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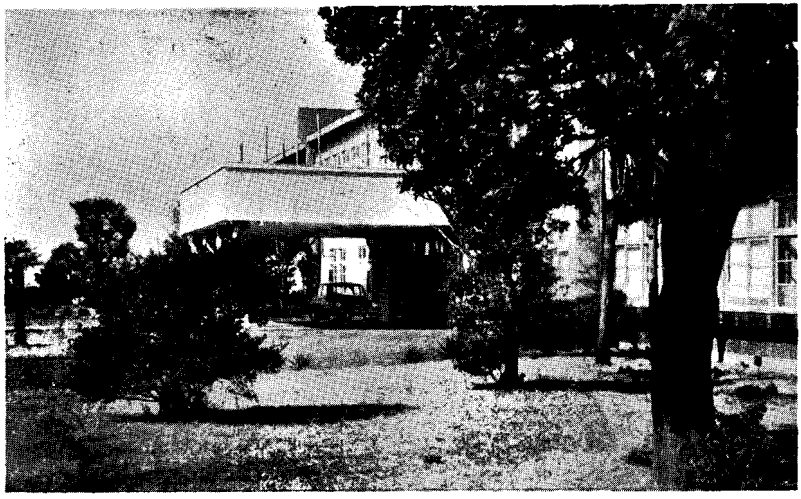

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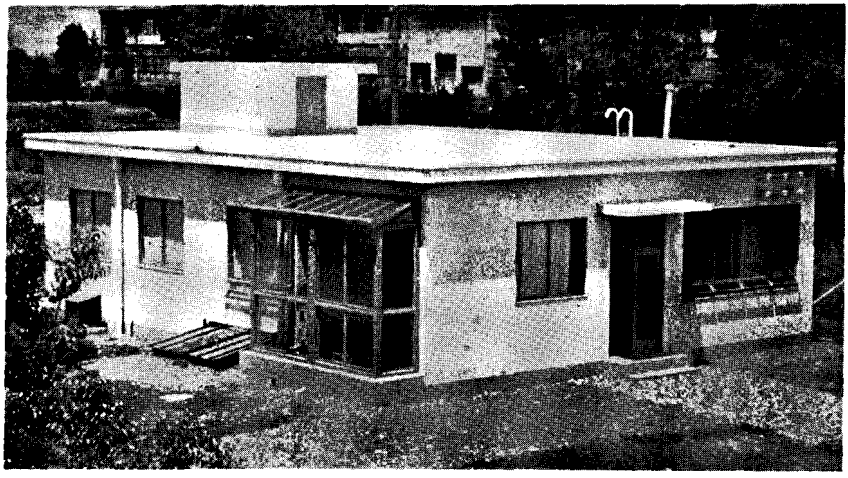

Radio Isotope Laboratory

GENERAL STATEMENT

In the current year the Institute has acquired a new department, the Department of Mutational Genetics, devoted to the study of mutations, especially those induced by irradiation and chemical agents. S. MATSUMURA has been appointed to take charge of this department, with two new staff members, T. SUGAWARA and S. KONDO, to work in cooperation with him. Thus, our Institute has now six departments with 36 research members including 9 part-time or associate members.

K. OGUMA retired in Oct. 1955 from his directorship, and was replaced by H. KIHARA, formerly professor of genetics in Kyoto University and also our part-time staff member. Oguma will continue at the Institute as a research associate.

T. KOMAI, K. SAKAI, as well as M. KIMURA who is on leave of absence at the University of Wisconsin, were invited to take part in the Symposium on Population Genetics held at Cold Spring Harbor, L.I., N.Y. June 6-13, under the sponsorship of the Long Island Biological Association, where they read papers. They also attended, by invitation, the Symposium on Mutation sponsored by the Biology Department of Brookhaven National Laboratory held at the Laboratory June 15-17. Before and after these symposia, both KOMAI and SAKAI travelled rather extensively in the United States visiting many laboratories and institutes of genetics.

A new concrete building, 65 tubo in floor dimensions, designed for

genetic studies with isotopes, is being constructed and will be completed by March 1956. Five residential houses for staff members, with land, were donated by Sizuoka Prefecture. A test barn for poultry breeding with accessory equipment was given to the Institute by the Whole-Japan Association of Poultry Genetics for the use of the Department of Applied Genetics.

The library has steadily expanded by acquisition of new periodicals, books and reprints, by purchase, donation or exchange. Dr. R. GOLDSCHIMIDT especially has continued to send many new reprints, books and periodicals which now total 1,138. These are of immense value and great help to our research.

The Institute was honored by the visit of H.I.H. the Crown Prince AKIHITO on April 13, 1955. The Prince, who is the heir of the Emperor with respect to both the throne and biological studies, spent more than four hours in the Institute, showing great interest in reports and demonstrations made by four staff members, and in equipment belonging to the Institute.

Among the foreign visitors during the current year, were: Dr. K. SUGIURA of the Sloan Kettering Institute for Cancer Research, New York, Drs. R. BRADFIELD and R. F. CHANDLER of the Rockefeller Foundation, Dr. and Mrs. D. C. WARREN of Purdue University, and Dr. and Mrs. F. J. RYAN of Columbia University and Fulbright Exchange Professor at Tokyo University.

The following grants were given during the current year to aid research by our staff.

From the Fund for Grants-in-Aid of the Ministry of Education to Researches in Institutions: to the Director, for Radiation genetics and its application to practical breeding ¥ 5,600,000.

From the Fund for Grants-in-Aid to Cooperative Investigations: to T. KOMAI and co-workers (including K. SAKAI), for: Studies on heterosis ¥850,000; to K. OGUMA and co-workers (including T. KOMAI, S. MAKINO, T. H. YOSIDA and K. TUTIKAWA), for: Cytological and genetical researches on tumors ¥ 600,000; to Y. TANAKA and co-workers (including M. TSUJITA), for: Studies on linkage in the silkworm ¥ 480,000; to K. SAKAI and co-workers (including T. KOMAI and H. OKA), for: Studies on polygenic inheritance ¥ 550,000; to S. MATSUMURA and co-workers, for: Researches of physiological genetics of crop plants under standardized temperature, humidity and day-light conditions ¥ 550,000; Y. TAKENAKA and co-workers (including K. HAYASHI), for: Genetics of the morning glory ¥ 400,000.

From the Fund for Grants-in-Aid to Investigations in Applied Sciences: to Y. TANAKA, for: Research on artificial control of diapause in the wild

silkworm, *Antheraea pernyi* ¥ 200,000.

From the Fund for Grants-in-Aid for Promotion of Improvement of Agricultural Techniques of the Ministry of Agriculture and Forestry; to K. SAKAI for: Comparative studies between pedigree method and bulk method in plant breeding ¥ 85,000; to M. TSUJITA, for: Studies on midgut polyhedrosis of the silkworm ¥ 90,000.

ABSTRACTS OF DIARY FOR 1955

Jan. 13. Meeting of Organization Committee of the International Genetics Symposia.
Jan. 19. Meeting of the Society of Biometrical Genetics.
Jan. 20. Meeting of the group of investigators on the wild silkworn *Antheraea pernyi*.
Jan. 27. Twelfth meeting of Board of Councillors.
Jan. 28. Thirty-third meeting of Misima Geneticists' Club.
Feb. 17. Symposium on Genetic Studies on Tobacco Plants.
Feb. 21. Meeting of Biology Group in the Special Committee of the Study of Effects of Radiation.
Feb. 28. Thirty-fourth meeting of Misima Geneticists' Club.
March 18. Ninth meeting of Biological Symposia.
March 21. Thirth-sixth meeting of Misima Geneticists' Club.
March 25. Thirth-seventh meeting of Misima Geneticists' Club.
March 25. Thirty-fifth meeting of Misima Geneticists' Club.
March 26. Tenth meeting of Biological Symposia.
April 13. The Crown Prince's visit.
April 20. Meeting of Organization Committee of the International Genetics Symposia.
May 10. Eleventh meeting of Biological Symposia.
July 1. General meeting of Whole-Japan Association of Poultry Genetics.
July 15. Thirth-eighth meeting of Misima Geneticists' Club.
July 26. Thirteenth meeting of Board of Councillors.
August 4. Meeting of Organization Committee of the International Genetics Symposia.
August 16. Meeting of poultry breeders to discuss the methods of improvement of poultry strains.
September 20. Thirty-ninth meeting of Misima Geneticists' Club.
October 7. Fortieth meeting of Misima Geneticists' Club.
October 29. Forty-first meeting of Misima Geneticists' Club.
November 7. Forty-second meeting of Misima Geneticists' Club.

November 9. Meeting of Committee of Exhibits for the International Genetics Symposia.
November 27. Twelfth meeting of Biological Symposia.
November 30. Forty-third meeting of Misima Geneticists' Club.
December 3. Thirteenth meeting of Biological Symposia.

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Yoshimaro TANAKA, Vice-President and Director of Researches

Association for Propagation of the Knowledge of Genetics

Kan OGUMA, President
Yô TAKENAKA, Managing Director
Seiji MATSUMURA, Managing Director

RESEARCH PROGRAM FOR 1955

Department of Morphological Genetics (TANAKA)
First Laboratory (TANAKA)

Studies on unstable genes in silkworm (TANAKA)
Linkage in silkworm (TANAKA)

Second Laboratory (KIHARA)

Studies on the ancestry of wheat (KIHARA)
Studies on substitution of nucleus in wheat (KIHARA)
Studies on *Agropyron*, a relative of *Triticum* (MATSUMURA and SAKAMOTO)

Nullisomic dwarfs among the offspring of pentaploid wheat hybrids (MATSUMURA)
Physiological genetics of the reaction of morning glory to day length (SAKAMOTO)

Third Laboratory (KOMAI)

Genetics of van der Hoeve's syndrome (KOMAI et al.)
Partial sex-linkage in man (K. TANAKA)

Department of Cytological Genetics (TAKENAKA)
First Laboratory (YOSIDA)

Cytology and genetics of tumors (OGUMA et al.)
Karyological characteristics of tumor cells (OGUMA, MAKINO and YOSIDA)
Genetics of tumor susceptibility (YOSIDA and ISHIHARA)
Histology and cytology of testes of male tortoiseshell cats (ISHIHARA and YOSIDA)
Determination of sex and sex-chromosomes in animals (YOSIDA and ISHIHARA)
Cytogenetics with tissue culture method (YOSIDA)

Second Laboratory (TAKENAKA)

