- and within-line mean squares as shown in Table 1. It was found from the results that radiation had induced little change in the mean values of the population except for days to flowering and plant height in the 20 kR treatment. Of noteworthy, however, was an increase in the inter-line variability due to irradiation and it was of interest to find that this trend was very distinct for all three measured characters in the 80 kR treatment. The estimated values of genetic variances were always larger in irradiated populations than in the control.

Unexpected, however, was an increase of the environmental variances in irradiated populations. In order to reconfirm whether or not this is a general trend, intra-line variability was investigated in the irradiated and control line groups. It was found that some lines from the irradiated populations were highly variable within themselves while no control line showed such

Table 1. Estimated mean ($\bar{x}$), genetic variance (σ_g^2) and environmental variance (σ_e^2) for three metric characters.

Treatment		Days to flowering	Plant height	Number of leaves
Control	$\bar{x}$	28.25±1.44	24.61±2.44	9.52±0.98
	σ_g^2	0.6959	1.3521	0.0189
	σ_e^2	2.4912	7.3786	1.1262
20 kR	$\bar{x}$	24.27±2.18	27.63±3.12	9.61±1.27
	σ_g^2	6.2231	3.9805	1.0087
	σ_e^2	6.0448	11.9730	1.7978
80 kR	$\bar{x}$	29.28±2.54	25.78±4.13	10.97±1.49
	σ_g^2	13.0520	15.7701	1.8514
	σ_e^2	5.8811	19.8423	2.7285

Table 2. Frequency distribution of standard deviations for within-line variation in M_4 generation

Character	Treatment	<1.0	1.0 ≀ 1.8	1.8 ≀ 2.6	2.6 ≀ 3.4	3.4 ≀ 4.2	4.2 ≀ 5.0	5.0 ≀ 5.8	5.8 ≀ 6.6	6.6 ≀ 7.0	No. of lines observed
Days to flowering	Control	3	8	3							14
	20 kR	1	8	6	2	1	2				20
	80 kR		6	6	7	1					20
Plant height	Control		3	5	4	1	1				14
	20 kR		1	8	6	3		1		1	20
	80 kR			2	4	8	2	2	1	1	20
No. of leaves	Control	11	3								14
	20 kR	4	14	2							20
	80 kR	4	12	2	2						20

variability (Table 2). This high variability might be due to intra-line segregation or developmental instability induced by irradiation. Recent investigations on this point, though not completed yet, suggest that increased developmental instability could be the major factor in this phenomenon.

Inbreeding Depression in Japanese Quail

Takatada KAWAHARA

Birds from semi-domesticated wild strain of Japanese quail, *Coturnix coturnix japonica*, those from a domestic strain, and their reciprocal F_1 hybrids were investigated with regard to the tolerance against inbreeding depression. The total number of matings with which the experiment did start was 467 of which 191 was from the wild, 85 from the domestic, 83 from domestic (♀) × wild (♂), and 108 from wild (♀) × domestic (♂). The birds were propagated by successive full-sib mating. Birds at the age of 12 to 20 weeks were used for mating, and the eggs were hatched twice per generation at a two-week interval to raise the progeny from eggs stored less than two weeks. As the control, the domestic strain was propagated by circular pair mating system in order to avoid inbreeding. No conscious selection for performance traits was practiced. Traits examined were fertility, hatchability, viability (up to 20 weeks after hatching), survival index (fertility × hatchability × viability), egg production rate up to 60 days after the first

egg, fitness index (survival index × egg production rate), age at sexual maturity, egg weight (average for first three eggs at sexual maturity) and body weight at 4, 8, 12, 16, 20 weeks of age and at sexual maturity.

The results of this investigation was summarized as follows:

1) The wild and two reciprocal F_1 hybrid strains were generally less tolerant to inbreeding than domestic strain. These three strains died out in the third or fourth generation of inbreeding, whereas the domestic strain survived until the fifth generation. No significant difference was found between the two reciprocal hybrids in this respect.

2) The degree of inbreeding depression was measured by the regression of a given trait on inbreeding coefficients (F). The mean amount of changes in the traits for a 10% increase in F was −9.10% in fertility, −11.34% in hatchability, −11.63% in viability, −10.64% in survival index, −7.39% in egg production rate, −8.99% in fitness index, +3.6 days in sexual maturity and −0.03 g in egg weight. In a similar manner, the body weight decreased with inbreeding, the decrease per 10% increase in F being −4.19 g for females and −2.99 g for males.