Determination and differentiation of sex in plants (TAKENAKA)
Induction of abnormal mitosis and inhibition of growth by substances extracted from certain plants (TAKENAKA)
Interspecific hybridization in *Nicotiana* (TAKENAKA, FURUSATO and LILIENFELD)
Genetics of *Pharbitis Nil* (TAKENAKA et al.)
Classification of Gramineae by karyotypes (TATEOKA)
Karyology of rice plant (TAKENAKA)

Third Laboratory (TSUJITA)

Studies on minute structures of cells with electron-microscope (TSUJITA, TSUDA and WATANABE)
Virus, especially its genetics (TSUJITA, TSUDA and YOSHIZAWA)

Department of Physiological Genetics (KOMAI)
First Laboratory (KOMAI)

Studies on heterosis (KOMAI et al.)
Problems on mouse genetics (KOMAI and TUTIKAWA)
Population genetics of the lady-beetle, *Harmonia axyridis* (KOMAI)
Population genetics of the land-snail, *Bradybaena similaris* (KOMAI)
Population genetics of *Drosophila rufa* (TAIRA)
The *T* locus in *Mus musculus molossinus* (TUTIKAWA)

Second Laboratory (OKA) and *Third Laboratory* (SAKAI)

Analysis of genes responsible for hybrid sterility in rice (OKA)
Analysis of genes controlling temperature response in germination of rice (OKA)
Heritability of various characters and genetic correlations in rice (SAKAI and OKA)
Population-genetic studies on wild rice plants (OKA)

Department of Biochemical Genetics (TSUJITA)
First Laboratory (TSUJITA)

Biochemical genetics of insects and microorganism (TSUJITA, NAWA and SAKAGUCHI)
Embryological and biochemical studies of silkworm (TSUJITA and SAKAGUCHI)
Biochemical studies of some mutants in silkworm and *Drosophila* (NAWA and TAIRA)

Second Laboratory (HAYASHI)

Biochemistry of the mechanism underlying variations in flower colors due to anthocyanins, especially blue colors (HAYASHI and ABE)
Biochemical genetics of flower colors in *Viola tricolor* (ENDO)
Genetic studies of anthocyanin pigments in the varieties of eggplant (ABE and GOTOH)

Department of Applied Genetics (SAKAI)
First Laboratory (TANAKA)

Breeding for high egg production in poultry (TANAKA and KAWAHARA)
Genetics of some hereditary nervous diseases in poultry (KAWAHARA)
Heritability and genetic correlation of economic characters in poultry (YAMADA)
Genetic study of long-tailed fowl (TANAKA)
Experiments on the control of diapause in *Antheraea pernyi* (TANAKA)
Studies on variation of quantitative characters in inbred mice and *Drosophila* and effect selection on them (YAMADA)

Second Laboratory (SAKAI)

Studies on polygenic inheritance (SAKAI et al.)
Studies on competition between plants of differents genetic constitution (SAKAI et al.)
Population-genetic studies of "red rice" growing among land rice (SAKAI et al.)

Comparative studies of pedigree method and bulk method (SAKAI)
Studies on the nature of "good" characters in tobacco plants (SAKAI)
Local differentiation of races in barley (GOTOH)
Genetic studies on quantitative characters of eggplant (GOTOH)
Polyploidy and sterility in fruit plants (FURUSATO and MIYAZAWA)

Department of Mutational Genetics (MATSUMURA)
First Laboratory (MATSUMURA)

Radiation genetics and its practical application (KIHARA et al.)
Relation between the quality of radiations and mutations (MATSUMURA, ETO and FUJII)
Radiation genetics of wheat and barley (MATSUMURA and FUJII)
Mutations induced by irradiation of tobacco plants (KIHARA, MATSUMURA and FUJII)
Cytogenetic studies of the effect of substances inducing mutations and of substances inhibiting mitosis (TAKENAKA)
Physiological and genetical studies on cultivated plants under controlled temperature, humidity and day-light conditions (MATSUMURA et al.)
Breeding of triploid plants and its practical applications (MATSUMURA et al.)

RESEARCH STUDENTS AND RESEARCH ITEMS

Kyôzo WATANABE: Cytology and genetics of infusorians
Yasuo SUZUKI: Population genetics of crop plants
Setsuji KATAOKA: Cytology of interspecific hybrids of lilies
Shin-ya IYAMA: Population genetics of upland rice
Yasu ONO: Cytogenetics of domesticated animals
Osamu YOSHIZAWA: Genetic studies of bacterial virus
Yoshito OGAWA: Cytogenetics of animals
Keiko SHIRATO: Genetics of *Drosophila*
Yasuo OTA: Cytogenetic studies of polyembryony in *Citrus*
Yutaka So: Karyological studies of inhibitory effect of various agents on tumorous growths
Yûichirô HIRAIZUMI: Polygenic inheritance

RESEARCHES CARRIED OUT IN 1955

A. HUMAN GENETICS

1. *Genetics of van der Hoeve's Syndrome*

(By Taku KOMAI)

A large kindred showing van der Hoeve's syndrome was discovered by Dr. H. KUNII who runs an ophthalmological hospital in Yamagata Prefecture. The members of this kindred were repeatedly examined by him and also by Dr. Y. OZAKI, research associate of our institute, and an extensive pedigree chart was completed. Based on this chart and accompanying records, KOMAI conducted a genetic study of this kindred. It has been concluded that the abnormalities found in many of the members, blue sclera, bone fragility, luxation of joints, deafness, as well as short stature and a large head, are probably due to pleiotropic manifestations of a single autosomal dominant gene. The manifestation of this gene, especially with regard to bone fragility, varies widely. It may cause "osteogenesis imperfecta congenita", which is a rare lethal or sublethal abnormality causing pre-natal death of the afflicted child, or milder defects which appear in the post-natal period. Among the latter, there is an almost complete series ranging from severe crippling appearing in babyhood to apparent normality throughout life. Based on the data of the frequency of "osteogenesis imperfecta congenita" recorded for Scandinavian people and defects of the milder type estimated for Japanese, the rate of mutation of the gene for this syndrome was estimated. The value 3.95×10^{-5} was obtained as a first approximation of the mutation rate of this gene.

2. *Tests for Partial Sex-linkage of the Gene for Achromatopsia*

(By Katumi TANAKA)

Among the parents of individuals showing total color-blindness in Japanese literature, the manner of relationship is known in 20 consanguineous matings. They include 1 half-sib, 17 first-cousin and 2 second-cousin matings. In 12 matings the husband was related to his wife through his father and their progeny were composed of (a) 15 normal males, (b) 18 affected males, (c) 17 normal females and (d) 12 affected females. The remaining 8 sibships, in which the husband was related to his wife through his mother, produced

as offspring (a) 11 normal females, (b) 11 affected females, (c) 15 normal males and (d) 6 affected males. The totals are thus: (a) 26, (b) 29, (c) 32 and (d) 18. The crossover value is calculated by using Haldane's formulas: $x=d/(b+d)=0.3830$ and $x=(2a-c)/(a+c)=0.3448$, where x is the estimate of crossover value. Combining these values we obtain $x=0.3786 \pm 0.0667$. The deviation is in the right direction for the hypothesis of partial sex-linkage, but it is not significant, since the difference between 0.5 and 0.3786 is only 1.82 times the standard error.

Excluding the above-mentioned cases, 26 complete sibships including individuals showing this defect were analyzed by use of the indirect method (Finney's first method), The total linkage score $S(\lambda)$ is $+15.55554$. The total information $S(\kappa)$ is 56.00002. Therefore the standard error is $\{S(\kappa)\}^{1/2}=7.483$, whence $t=15.55554/7.483=2.08$, which corresponds to a probability lower than 1.9%, thus being significant. The estimate of the crossover value is given by

$$x=\frac{1}{2}\left\{1-\left(\frac{S(\lambda)}{S(\kappa)}\right)^{\frac{1}{2}}\right\}$$

whence we obtain $x=0.2365$.

3. *Sex Ratios among Sibships showing Xeroderma Pigmentosum and Achromatopsia*

(By Katumi TANAKA)

Macklin (1944, 1952) has found in her analysis of the data on xeroderma pigmentosum that there are more affected males than females in which the inheritance is through the paternal line of the husband, and more affected females than males in sibships in which the hushand is related to his wife through his mother, in agreement with the expectation on the hypothesis of partial sex-linkage. However, she has noticed a significant excess of normal males over normal females in the paternal relationship and an excess of normal females over normal males in the maternal relationship, and she expresses scepticism of the interpretation of the data by assuming partial sex-linkage.

In the 20 Japanese sibships showing achromatopsics in which the parental relationship is fully known, the sex ratio has been found to be nearly 1:1, i. e. 33 males to 29 females in the families with a paternal relationship and 21:22 in the families with a maternal relationship.

The remaining 26 complete sibships in which the parental relationship is not fully known are divided into 3 groups: (A) those in which only males are affected, (B) those in which only females are affected and (C) those in which both sexes are affected. The number of boys expected

per family of a given size in group (A) and the number of girls expected in group (B) may be given by the formula:

$$r=\frac{s\{2(4-x)^{s-1}-(1+x)3^{s-1}\}}{(4-x)^s-3^s} \quad \cdots\cdots\cdots\cdots\cdots\cdots(1)$$

if the patients are non-crossovers, and by the formula:

$$r'=\frac{s\{2(3+x)^{s-1}-(2-x)^{s-1}\}}{(3+x)^s-2^s} \quad \cdots\cdots\cdots\cdots\cdots\cdots(2)$$

if the patients are crossovers, where s is size of sibship and x the crossover value. The value r is always smaller than r' as long as the crossover value is smaller than 0.5.

Assuming $x=0.5$, the numbers of children to be expected in Macklin's xeroderma families and in the present author's achromatopsia families were calculated, and compared with the observed numbers. As shown in the table, the expected numbers are in good agreement with the observed.

	Number of boys in group (A)		Number of girls in group (B)	
	Observed	Expected	Observed	Expected
Achromatopsia	40	41.650	26	23.704
Xeroderma pig.	125	124.061	96	91.145

If it is assumed that $x=0.2$ or 0.3, and that all patients in groups (A) and (B) are non-crossovers, the numbers of children calculated by using formula (1) are smaller than the observed numbers listed above, but the deviation is not significant in either case. The deviation would become smaller if the expected number were larger as there would be some crossovers, and, in these cases, the numbers of children should be calculated by formula (2).

Thus there is no evidence of disturbance of the sex ratio among total sibs which might cause an abnormal sex distribution among affected sibs in the families with these diseases.

B. GENETICS AND CYTOLOGY OF MAMMALS AND SOME OTHER ANIMALS

4. *Studies on a New Mutant "Tremor" in the House Mouse*

(By Kiyosi TUTIKAWA)

A new anomaly appeared as a spontaneous mutation in 1953, in a mildly inbred "deformed pinna" strain maintained in our laboratory. Its

origin probably had been within the few previous generations. It was named "Tremor" (Mouse News Letter, 12:44).

Tremor (symbol *tm*) is due to a recessive gene with a regular manifestation. As no tremor males breed, most data are from matings between two heterozygotes, which produced altogether 88 normals and 27 tremors.

The syndrome is rather complex, and needs minute pathological examination for any detailed description.

The abnormality is hard to recognize with certainty until about 17 days of age. By careful daily examination of young mice about two weeks old, however, the abnormality may be detected. At this stage, the affected animals show a slight tremor of head and body. At three weeks, they are apparently more active than their normal sibs, and run about vigorously. They can climb up the wire gauze wall, and walk across a narrow bridge without any difficulty. At this stage, however, these mutants show a pronounced tremor of the hind legs when initiating purposeful movements or when excited. Later, in the fifth or sixth week of life, a progressive paralysis of the hind legs appears, with tremor and loss of coordination; the animal can no longer sit up on its haunches, the gait becomes progressively slower, and overbalancing of the hindquarters begins. The affected males seem to be sterile, probably because of mechanical difficulties in copulation due to the inability of coordinated movements. The females are fertile and nurse their own litters.

This new mutation differs in its manifestation from the well-known defects deaf, choreic or waltzing. It has some features in common with agitans, *ag*, ataxia, *ax*, reeler, *rl*, vacillans, *vc*, and wobbly, *wb*, but it is relatively closer to quivering, *qr*. No genetic test has been made by crossing *tm* and *qr*, but tests for the linkage relationships of the gene for tremor are in progress.

5. *Studies on the t-alleles in Populations of a Japanese Wild Mouse*

(By Kiyosi TUTIKAWA)

Samples of populations of the wild mouse, *Mus musculus molossinus*, caught in three different localities, Misima, Niigata and Fukuoka, were tested for the *t* gene by mating them to a standard testing stock *T*/+(Brachy) of *M. m. musculus*. Some wild mice from these three sources when so tested produced, in addition to the expected Brachy (*T*/+) and normal offspring, tailless offspring which on further testing proved to be T/t^m, and gave rise to seven new tailless lines.

The t^m alleles obtained from these populations of *Mus m. molossinus*

resemble in their genetic properties the t- or t^w-alleles of *Mus m. musculus* reported by Dunn. Wild males, $+/t^m$, seem to produce a great excess of t^m sperm. The ratio of t^m to + sperm is approximately the same as the ratio of tailless T/t^m to Brachy $T/+$ offspring, when a $+/t^m$ male is mated to a $T/+$ female. For all the 6 $+/t^m$ wild males tested, this proportion was 77:7 (table 1), a ratio of 11 t^m to 1+. A similar apparent excess of t sperm in males heterozygous for t-alleles has been found in *M. m. musculus* (t^0, t^1, t^2, t^9, t^{12}—Dunn and Gluecksohn—Schoenheimer, 1950; t^{w1}, t^{w2}, t^{w3}, t^{w4}, t^{w5}—Dunn and Morgan, 1953).

The lethality of the t^m alleles from the wild *molossinus* was also studied. Each F_1 tailless male which had been proved to be T/t^m was crossed with an F_1 tailless female, similarly tested, and derived from the same wild male. The F_2 offspring should reveal whether the t^m allele in each case is lethal or viable. If lethal, the cross $T/t^m \times T/t^m$ should produce only tailless young, since TT and t^mt^m would die before birth. If viable, t^m homozygotes would survive, and give young with normal tails. When this cross was made, the F_2 progeny of one male (k-1, Fukuoka) consisted of 28 tailless and 18 normal animals. This result indicates that the t^m allele is viable and gives a normal tail when homozygous. On the other hand, the F_2 progeny of a female (m-4411) and three males (m-306, m-802, m-9515) derived from the same locality (Misima) consisted of 56 tailless and 13 normal animals, beside 8 animals with very short tails. No individual of this last type was found in the previous tests, F_1 tailless crossed with a known T/t^n stock. The tentative interpretation is that the homozygote for this t^m allele is also viable, but that it is occasionally manifested as a very short tail. Similar breeding results were obtained for the viable allele derived from two males (n-63, n-65) from Niigata; namely 21 tailless, 9 normals, and one with a very short tail.

Table 1

Wild parent		Source	F_1 progeny		
			Normal $+/+$; $+/t^m$	Brachy $T/+$	Tailless T/t^m
Female					
m-4411	$+/t^m$	Misima (1)	7	1	1
Male					
m-306	$+/t^m$	Misima (1)	4	—	6
m-802	$+/t^m$	Misima (1)	10	—	14
m-9515	$+/t^m$	Misima (2)	16	1	23
n-63	$+/t^m$	Niigata	14	2	8
n-65	$+/t^m$	Niigata	7	—	10
k-1	$+/t^m$	Fukuoka	13	4	16
Totals of 6 males	$+/t^m$		64	7	77

It is not known whether the t^m alleles found in the wild mice from different localities are identical or not. Other questions to be answered concern: 1) the relative viability and fertility of various t^m/t^m individuals and 2) the relationship between the t^m-allele found in *molossinus* and the t or t^w-alleles known in *musculus*.

6. *Cytological and Genetical Studies on Sterility in Mammals*

I. Spermatogenesis of Male Tortoiseshell Cats

(By Takaaki ISHIHARA)

It is well known that the male tortoiseshell cat is sterile. This sterility is due to the entire absence of spermatogenesis in the seminal tubules. According to cytological observations on the testes of six male tortoiseshell cats, there is a great deal of individual variation in the rate of degeneration of the male cells. One of these cats entirely lacked spermatogenesis, two contained a few spermatogonial cells in the seminal tubules, one had its male germ cells developed to the secondary spermatocyte stage, while two tortoiseshell cats showed apparently normal spermatogensis. Thus, among the six male tortoiseshells examined, four showed distinct histological signs of sterility (Fig. 1), while two had apparently normal testes (Fig. 2).

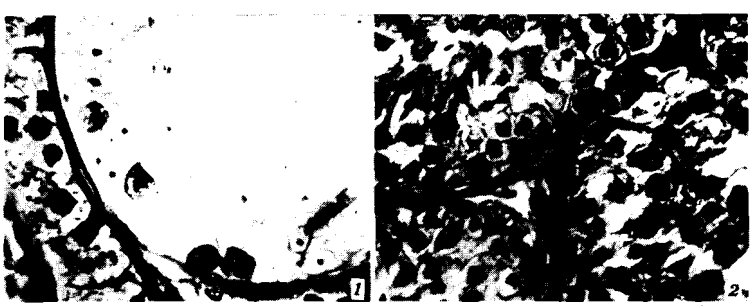


Fig. 1. A seminal tubule in the testis of an apparently sterile male tortoiseshell cat. Fig. 2. Seminal tubules in the testis of an apparently fertile male tortoiseshell cat.

It is not quite certain whether or not these two male tortoiseshell cats could have been fertile, but as far as can be judged from the morphology of their testes, they appear to be perfectly normal. In the testes of these two cats, spermatogonial cells have been found to contain 38 chromosomes including an X-Y set. In the primary spermatocyte, there are 18 pairs of autosomes and a pair of sex-chromosomes. The X-Y complex consists

of a long X and a small Y which are associated at their ends. Thus, these tortoiseshell males have exactly the same chromosome constitution as the normal male cat.

7. *Cytological and Genetical Studies on Sterility in Mammals*

II. Note on a Hydrotestis found in the Wistar-King-A rat Strain

(By Takaaki ISHIHARA)

Some rats of the Wistar-King-A strain kept in the Institute have developed hydrotestis (Table 1). This abnormality is found mostly in rats of ten months or older, and seldom in rats younger than six months of age. In order to examine whether hydrotestis is a heritable character hybrids between Wistar-King-A and Wyne-pink-eyed yellow strains were examined. As shown in the table, some of these F_1 rats developed the defect. This observation suggests that the hydrotestis occurring in the Wistar-King-A strain is a heritable character.

Table 1. Incidence of hydrotestis in rats

Strain	Age (in months)	Hydro-testis	Doubt-ful cases	Normal testis	Total number of individuals observed	Frequency
WKA	10–20	36	3	5	44	81.81
WKA	4–6	0	2	9	11	18.18
Wyne	10–20	0	0	25	25	0.0
W	10–20	0	0	5	5	0.0
Mixed strain	10–20	0	0	10	10	0.0
F_1(WKA♂ × Wayne♀)	10	2	0	2	4	50.0

The obnormal testis is five or six times as large as normal. The seminal lobes appear to be suspended in liquid accumulated in the organ. Histological examination of affected testes reveals remarkable degeneration of the germ cells, so that all rats which develop hydrotestis seem to be completely sterile.

8. *A New Micromelic Mutant Domestic Fowl.*

(By Takatada KAWAHARA)

In the hatching season 1955, seven abnormal embryos called micromelia, were found among the offspring of full brother-sister matings of White

Fig. 1. A normal (left) and micromelic (right) embryos, 21 days of incubation.

Leghorn. The great majority of these embryos survived to the end of the incubation period, but none hatched. Some individuals died after 15 to 18 days of incubation. The characteristics of these abnormal embryos are as follows: 1) The long bones of both extremities are very short; 2) the beaks, especially the upper beak, are shortened; 3) the body is soft owing to imperfect calcification of the bones; and 4) the amniotic and allantoic fluids are retained as highly viscous fluids until the end of the incubation period. The measurements of the long bones in normal and micromelic embryos are shown in Table 1.

Table 1. Average length of long bones in normal and micromelic embryos, 21 days of incubation.

Description of embryos	Femur	Tibia	Tarso-metatarsus	Humerus	Ulna
Normal embryos	19.00 ±0.54	24.14 ±0.30	17.00 ±0.28	11.50 ±0.21	10.07 ±0.21
Micromelic embryos	7.07 ±0.39	9.50 ±0.33	7.43 ±0.27	4.76 ±0.33	4.16 ±0.19
Difference	11.93 ±0.52	14.64 ±0.33	9.57 ±0.69	6.74 ±0.33	5.91 ±0.23

Among the offspring of several matings of parents presumably heterozygous for the gene for micromelia, 339 normal and 99 micromelic embryos were obtained, a ratio approximating 3:1 (expectation 328.5:109.5). Among the normal progeny, about two-thirds were heterozygous, and there was no indication of any lethality of the mutant gene. The sex ratio of the micromelic embryos was normal. These results indicate that this defect is due to a simple autosomal recessive gene. Some biochemical studies of this mutant were carried out by means of paper-chromatography. The most interesting results thus obtained is the finding of an exceedingly large quantity of magnesium and very little calcium in the micromelic bones, a relation which is just the opposite in the bones of normal chickens.