3) The lethal equivalents as described by Morton *et al.* (1956) were estimated. The total load ($A + B$) of fitness index in terms of lethal equivalent was between 6.5 and 9.0 per gamete or 13 and 18 per zygote. Thus, the estimates of B and B/A statistics in Japanese quail were found to be higher than those reported by Sittmann *et al.* (1966) using domestic quails.

A Genetic Analysis of Skeletal Characters in Japanese Quail

Takatada KAWAHARA

Genetic and phenotypic correlations among traits and heritability values of skeletal characters were estimated in a random-bred population of domestic Japanese quail. Investigated were total body weight, eviscerated body weight, total bone weight, keel length, keel depth, lumbosacral bone length and skull width. In addition to them, length and weight were measured in the following nine bone characters, *i.e.*, coracoid, scapula, humerus, ulna, carpometacarpus, femur, tibiotarsus, tarsometatarsus and thoracic vertebrae. Data were collected from 584 progenies (305 female and 279 male birds) originated from 69 pair matings. The birds were killed at the

age of 25 weeks and autopsied.

The results of this study are summarized as follows:

1) The female birds tended in many characters to be significantly larger than the males except for keel length and depth, skull width, coracoid weight and eviscerated body weight, in which the males were significantly larger than the females. Weight of scapula and carpometacarpal bones did not significantly differ between sexes.

2) Heritability values were estimated from the analysis of variance of the data for various bone characters. In the males, bone length generally had higher heritabilities than bone weight. Females showed lower heritability values than males and in females, the values for bone length were not higher than those for bone weight. When the data for females and males are pooled, the heritability for the hind-limb bone complex including femur, tibiotarsus and tarsometatarsus, was 0.819 for length and 0.789 for weight; the fore-limb bone complex consisting of humerus, ulna and carpometacarpus, had somewhat lower values, *e.g.*, 0.726 for length and 0.637 for weight. Lower heritability values were found in lumbosacral bone length (about 0.35 for both male and female) and in skull width (0.378 for male and 0.479 for female).

3) Genetic and phenotypic correlations among various traits were generally higher in the male than in female. The phenotypic correlation between length and weight of the same bone estimated among nine different bones was 0.431 in male and 0.266 in female. The genetic correlation also showed a similar trend, namely, 0.595 in male and 0.273 in female. Correlations of body weight or eviscerated body weight with bone characters were higher in male than in female. The body weights were strongly correlated with bone length but weakly correlated with bone weight. Bones in the same body part were strongly inter-correlated, but those in different body parts showed lower correlations.

A Note on the Breeding Behavior and Genetic Variations of Sunflowers Observed in Central Luzon

Hiko-Ichi OKA

Two sunflower (*Helianthus annuus* L.) varieties, Arrowhead and Armaveric,

were used for determining self-fertility, outcrossing rate and other genetic parameters. Isolation of single plants by a distance reduced seed fertility to about 20 per cent indicating their tendency to self-incompatibility. Outcrossing rate was estimated to be 75 per cent from the frequency of plants with striped seed (a dominant character) in the progeny of Armaveric (with black seed) raised together with Arrowhead (with striped seed). The heritability values estimated from parent-offspring regression were 52% for number of days to flowering, 68% for plant height, and 22% for head diameter. The number of days to flowering and plant height were genetically correlated, but head diameter was not correlated with the former two characters.

Performance in Central Luzon of Soybean Varieties Selected in Taiwan for Wide Adaptability

Hiko-Ichi OKA

Ten soybean varieties from Taiwan and two local ones were tested in three different seasons at the Central Luzon State University, Muñoz (15°N), Philippines. Of the ten Taiwan varieties, eight had been selected for yield stability following the breeding scheme of "disruptive seasonal selection". The results proved that, if planted at a proper density, at least two Taiwan varieties (CH–2 and Fall-17) could have a high yielding potential (about 2.5 ton/ha) and a small yield variance among the six seasons compared (spring, summer and fall in Taichung and rainy, early dry, and late dry at Muñoz). Generally, varieties adapted to the fall cropping in Taiwan appeared to be adaptive in the tropical conditions in Central Luzon. Correlation studies among yield characters indicated varietal differences in the physiological responses to seasonal conditions, yet yield stability was recognized to be a genotypic character established in certain Taiwan varieties. This may serve as an evidence for the usefulness of disruptive seasonal selection for obtaining wide adaptability. (Published in SABRAO Newsletter **5**: 29–38, 1973).

Variation in the Response to Planting Density among Sunflower Varieties

Hiko-Ichi OKA

Ten sunflower (*Helianthus annuus*) varieties were tested at four different planting densities. They generally responded to close spacing by reducing their height and organ size, but differed in the magnitude of their responses. Logistic functions were computed from the data of plant height to estimate the number of days to half-size time and maximum growth rate. Further, regressions of these growth parameters and other character values on planting density (linear and quadratic) were computed in each variety. The results indicated significant varietal differences in the mode of developmental plasticity. This work was carried out at the Central Luzon State University, Philippines, in the 1971/72 dry season.