9. *The Sex Chromosomes in Hyla arborea japonica* GUENTHER

(By Tosihide H. YOSIDA)

Since the early works by von RATH (1895) and Carnoy and LEBRUN (1900)

several papers have been published dealing with the cytology of amphibians, yet the problem of sex chromosomes in this group remains unsolved to this day. The chromosome complex of the frog *Hyla arborea japonica* was observed by IRIKI (1939), who reported that the 2*n* chromosomes of this frog are 24 in number, and include a pair of large V-shaped elements which are apparently X and X; he believed the male to be homogametic.

My observation of the chromosomes of male germ-cells of this frog with the squash technique has revealed the following facts. Most spermatogonial cells contain 24 chromosomes (12-pair), of which 3 pairs are large and V-shaped, 3 pairs large and J-shaped, and the remaining 6 pairs small and J-or V-shaped (Figs. 2–4). The No. 9 pair (shown by "i" in Fig. 4) are unequal one member being somewhat smaller than the other, and the two arms of the larger member being of different length. This observation suggests that the unlike pair is the sex chromosomes, the larger chromosome is X and the smaller one, Y.

In the prophase of the spermatogonial cells there is a chromosome

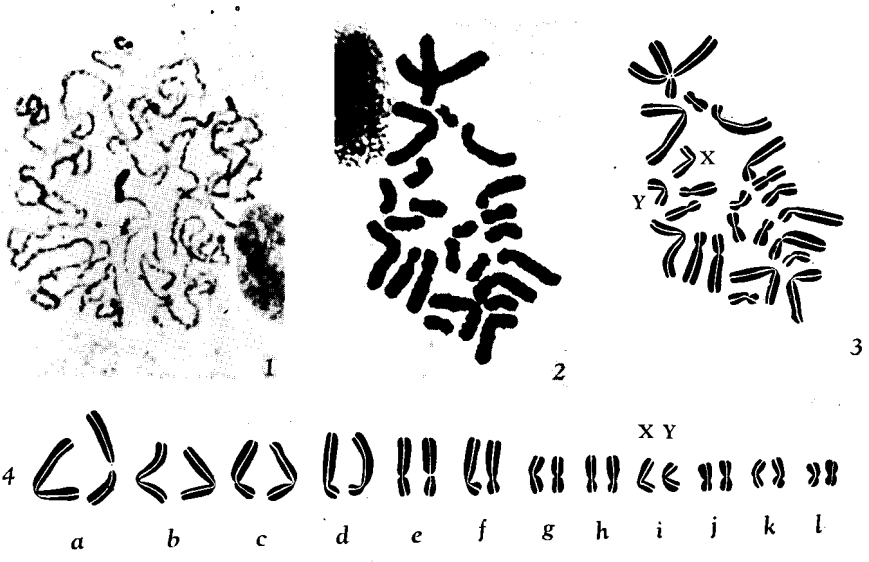


Fig. 1. Chromosomes in prophase in a spermatogonial cell of *Hyla arborea japonica*.
Fig. 2. Chromosomes in metaphase in a spermatogonial cell.
Fig. 3. Drawing to clarify Fig. 2.
Fig. 4. Serial alignment of chromosomes in the cell drawn in Fig. 3. X=X-chromosome, Y=Y-chromosome.

which is remarkable for its behavior, different from that of other chromosomes. When other chromosomes become indistinct by uncoiling and swelling, this particular one remains quite distinct (Fig. 1). This chromosome is probably X, as it is similar in shape and appearance to the X chromosome observed in metaphase cells. Thus, it seems justifiable to conclude that the male of this frog is of the X-Y type, and that the male is the heterogametic sex.

10. *Karyotype of Apus aecqualis* PACKARD (*Phyllopoda, Crustacea*)

(By Tosihide H. YOSIDA and Hiroyuki HIRUMI)

Among species belong to the Apodidae, one (*Apus* sp.) has been cytologically investigated by MOORE (1894). This author reports the striking fact that the ovarian cells in this species probably contain only one chromosome.

Material was gathered from a paddy field in the region of Numazu. Of 43 individuals, collected, 34 were males, and 9 females. According to UENO (1925) all of the 45 individuals he gathered in the suburbs of Wakayama were females. It is well known that males are rare in animals belonging to this family, so it is rather noteworthy that in the present lot, there were more males than females.

The chromosomes revealed by the squash technique were all very small. The spermatogonial cell contained 12 chromosomes consisting of a large V-shaped pair and 5 small rod or V-shaped pairs.

C. CYTOLOGY OF TUMORS

11. *Utility of a Pretreatment Technique Using Hypotonic RINGER's Solution for Observation of Chromosomes of Various Animals*

(By Tosihide H. YOSIDA and Takaaki ISHIHARA)

YOSIDA (1955) has found that the metaphase chromosomes in ascites tumor cells and in male germ cells of the rat may be spread apart from one another by pretreatment with hypotonic RINGER's solution: the chromatids of each chromosome also become distinguishable by this treatment. We have utilized this technique for the observation of chromosomes in various animals. It consists of three successive procedures: (1) pretreatment with hypotonic RINGER's solution (10 min.), (2) staining with acetic orcein, and (3) squashing. Our experiments have shown that the concentration of RINGER's

solution should be adjusted according to the type of tissue and the species of animal. The proper concentration for each of the species tested is shown in Table 1.

Pretreatment with a hypotonic solution has been utilized for the examination of animal chromosomes by several previous workers (MAKINO and NISHIMURA 1951, with water: HUGHES 1952, with hypotonic TYRODE's solution for tissue culture material: HSU and POMERAT 1953, with hypotonic GEY's solution for tissue culture material: WAHMAN and ZAHAVI 1955, with hypotonic TYRODE's solution: SCHMIDTKE 1955, with hypotonic NaCl solution:

Table 1. Appropriate concentrations of RINGER's solution for observations of chromosomes in various materials

Material used		Appropriate concentration of RINGER's solution (Ringer: Water, v/v)
Species	Type of tissue or tumor	
Rattus norvegicus	Male germ cells	1 : 10–100
	Hepatic cells	1 : 100
	YOSHIDA sarcoma	1 : 10–100
	MTK-sarcoma I	〃
	MTK-sarcoma II	〃
	Hirosaki sarcoma	1 : 10–20
Mus musculus	Male germ cells	1 : 10–20
	Hepatic cells	1 : 100
	Ascites tumor (MORI)	1 : 100
	Ehrlich ascites carcinoma	1 : 10–20
Lupus cuniculus domesticus	Male germ cells	1 : 5
Felis domestica	Male germ cells	1 : 20–30
Anas platyrhyncha domestica	Kidney cells	1 : 50
Hyla arborea japonica	Male germ cells	1 : 100
Various species of grasshopper	Male germ cells	1 : 100

and YOSIDA 1955, with hypotonic RINGER's solution). Our studies have revealed the necessity of selecting a suitable concentration of RINGER's solution for each material.

Reference: YOSIDA, T. H. and T. ISHIHARA 1955. A simple squash technique for observations of chromosomes in animals. La Kromosomo 27–28: 1005–1009.

12. *Utility of Various Concentrations of Chloride Solutions for Chromosome Study*

(By Tosihide H. YOSIDA and Yoshito OGAWA)

To attempt to find the mechanism inducing spreading and splitting of chromosomes by hypotonic RINGER's solution, the effects of various concentrations of chloride solutions, such as KCl, NaCl, $CaCl_2$, $MgCl_2$, $FeCl_2$, $FeCl_3$ and $AlCl_3$ were examined. The materials used were YOSHIDA sarcoma, ascites hepatoma No. 7974 and grasshopper gonads. The results are summarized in Table I.

Table 1. Spreading and separating effects of various chlorides on chromosomes (treatment with each solution for ten minutes)

Materials	Concentration	KCl	NaCl	$CaCl_2$	$MgCl_2$	$FeCl_2$	$FeCl_3$	$AlCl_3$
YOSHIDA sarcoma	N/10	−	−	−	−	−	−	−
	N/100	−	−	±	+	−	−	−
	N/1.000	−	±	+	−	−	−	−
	N/10.000	−	+	+	−	−	−	−
Ascites hepatoma	N/10	−	−	−	−	−	−	−
	N/100	−	−	±	±	−	−	−
	N/1.000	−	−	+	+	−	−	−
	N/10.000	−	−	+	+	−	−	−
Grasshopper gonads	N/10	−	−	−	−	−	−	−
	N/100	−	−	−	−	−	−	+
	N/1.000	−	−	−	−	+	−	±
	N/10.000	−	−	−	−	±	−	+

It is clear that KCl, which is the main constituent of RINGER's solution, is not an important agent for spreading and splitting chromosomes. Good results were obtained using N/1.000 NaCl and N/100 $CaCl_2$ solutions for YOSHIDA sarcoma cells, N/100 $CaCl_2$ and N/100 $MgCl_2$ solutions for ascites hepatoma cells, and N/10.000 $FeCl_2$ and N/1.000 $AlCl_3$ for grasshopper germ cells.

Based on these results we have made new mixtures of salt solutions which seem to be suited for the purpose. They are:-

YO I solution (for YOSHIDA sarcoma cells)
- N/1.000 ..... NaCl
- N/100 ..... $CaCl_2$

YO II solution (for ascites hepatoma cells)
- N/100 ..... $CaCl_2$
- N/100 ..... $MgCl_2$

YO III solution (for germ cells of grasshopper)

N/10.000..... $FeCl_2$
N/1.000 ..... $AlCl_3$

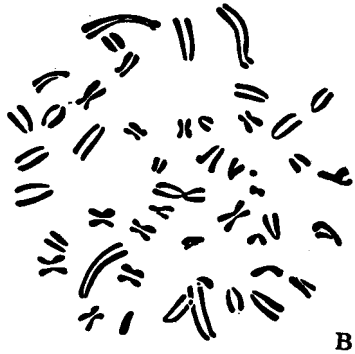
13. *Comparative Study of Chromosomes in Normal Hepatic and Ascites Hepatoma Cells of the Rat*

(By Tosihide H. YOSIDA and Takaaki ISHIHARA)

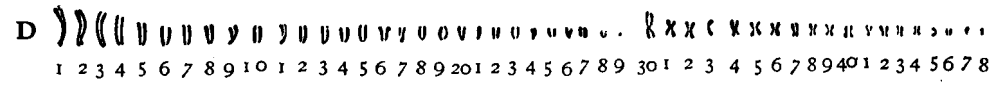
YOSIDA (1955) has found that the characteristic V-shaped chromosomes occurring in the tumor cells of the YOSHIDA sarcoma, the MTK-sarcoma II and III, and the Hirosaki sarcoma originate by fusion of two different chromosomes. To know whether such V-shaped chromosomes may be found in the course of development of the tumor, we have studied chromosomes of hepatic cell in various stages of developing rat hepatoma.

First, the chromosomes of normal hepatic cells and of the transplantable ascites hepatoma of the rat were observed utilizing our squash technique. Hepatic cells of new-born rats and in the regenerating liver

A



B



1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6 7 8 9 20 1 2 3 4 5 6 7 8 9 30 1 2 3 4 5 6 7 8 9 40 1 2 3 4 5 6 7 8

Figs. A and B. Chromosomes of two cells in ascites hepatoma No. 7974.

Figs. C and D. Serial alignments of these chromosomes. Fig. C corresponds with Fig. A. Fig. D corresponds with Fig. B. Out of 48, 29 are rod-shaped, and 19 V- or J-shaped. Chromosome No. 30 is of a large V-shaped and No. 29 is dot-shaped.

of adult rats were used as the normal somatic cells, while, the transplantable ascites hepatoma No. 7974, which originated in the SASAKI Institute of Tokyo, was used for the tumor material.

In the hepatic cells of new-born rats the chromosome number is highly variable (36–84). The majority of the cells have 42 chromosomes exactly like normal diploid cells, and the karyotype coincides with that of the spermatogonial cells (YOSIDA 1955). In the regenerating rat liver the majority of the hepatic cells are of polyploid or alloploid type. Determination of the chromosome numbers in these polyploid cells is very difficult because of clumping of the chromosomes. However, soms cells are distinctly of the diploid type, and have 42 chromosomes.

The tumor cells of the ascites hepatoma No. 7974 also show a wide range of variation in chromosome number (30 to 72), with the mode at 48. Based on a karyological analysis of these cells, it was found that out of the 48 chromosomes, 29 were rod shaped, and 19 V- or J-shaped (Figs. A–D). It is a noticeable fact that chromosome No. 30 is large and V-shaped and No. 29 is dot-shaped.

Based on the above investigations, it can be said that the tumor cells of this hepatoma have a characteristic chromosome complex which is never found in normal hepatic cells.

14. *Karyological Observation on a Hepatoma Developed in the White Rat*

(By Tosihide H. YOSIDA and Takaaki ISHIHARA)

In the course of experiments inducing hepatoma by the administration of p-Dimethylaminoazobenzen (D.A.B.) in the white rat, a hepatoma developed in a male. A karyological study of this hepatoma has yielded the interesting results described below.

The hepatoma developed after the administration of D.A.B. for 150 days. At autopsy, four tumorous masses were found in four regions of the hepatic lobes, two in the second lobe and two in the third lobe. The chromosome constitution of these tumors is summarized in Table 1. The majority of the cells in the No. 1 tumor had a subdiploid complex, while in the No. 3 tumor, cells of subdiploid and subtetraploid complex were included with almost equal frequency. On the other hand, the No. 4 tumor consisted entirely of cells containing a subdiploid chromosome complex. Karyological analysis of these tumor cells has revealed distinct V-shaped chromosomes in the cells of the No. 2 tumor. No such chromosome was observed in cells of the other tumorous masses. From these observations it may be said that the peculiar V-shaped chromosome is not a necessary constituent of tumor cells.

Table 1. Frequency of diploid, tetraploid and hyperploid cells occurring in the hepatoma.

No. of tumor	Hepatic lobe	Frequency			No. of cells observed	Variation in chromosome number	No. of cells observed
		$2n$	$4n$	$8n$–$32n$			
		%					
No. 1	2nd	29.0	52.0	19.0	100	25–151 (?)	20
No. 2	2nd	75.0	18.0	7.0	100	27– 78 (41)	37
No. 3	3rd	40.0	38.0	22.0	50	34–168 (81)	16
No. 4	3rd	96.6	3.3	0	30	—	—

15. *Observations on Hyperploid Cells in Regenerating Liver after Partial Hepatectomy of the Rat*

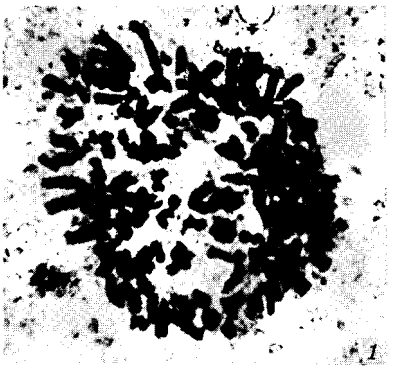
(By Takaaki ISHIHARA and Tosihide H. YOSIDA)

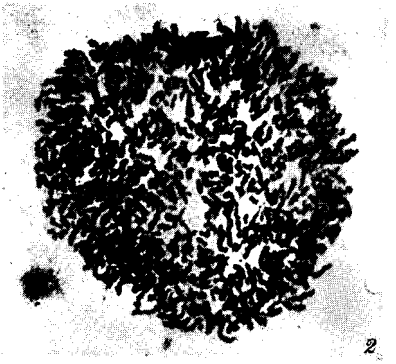
As is well known, after partial hepatectomy the remaining portion of the liver undergoes cell regeneration by mitotic divisions. The chromosomes of such regenerating hepatic cells were observed, using our squash technique.

Nine individuals of pure-strain (W, Wistar-King-A, Wyne) and mixed-strain rats were used in this study. The methods and results are shown in Table 1. There are more polyploid than diploid cells. Though the exact chromosome number in the hyperploid cells could not be determined, it apparently ranged from $8n$ to $32n$.

Table 1. Frequency of polyploid cells in the course of liver restration after partial extirpation

Ind. No.	Sex	$\pm 2n$	$\pm 4n$	$>\pm 4n$	Total	Strain	Age (mon.)	Hrs. after operation
1	♂	21	22	7	50	W	12	48
2	♀	45	110	87	242	W	8	48
3	♂	22	19	9	50	Wyne	5	24
4	♂	20	21	9	50	Wyne	5	72
5	♂	16	12	9	37	WKA	12	72
6	♂	17	23	10	50	WKA	18	72
7	♂	54	91	30	175	WKA	12	48
8	♀	9	22	19	50	N	3	48
9	♀	15	22	13	50	mix.	4	48
Total		219	342	193	754			
%		29	45	26	100			





Figs. 1, 2. Hyperploid cells occurring in regenerating liver after partial hepatectomy. 1, $8n$ cell. 2, $32n$ cell. The two photomicrographs were taken with a "Leitz Mikam" under the same magnification ($\times 2000$).

The relationship between the size of the cell-body and the number of chromosomes was examined in the regenerating hepatic cells. In $2n$, $4n$ and $8n$ cells, increase of cell size is roughly proportional to the chromosome number (Fig. 1). On the other hand, in the case of hyperploid cells (higher than $8n$), no further increase of cell size can be perceived. The chromosomes in these hyperploid cells are slender in shape, something like daughter chromatids in diploid cells (Fig. 2). These hyperploid cells are almost the same in size as tetraploid or octoploid cells. It is a noticeable fact that the increase of chromosome number in regenerating hepatic cells is not always followed by an increase in cell size.

The observations suggest that in a regenerating rat liver there are two types of polyploid cells, one in which the increase of chromosome number is accompanied by an increase in cell size and the other in which this parallelism is not found.

D. GENETICS AND BIOCHEMISTRY OF THE SILKWORM AND OTHER INSECTS

16. *Bi-and Uni-lateral Variations in Certain Characters of the Silkworm due to Unstable Genes*

(By Yoshimaro TANAKA)

The author has already revealed that the major genes *L* (for multilunar marking) and *K* (for knobbed) are highly stable, while the modifiers which control the numbers and positions of the multilunar spots and knobs are very unstable and variable. The most important characteristic

of these modifiers is that they cannot be fixed even after a long-continued selection, although selection may be effective in many cases. As previously mentioned (TANAKA 1951), the inheritance of unstable genes is difficult to analyse on a polygenic basis. The case to be described here is, it seems, another example which demonstrates the peculiar nature of the unstable gene.

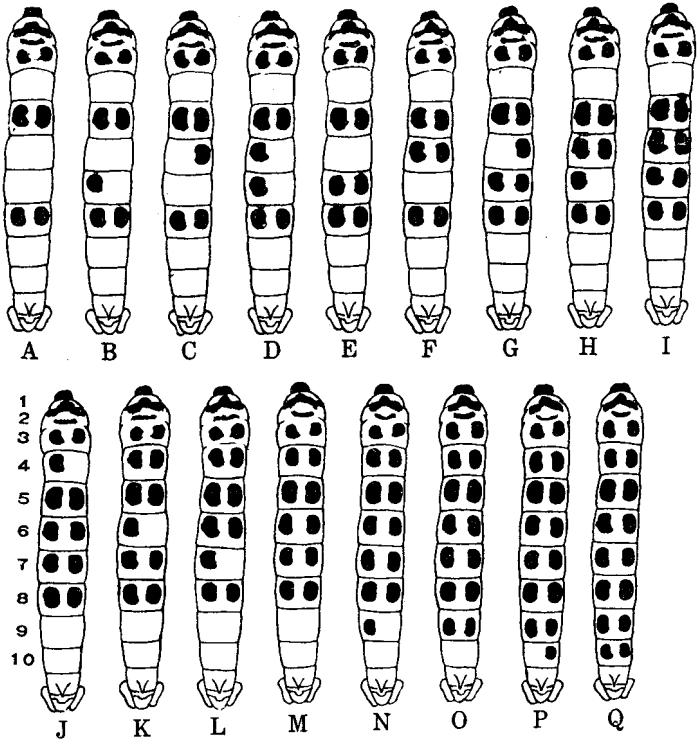


Fig. 1. Semi-diagramatic representation of the more frequent types classified by the numbers of large brown spots of multilunar markings. In counting the numbers of spots, those on the third segment are excluded as they are common to all types. The numerals on the left denote the segment numbers of the larvae.