Variations in the Growth Pattern of *Oryza Perennis* Strains

Hiroko MORISHIMA and Hiko-Ichi OKA

As previously reported (1969 and 1970), we have continued investigations on the growth pattern of wild (*Oryza perennis*) and cultivated (*O. sativa*) rice strains for six years to look into its role in adaptation. Six wild and three cultivated strains were tested in an experimental field, automatic shortday plots (latitudinally controlled by using astrodials), and in gravel culture. In each condition, a strain was represented by about 20 plants and four to five plants were sampled several times in the period from floral initiation to maturity for recording plant weight and length of certain organs. A part of plant weight data were, however, obtained by the use of an electronic "grass meter". The growth pattern in dry matter weight and elongation patterns of individual organs were represented by logistic equations to compute certain parameter values, *i.e.*, days from maximum growth to heading (early *vs.* late vigor) and to the time when growth rate declines to half of the maximum (growth persistence), and growth rates 25 days before heading, at heading, and 10 days after heading; as to the elongation patterns of panicle and first to fourth internodes (from the top), the number of days from half-size time ($t_{1/2}$) to heading was computed for each organ.

Variance analysis of the data proved that most of these values were genotypically controlled.

In cultivated as well as perennial wild strains, the elongation of third internode and that of panicle were synchronous ($t_{1/2}$ being about one week before heading) and the following elongation of first and second internodes resulted heading. In contrast, in wild strains of annual habit, the panicle elongated earlier than the third internode, and the elongation of the latter resulted in heading. Then, the first and second internodes elongated after heading to lift up the panicles high. This behavior seems to help the plants to disperse seeds.

From the growth curves of dry matter weight, wild strains were generally found to have lower growth rate than cultivated ones. The annual wild strains showed high vigor around the heading time and declined rapidly, particularly under shortdays, while the strains of perennial habit continued growth longer and were characterized by vegetative growth after heading.

It was found further that wild strains generally had higher developmental plasticity as to organ size than cultivated ones, particularly in the length of panicle, third internode and leaf blade. Among the wild strains, perennial ones were more plastic than annuals. This suggests that the wild plants of perennial habit can survive varying adverse environments, as they are resistant to drought and submersion. In contrast, annual plants may be forestallers.

Variations in the Mortality of Perennial Rye Grass and Orchard Grass Varieties

Hiroko MORISHIMA and Hiko-Ichi OKA

An observation of differential survival in different environmental conditions was initiated in 1970 using three varieties of perennial rye grass (*Lolium perenne*) and also three of orchard grass (*Dactylis glomerata*). As reported last year, 400 viable seeds per plot (1 m×1 m) were sown in 1970 September in pure stand (6 varieties) and species mixture (1 : 1, 9 varietal combinations). The 15 populations were tested in an experimental field (4 replications differently managed later) and on road side (2 replications).

In general, many seedlings died in two months after germination. Then, the established plants gradually decreased in number. The infant mortality

was higher on road side than in field, and higher in orcharad grass than in perennial rye grass. However, the mortality of established plants tended to be higher in the field than on the road side, and higher in perennial rye grass than in orchard grass. Variance analysis of the survival data (after arc-sine transformation of percentages) showed that not only the difference between the two species but also those among varieties of the same species were significant. This indicates that mortality under a certain condition is genetically determined. The heritability estimates for the within-species differences in survival were about 15% for infant mortaility and about 8% after establishment. Negative correlations between infant and adult mortalities were found within perennial rye grass varieties. This seems to suggest an adaptive role of the death of young plants.

In the summer of second year, most of the experimental plants on road side were driven out by weeds which were better adapted to the warm climate in Misima. In the experimental field, the effects of cutting and herbicide application were tested. Contrarily to expectation, these environmental stresses tended to increase the survival rate possibly because of reduction of mutual shading of the plants. This tendency was more pronounced in orchard grass than in perennial rye grass.