A: 4-spot type B, C: 5-spot type D, E, F: 6-spot type
G, H: 7-spot type I: 8-spot type J, K, L: 9-spot type
M: 10-spot type N: 11-spot type O: 12-spot type
P: 13-spot type Q: 14-spot type

Among different multilunar strains, there are some lines in which 10-spotted larvae are usually most abundant (Fig. 1). One of these lines was selected for the 10-spot type through many generations, and the bi-lateral variation of spot number has been followed (Table 1). This is a typical case of unstable inheritance.

In another 10-spot line, a similar selection was practiced through a number of generations, except that some 14-spot individuals were introduced to ensure reproduction at two different times. This line gave almost exclusively, however, offspring with more than ten spots, showing a uni-lateral variation towards the plus side (Table 2).

A line of the "knobbed" strains showed for many generations a uni-lateral variation towards the minus side, when the 10-spot type only, with a single exception, was selected for propagation (Table 3.).

These facts seem to suggest a partial inactivation of variability of unstable genes, resulting in a stable condition in a plus or minus direction.

Table 1. Bi-lateral Variation of L-Spots

Lot No.	4	5	6	7	8	9	10	11	12	13	14	Total
291s271 (26)				1	24	97	**512**	68	12	1		723
301s271 (27)					51	136	**418**	157	57			829
311s271 (13)					31	133	**463**	55	5			694
321s271 (6)				1	70	134	**315**	41	12	1		581
331s271 (13)					26	82	**396**	25	2	1		542
341s271 (29)			1		54	68	**26**	50	28	5	2	299
351s271 (4)			1	5	84	159	**168**	5	1			426
361s271 (14)				1	112	108	**72**	60	26	2	1	421
371s22 (5)					6	40	**165**	12				227
381s22 (15)					3	34	**119**	59	17		2	238
391s22 (12)					30	89	**270**	40	13		1	449
401s25 (14)					22	77	**323**	12				434
411s25 (9)					12	38	**109**	43	20	8	3	239
421s25 (14)					31	45	**99**	10	6		1	195
431s25 (7)							**176**	25	4			205
441s25 (13)							**185**	19	3			207

Note: 1) The numerals in the top row represent the spot types.
2) The numbers in heavy type connected by lines show the spot types selected for reproduction in successive generations.
3) The figures enclosed by parentheses are the numbers of egg-batches from which the larvae hatched out.

Table 2. Uni-lateral Variation of L-Spots

Lot No.	4	5	6	7	8	9	10	11	12	13	14	Total
401 1 61 (14)						4	**133**	23	11	1		172
411 1 61 (14)					3	7	**190**	8				208
421 1 61 (14)					1	5	**90**	15	4			116
431 1 61 (13)							**194**	29	6			229
441 1 61 (15)							**147**	25	4			176
451 1 61 (16)			1		1	1	**194**	35	10			242
461 1 61 (14)							**97**	7	3	2		109
471 1 61 (7)			1				**125**	25	3		1	155
481 1 61 (14)							**20**	1	1			22
491 1 61 (5)						3	**69**	6	3	1 62(12)	**240**	321
501 1 61 (9)							**77**	5	1	1 62(4)	**186**	269
502 1 621 (1)							**65**	42	36	24	66	233
503 1 621 (1)							**37**	20	52	46	116	271
504 1 6212 (1)							**124**	15	28	11	49	227
511 1 6212 (1)							**27**	33	66	47	80	253
512 1 6212 (1)							**232**	35	18	2		287
513 1 6212 (1)							**60**	12	18	4		94
514 1 6212 (1)							**180**	28	9	3	1	221
521 1 17 (1)							**47**	61	103	108	187	506
531 1 17 (5)							**319**	112	55	29	17	532
532 1 17 (1)							9	20	46	32	**18**	125
533 1 171.. (1)							**29**	21	55	49	14	168
541 1 172 (2)							**86**	102	212	65	79	544

For legend see Table 1.

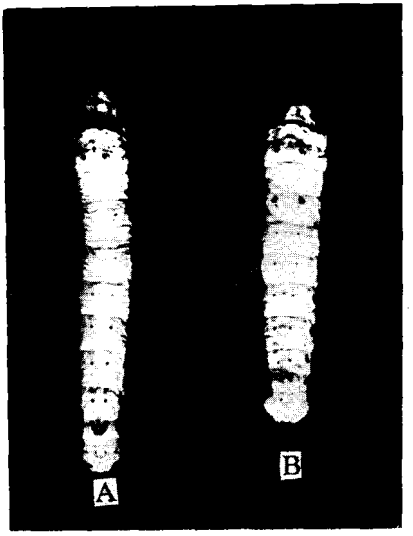
Table 3 Uni-lateral Variation of Knobs

Lot No.	4	5	6	7	8	9	10	11	12	13	14	Total
512 s 312 (1)	15	26	82	12	4	4	**3**					146
513 s 313 (1)	2	4	18	14	10	3	**6**					57
514 s 313 (2)		1	12	15	23	41	**89**					181
521 k 6 (2)			4	10	11	39	**434**					498
531 k 6 (3)		4	47	3	59	117	**210**					440
541 k 6 (3)			12	7	20	53	**217**					309
542 k 6 (2)			**5**		15	28	**250**					298
543 k 6′ (1)			5	8	12	30	162	**9**	**3**			229
544 k 6 a (2)						3	**55**					58
551 k 6 a (4)						4	**464**					468
552 k 6 a (1)					2	30	**229**					261

For legend see Table 1.

17. *A new E^{Ca} Strain of the Silkworm*

(By Mitsuo TSUJITA)



A: Larva without crescent or star patterns
B: Larva with rudimentary crescent patterns on the 5th segment

Among the F_1 silkworms from the cross $E^{Ms}/+ \times +$ reared in the third rearing season of 1951, one apparently new mutant male was found. This larva had supernumerary crescent patterns on the dorsal side of the 6th segment and lacked the star patterns on the 8th dorsal segment. At first this was suspected to be an $+/E^{Lc}$ larva, a type which occasionally appears by segregation from such a cross. However, genetic analysis of

this larva has shown that it was not an E^{Mc} but an $E^{Ca'}$ (a type of E^{Ca}) mutant.

This mutant was crossed to a wild type female, and segregation occurred in F_1 generation as shown in Table 1.

Table 1. Segregation in the cross + × double crescents

Mating type	+	Triple crescents*	Double crescents (II)**
+ × double crescent	267	7	258

* larvae with paired crescent patterns on the 5, 6 and 7th segments.

** larvae lacking the crescent patterns on the 6th segment or having rudimentary crescent patterns on the 5th segment (Photo. B).

The larvae with the double crescents were sib-mated and three batches were reared the next spring. Segregation took place as in Table 2.

Table 2. Segregation in the sib-mating of double crescents

No.	Mating type	+	Triple crescents	Double crescents (I)*	Double crescents (II)	Non- crescents**
1	Double crescents × double crescents	100	13	157		
2	〃	48		11	73	25
3	〃	90		49	115	

* Larvae with paired crescent patterns on the 5th and 6th segments.

** Larvae lacking crescent patterns on the 5th and 6th segments (photo. A).

As shown in Table 2, from a batch of sib-matings of double crescents (No. 2), three types of larvae appeared; namely, 11 with double crescents (I), 73 with double crescents (II) and 25 without crescents.

Next, sib-matings of the non-crescent individuals yielded a number of larvae without any crescents or star-shaped markings and also larvae with rudimentary patterns on the 5th segment. By selection of unmarked individuals, a line was established in which most of the larvae were of the same phenotype, lacking in the crescent patterns on the 5th and 6th segments. So far, however, no strain in which the phenotype of larvae is entirely uniform, has been obtained.

The larvae without the crescent markings resemble the larvae of strain *Nl*, and the larvae with the double crescents (II) resemble the larvae of E^{Cr}. However, homozygous individuals of both types die in a late embryonal stage, exhibiting the characteristics of an E^{Ca} embryo.

The $E^{Ca}/E^{Ca'}$ embryo produced from the cross $E^{Ca}/+ \times E^{Ca'}/+$ shows

the same abnormarity as the E^{ca}/E^{ca} embryo.

From these observations, it is clear that the genic constitution of the non-crescent type is identical with that of E^{ca}. However, there must be some reason why most of the larvae of this strain lack the crescent patterns. One possible explanation is that $E^{ca'}$ is somehow different from E^{ca} which has a characteristic for suppression of the crescent patterns on the 5th and 6th segments, and if these patterns appear, it is apparently due to the presence of several recessive modifiers. Another possibility is that $E^{ca'}$ has the same genic constitution as E^{ca}, but that the presence or absence of the crescents depends on modifiers which act as suppressors or enhancers of the patterns.

18. *A No-lunule Mutant in the Silkworm Induced by X-ray Treatment*

(By Mitsuo TSUJITA)

Female pupae of the genotype *HKp/HKp* were irradiated with X-rays of 8000 r, and the imagines were mated to normal males. Two exceptional larvae lacking crescent and star markings and having the phenotype *Nl* (No-lunule), were found among 7346 F_1 individuals. The female moths emerging from these larvae were crossed with normal males, and the following segregation was observed:

+		*Nl'*		Total
HKp	+	*HKp*	+	
79	95	80	100	354

Genetic analysis of this mutant has disclosed that it is homolethal and death occurs in the embryonal stage. The heterozygous larvae are viable and show the characteristic phenotype. In appearance this mutant resembles *Nl*/+ discovered by Y. TANAKA, therefore the same symbol, *Nl'* may be used. It has been revealed that the gene for this mutant is completely linked to the *U* locus. No recombinants were obtained from either the cross $U/Nl' \times +$ or $+ \times U/Nl'$.

The recombination value between *odk* and *Nl'* is 4.9, which is higher than the 2.9 which was estimated for *odk* and *Nl* (Tsukushi 1955).

In another experiment the recessive gene *oa* was used. This is located on the XIV chromosome and larvae homozygous for *oa* have a translucent integument. Two types of larvae, *Nl'*/+ and normal, segregated from

the cross *Nl'*/+ × *oa*/*oa*. All of the larvae of the former genotype have translucent integument and all of the latter genotype have normal integument. It may be inferred from this result that *Nl'* is a deficiency of the region of the *oa* locus on the XIV chromosome.

The results of the genetical analysis of *Nl'* stated above are nearly identical with the results obtained for *Nl* by TSUKUSHI (1955). To examine the genetic relation between *Nl* and *Nl'*, the cross +/*Nl* × +/*Nl'* was made, giving some F_1 individuals of the genotype *Nl*/*Nl'*. These showed lethality in the embryonal stage. Thus, in genetic constitution *Nl'* can not be discriminated from *Nl*.

19. *Induction of the Mutants E^{ca} and Nl by X-ray Treatment*

(By Mitsuo TSUJITA)

The mutant E^{ca} appears spontaneously, though extremely rarely. *Nl* was discovered first by Y. TANAKA (1925) as a spontaneous mutant. However, as far as I know, it has never since been found as a spontaneous mutant. Both of these mutants can be easily induced by X-ray treatment.

In my experiments, some female pupae of the genotype *HKp*/*HKp* were irradiated by X-rays of various dosage as shown below (Table 1).

Table 1. Details of X-rays utilized for experiments

Season	Kvp	mA	Distance cm	r/min	Duration min.	Total dosage r
1954 Summer	180	3	20	162.44	49.3	8000
1955 Spring	180	3	40	40.61	194.6	8000
1955 Summer	180	3	20	162.44	49.3	8000

The experiments were repeated three times: the results are shown in Table 2.

Table 2. Progeny of X-rayed *HKp*/*HKp* (♀) × +(♂)

Season	No. of treated moths	Exceptional types				*HKp*/++	Total
		E^{ca}	*Nl*	Several other mutants	Total of mutants		
1954 Summer	167	10	2	21	33	7346	7379
1955 Spring	230	16	20	29	65	29568	29633
1955 Summer	31	2	1	1	4	204	208

Mutants were looked for in the stages from the 2nd to 4th instars. It is possible that some exceptional individuals escaped our notice because the characteristics of the mutants studied are hard to discriminate in larvae that are too young.

As shown in the above table, several exceptional types were found. Among these, E^{ca} and *Nl* individuals are commoner than any other mutant. Moreover, these mutants were produced in all three experiments.

Nl is a deficiency in the XIVth chromosome. E^{ca} may be also some chromosomal aberration, perhaps a deletion of the *E* region of the VIth chromosome. It is noteworthy that most of the mutants induced by X-ray treatment are located on either the VIth or XIVth chromosome. This fact suggests some affinity between these two chromosomes.

20. *Studies on Maternal Inheritance of the Lethal Yellow in the Silkwarm. Exchange of Ovaries between +/lem^l and lem/lem^l Larvae.*

(By Bungo SAKAGUCHI and Mitsuo TSUJITA)

The maternal inheritance of the yellow lethal in the silkworm has been described by TSUJITA ('53, '54, '55). To make clear the mechanism of this peculiar inheritance the present experiments were undertaken.

Fifth-instar larvae with genotypes +/*lem*^{l} and *lem*/*lem*^{l}, which had segregated from a cross between +/*lem* and *lem*/*lem*^{l}, were used as material. The ovaries of larvae of the different types were exchanged. Of 630 larvae thus operated on, only 3 survived and had progeny.

The female moths produced from these larvae were mated with male moths of the genotype *lem*/*lem*^{l}. The eggs laid were incubated after treatment with heated dilute hydrochloric acid, and the larvae homozygous for the *lem*^{l} gene were examined. The results may be summarized as follows.

When the +/*lem*^{l} larvae were implanted with ovaries of *lem*/*lem*^{l}, the homozygous *lem*^{l} embryos which develop from these transplanted eggs hatch out as apparently normal, black-colored larvae, but they change into distinctly yellowish larvae immediately after the first moulting and starve to death. On the other hand, the *lem*^{l} homozygous larvae emerging from eggs laid by the *lem*/*lem*^{l} moth which had received a +/*lem*^{l} ovary by transplantation develop into yellowish brown larvae: they, however, die in the egg as they are unable to chew through the chorion.

From these experimental results it may be safely concluded that lethality is not determined by the genotype (+/*lem*^{l} or *lem*/*lem*^{l}) of the

embryo but by the genotype of the mother's body in which the eggs develop.

It seems that the maternal inheritance of the yellow lethal character is due to the presence or absence of some diffusible chemical substance in the oöplasm of eggs produced by the mother moth. This substance is closely related to xanthopterin-B according to our genetical and biochemical studies of this lethal strain already published.

21. *Genetical and Biochemical Studies on the Yellow Lethal Silkworm. IV. Changes in Quantity of Pterin Compounds in Explanted Ovaries.*

(By Bungo SAKAGUCHI and Mitsuo TSUJITA)

In another experiment it has been disclosed that the lethality of larvae of this type depends on the genotype of the mother, and not on the genotype of the egg. In order to clarify what substance is directly concerned with this phenomenon, the following experiments were carried out.

Wild type ($+/lem^l$) and lemon (lem/lem^l) individuals, segregated from the cross between $+/lem^l$ and lem/lem^l, were used as material, transplantions were carried out on larvae in the first or second day of the 5th instar stage. The ovaries were removed from individuals of genotypes $+/lem^l$ and lem/lem^l and exchanged by transplantation. When the individuals thus operated on developed to adults, their ovarian tubules were removed and chemically examined.

The pterins contained in the ovarian tubules were extracted with 30% acidified ethanol. The solution was fractioned by paper chromatography, A methyl-enthyl-ketone+propionic acid+H_2O solvent was used as a developer for the fraction. The quantitative analysis of pterins was made with a fluorophotometer.

By this procedure, several fluoresecent substances were identified. A striking difference was detected in the quantities of isoxanthopterin and xanthopterin-B contained in the ovarian eggs of the operated individuals of the two types as compared with the ovarian eggs of control individuals of the corresponding genotypes. The results are shown in Table 1.

As shown in the table, the effect of the maternal body upon the production of pterins is very remarkable. When ovaries of the genotype lem/lem^l were transplanted into individuals of the genotype $+/lem^l$, the amount of isoxanthopterin in the ovarian eggs increased remarkably, but the xanthopterin-B decreased. On the contrary, in the ovarian eggs of the genotype $+lem^l$ transplanted into larva of the genotype lem/lem^l, the

Table 1. Quantitative analysis of pterins in the transplanted ovaries (relative value).

	$+/lem^l$	lem/lem^l	Ovaries transplanted from lemon (lem/lem^l) to wild type ($+/lem^l$)	Ovaries transplanted from wild type ($+/lem^l$) to lemon (lem/lem^l)
isoxanthopterin	57.0	15.0	42.8	24.2
xanthopterin-B	17.0	30.0	22.0	29.8

amount of isoxanthopterin decreased significantly and that of xanthopterin-B increased.

It seems probable from this experiment that the maternal inheritance of the yellow lethal depends upon the relative amount of isoxanthopterin and xanthopterin-B present in the oöplasm and that this is controlled by the somatic cells of the mother.

22. *Genetical and Biochemical Studies on the Yellow Lethal Silkworm*

V. On Metal Metabolism of this Mutant

(By Mitsuo TSUJITA and Bungo SAKAGUCHI)

For the purpose of determining the relation between the phenomenon of maternal inheritance of the yellow lethal character and metal metabolism, further experiments were carried out.

Materials and Methods: Eggs, larvae and imagines of the genotypes $+/+$, $+/lem^l$, lem/lem^l and of the yellow-lethal eggs which segragated from the cross $lem/lem^l \times +/lem^l$, were used. The material was ashed, and an analysis of metals was made by paper chromatography. In order to detect the metals, such reagents as rubeanic acid, potassium ethylxanthogenate, pyrochatechol and dithyzone were sprayed on the chromatogram.

Experimental results:

1. Detection of metals in various developmental stages. Fe, Cu, Co and Ni were found throughout the developmental stages up to metamorphosis in the normal strain, and Ti was also detected in the lemon strain. It was found that throughout development there was a larger amount of Ti in individuals of the genotype lem/lem^l than in individuals of the genotype lem/lem. In the former Cu was not detectable. Thus, among the $+$, lem, and lem^l strains, the following relations hold regarding the relative amount of Cu and Ti: For Cu there is the relation $+ > lem$ and for Ti $lem^l > lem$.

2. Experiments with metal injection. Chlorides of metals such as Fe, Cu, Ni, Co, and Ti were injected into pupae of +, *lem* and *lem*^{l} strains, and the metal elements contained in the eggs laid by the moths emerging from the injected pupae were examined. The results show that all metals injected into the pupae may be detected, but that they have produced no effective change on the phenomenon of maternal inheritance of the yellow lethal.

Conclusion: From these experimental results, it may be seen that a much larger amount of Ti is accumulated in the *lem*^{l}/*lem*^{l} individuals than in individual of any other genotype. This fact seems to indicate that there is a close relationship between Ti metabolism and the phenomenon of maternal inheritance of the yellow lethal. However, the experiments with metal injection put in doubt the interpretation that the maternal inheritance of the yellow lethal is only due to the presence of Ti in a large quantity. More probably, the coexistence of Ti and some other substances, and their co-operative action, is necessary for this phenomenon.

23. *Photoperiodic Effects of Alternated Dark and Light Phases of Diapause of Antheraea pernyi*

(By Yoshimaro TANAKA)

In previous papers (TANAKA 1950, 1951) the author reported that subjected to a short day (less than 13 light hours) Antheraean pupae hibernate, while with a long day (more than 15 light hours), if the whole larval life is exposed to the same photoperiod, all pupae become non-dormant without exception.

In recent experiments larvae were exposed to intermittent light and dark phases of fixed duration during the period from hatching to cocooning in the second culture of 1955.

Experiment I. 2-hour light and 1-hour dark phases alternated.

Experiment II. 1-hour light and 2-hour dark phases alternated.

In Exp. I, where the total light hours per day were 16, 2.5% pupae hibernated; thus the long-day effect was apparent. In Exp. II, there were 8 total light hours per day, but the hibernation frequency was only 12.15%. This seems to mean that the short-day effect cannot be perfect when the dark phase is interrupted by a light phase so frequently.

Similar experiments with different durations of light and dark phases were made in the third culture.

Experiment I. $2^{1}/_{2}$-hour light and $^{1}/_{2}$-hour dark phases alternated.

Experiment II. $^{1}/_{2}$-hour light and $2^{1}/_{2}$ hour dark phases alternated.

The total light hours were 20 in Exp. I, and 4 in Exp. II, the hibernation frequencies were 8.55% and 42.3%, respectively.

From these results we may conclude that a continuous illumination of 4–13 hours is indispensable for a perfect short-day effect, but the long-day effect is not much affected by intercalary darkness.