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ABSTRACTS OF DIARY FOR 1972

March	17	195th Meeting of Misima Geneticists' Club
	31	196th Meeting of Misima Geneticists' Club
May	16	99th Biological Symposium
	29	197th Meeting of Misima Geneticists' Club
June	2	198th Meeting of Misima Geneticists' Club
July	7	199th Meeting of Misima Geneticists' Club
September	22	200th Meeting of Misima Geneticists' Club
October	27	201st Meeting of Misima Geneticists' Club
November	14	100th Biological Symposium
December	1	202nd Meeting of Misima Geneticists' Club
	15	203rd Meeting of Misima Geneticists' Club

FOREIGN VISITORS IN 1972

May	16	DOI, Roy H., University of California, U.S.A.
July	12	SCHEIN, M. W., West Virginia University, U.S.A.
August	22	de SERRES, F. J., NIEHS-NIH, U.S.A.
		FREESE, E., N.I.H., U.S.A.
		NICHOLS, W. W., Institute for Med. Research, Camden, U.S.A.
October	3	BOUNHIL, J. J., Biological Laboratory of the University, Bordeaux, France
December	19	HERNANDEZ-ARAGON, L., Centro de Investigacions Agricolas de Sinaloa, Mexico

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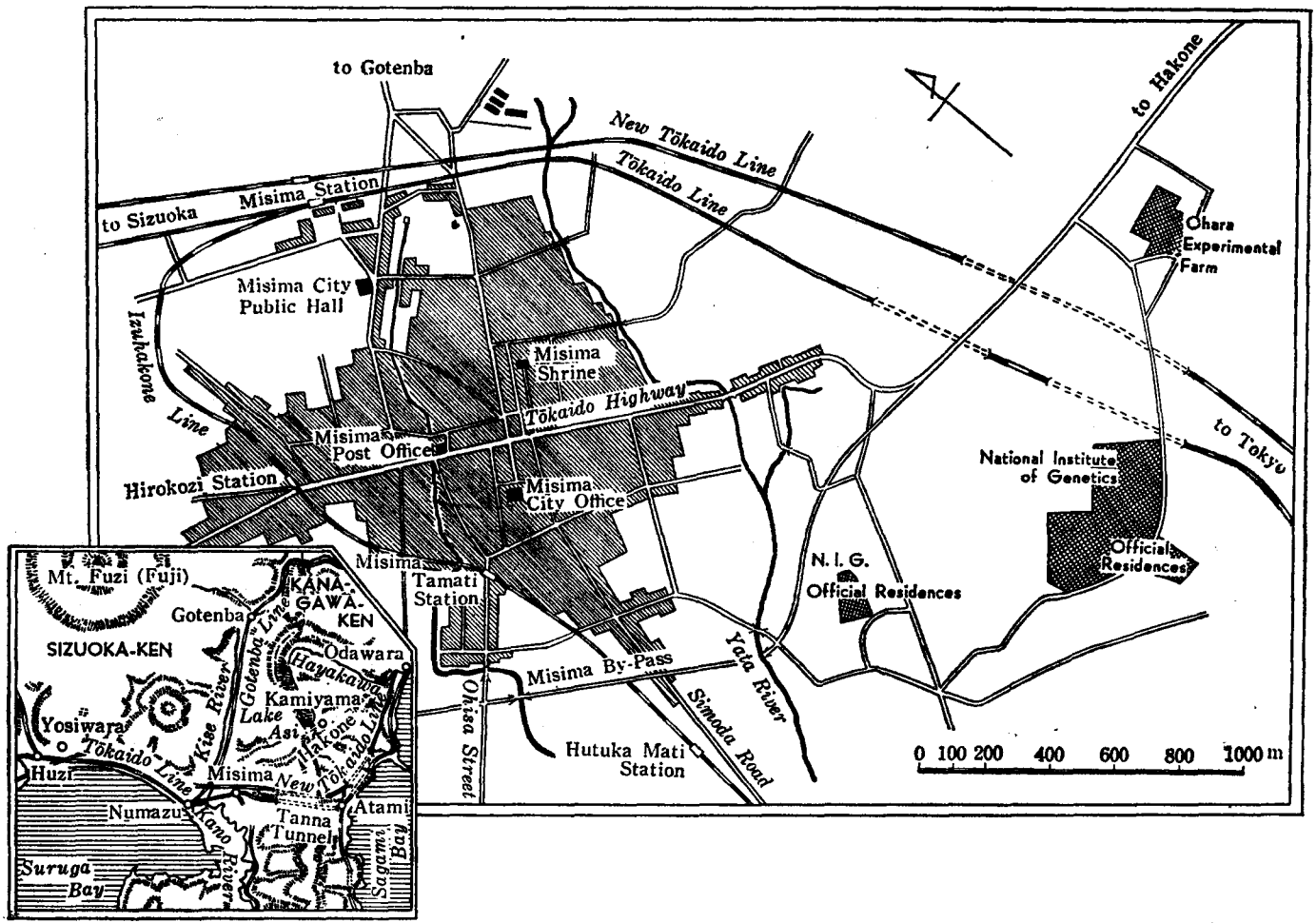
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