24. *Genetical and Biochemical Studies on Pteridine Metabolism.*

(By Saburo NAWA and Toshifumi TAIRA)

It has been found previously that the red eye pigments of *Drosophiia melanogaster* are closely related to pteridine[1]. The yellow pigment found in the eye of the mutant strains *se* and *cl* has been identified as xanthopterin-B[1] which has the structure N^8-lactyl-7, 8-dihidro-2-amino-4-hydroxypteridine-6-carboxylic acid[2].

In the eyes of the double mutants *v*; *se* and *v*; *cl* there is accumulation of xanthopterin-B, so that the scheme:

$$\text{xanthopterin-B} \xrightarrow[(se,\ cl)]{} \text{red pigments}$$

can be suggested.

The body of strain *sed* contains a larger amount of yellow pigment and a smaller quantity of isoxanthopterin than do the bodies of other strains. This yellow pigment in *sed* has been identified as xanthopterin-B by examining the Rf value, fluorescence, ultraviolet absorption spectra and chemical properties. It has been found that there is an accumulation of xanthopterin-B and an inhibition of isoxanthopterin production in the body of the double mutant, *cn*; *sed*. Such a relationship has also been found in the *lem* mutant of the silkworm. Thus the scheme:

$$\text{xanthopterin-B} \xrightarrow[(sed,\ lem)]{} \text{isoxanthopterin}$$

may be proposed.

To examine the interrelationships among xanthopterin-B, isoxanthopterin and the red pigments some *in vitro* experiments have been carried out, but no clear results have been obtained so far.

1) NAWA, S. and TAIRA, T. 1954. Proc. Japan Acad. 30:632.
2) FORREST, H. S. and MITCHELL, H. K. 1954. J. Am. Chem. Soc. 76:5658.

25. *Biochemical Studies on the Hibernating Character in the Chinese tussah Silkworm, Antheraea pernyi. II. The Role of Sugar Metabolism in Hibernation.*

(By Bungo SAKAGUCHI)

TANAKA ('41–'43, '50–'51) has found that photoperiodism plays the most important role in inducing hibernation of the Chinese tussah silkworm, *Antheraea penyi* Guer. As reported in the first paper of this series, there is slight difference in the respiratory emzyme system between hibernating and non-hibernating pupae of this insect.

a) The effect of an artificial supplement of sugar on hibernation.

The present experiments were undertaken to explore the response of hibernating and non-hibernating pupae to sugar supply. Hibernating larvae in the middle stage of the 5th instar, obtained by photoperiodic treatment of the eggs and larvae, and non-hibernating larvae were kept for four days in the presence of an artificial supply of 0.88 M sucrose solution. Then the number of days required to pass from pupa to imago was recorded for hibernating and non-hibernating larvae. Of the 20 pupae derived from hibernating larvae, 9 developed imagines in 30 to 40 days, 4 to 60 days, and 3 remained in hibernation. On the other hand, the non-hibernating larvae thus treated did not develop into hibernating pupae in spite of the sugar supplied. Consequently, it may be concluded that sucrose has some effect on the hibernating character.

b) Detection of sugar in the blood of larvae with different hibernating characters.

On the basis of the above results, and to further examine the relation between the hibernating character and sugar metabolism, further experiments were undertaken. Sugar was obtained by acid hydrolysis of the blood of 5th instar larvae and examined by paper chromatography. The chromatograms were run with butanol+acetic acid+H_2O or phenol and sugars on the paper were detected by the reaction of an aniline chloride solution.

Sucrose, galactose and glucose were distinctly detected in the blood of non-hibernating larvae, while only traces of these sugars were found in hibernating larvae. In the latter some unknown substances related to sugars with an Rf value of 0.2 were detected by a solution of phenol or butanol+acetic acid+H_2O.

It is likely that there is a close relationship between hibernation and sugar metabolism, and that sucrose is factor effective in controlling the hibernating character. LIVERMAN and BONNER (1953) reported that sugars

(glucose, sucrose, galactose) and Krebs cycle intermediates (citrate, malate), even without illumination of high intensity, affected the photoperiodism of *Xanthium pensylvanicum*. My previous (1955) and present results, which show a physiological relation between photoperiodicity and the Krebs cycle or sugar metabolism, confirm these findings.

E. POPULATION GENETICS OF SOME INSECTS AND A LAND SNALL

26. *Population genetics of a ladybeetle and a land snail.*

(By Taku KOMAI)

A short paper on an actual instance of microevolution observed in a population of the ladybeetle, *Harmonia*, inhabiting Suwa (*Ann. Rep. Nat. Inst. Genet. 5*: 37–38) was read before the Symposium on Population Genetics held at Cold Spring Harbor, June 6–13. A review, "Genetics of ladybeetles" was completed for *Advances in Genetics* vol. 8. A paper by Komai and Emura, "A study of population genetics on the polymorphic land snail, *Bradybaena similaris*", was published in *Evolution* (vol. 9, no. 4: 400–418).

The records of the results obtained by studies of population genetics with various materials, which had been carried on during the period 1951–1953 in cooperation with a group of investigators, were completed in the current year and published in a book entitled "Syûdan Idengaku (Population Genetics)" from Baifûkan, Tokyo. The book contains the following articles by Komai:—

"Population genetics of the lady-beetle, *Harmonia axyridis*" (pp. 46–60),

"Population genetics of the land-nail, *Bradybaena similaris*" (pp. 61–76),

"Population genetics of two species of butterflies" (pp. 77–83).

Some supplementary experiments on the capacity of tolerance for adverse temperature and humidity of *Harmonia* and *Bradybaena* were conducted. A small sample of a population of *Harmonia* was obtained from Taipei, Formosa. This consisted entirely of the type *conspicua*, and suggests a closer affinity of this population to populations of the same species in Kyûsyû, rather than to those of Middle China.

27. *Population Genetics of Balanced Polymorphism in Drosophila rufa.*

(By Toshifumi TAIRA)

The female of this species is distinctly dimorphic. The "dark" type has a dark band on each abdominal segment, much like the male *melanogaster*. The "light" type has no such band, so that it resembles the female *melanogaster*. The male is monomorphic. The dark marking in the female shows complete dominance over the light marking.

The result of analysis of this polymorphism is reported in *Syudan-Idengaku* (edited by T. KOMAI and K. SAKAI, 1956). The relative adaptive value of different genotypes in natural populations has been shown to be $D/d > D/D > d/d$. In laboratory populations in an equilibrium state, started at various initial proportions of each genotype and kept at 25°C, the relative adaptive value is $D/d > d/d > D/D$. This relationship is kept when the population making up an equilibrium state is moved from 25°C to 18°C.

Our experiments have revealed that the difference in the relative adaptive values of the two homozygotes in the populations studied is apparently due, in part, to the fact that D/D has an advantage over d/d in mating, while d/d is superior to D/D in the competitive viability of the larva. The experimental results, however, are not sufficient to completely explain the difference between these relative adaptive values in the two populations in natural and laboratory. Our observations on natural populations have also shown that d/d has some advantage over D/D in the spring time, while it has some disadvantage in the summer, suggesting that d/d is better fitted to low temperatures than is D/D.

Further experiments, one concerning the effect of population density, the other on the effect of low temperature on the two homozygotes, were carried out. The results are presented in Tables 1 and 2. The initial populations contained the dominant (D) and recessive (d) genes in equal proportion. The process of change of the proportion with time is shown in these tables.

Table 1. Frequency of d/d individuals in large populations

Days	0	61	122	153	187	240
25°C	.50	.34±.014	.28±.013	.25±.027	.29±.014	.32±.016
18°C	.50	.66±.014	.51±.018	.62±.019	.42±.049	.49±.016

Table 2. Frequency of *d*/*d* individuals in small populations

Days	0	24	38	45	75	155
25°C	.50	.34±.025	—	.27±.016	—	.25±.008
18°C	.50	—	.36±.022	—	.41±.013	.49±.012

The large populations, one at 18° and the other at 25°C, differ in the frequency of *d*/*d* individuals. A similar difference may be found between two smaller populations, one at 18° and the other at 25°C also.

These data suggest that *d*/*d* has an advantage over *D*/*D* either in vitality or competitive ability, or both. This seems to be the main reason why *d*/*d* is always maintaind in a frequency higher than *D*/*D*, in spite of the inferiority of *d*/*d* in mating, both in natural spring populations and in laboratory populations.

28. *Variation in the Elytral Pattern of Convergent Lady-birds (Hippodamia convergens) collected in Colorado*

(By Tosihide H. YOSIDA)

Specimens of the convergent lady-bird (*Hippodamia convergens*) were collected in Fort Collins, Colorado, in June, 1952 by Dr. S. MAKINO of Hokkaido University, who was then travelling in America. The specimens were sent to me for examination of variation in the elytral pattern.

Table 1. Variation of the elytral pattern in convergent lady-birds (*Hippodamia convergens*)

Author	Class				Total
	A	B	C	D	
YOSIDA (1955)	498 (86.1%)	1 (0.2)	79 (13.6)	0	578 (99.9)
KELLOGG & BELL (1904)	900 (86.8)	27 (2.6)	70 (6.8)	38 (3.7)	1035 (99.9)

Remarks: Class A represents the normal type having 12 modal spots on each elytron. Class B is a type having some coalesent spots. Class C is of type with some spots lacking. Class D is of a type having more than 12 modal spots.

The results obtained are summarized in Table 1, together with the data obtained by KELLOGG and BELL (1904) on material collected at Palo

Alto, California. Considerable difference in the mode of variation of the elytral spots may be found in the two sets of data. Generally speaking, the beetles inhabiting Colorado have smaller spots than those from California. It is likely that this difference is correlated with the difference in climate in the two localities. SHULL (1944) has carried out crossing experiments between spotless and spotted convergent lady-birds. From the data obtained, he has concluded that there is a spotless gene which is almost completely dominant over the gene for spotting, as well as some modifying genes. The difference in the frequency of spotless individuals is mainly due to the difference in the incidence of the gene. He has found that the material from Colorado has the spotless gene in 6–7 per cent of the individuals while the California material has it in only 2 3.5 per cent, and the modifiers are nearly three times as frequent in Colorado as in California beetles. The present material confirms SHULL'S findings in the frequency of spotless individuals.

KELLOGG, V. L. and R. B. BELL 1904. Studies of variation in insects. Proc. Wash. Acad. Sci. 6: 203–332. SHULL, A. F. 1944. Inheritance in lady beetles II. The spotless pattern and its modifiers in *Hippodamia convergens* and their frequency in several populations. Jour. Hered. 35: 329–339. YOSIDA, T. H. 1956. Variation in elytral pattern of the convergent lady-birds (*Hippodamia convergens*) collected in Colorado. Annot. Zool. Japon. 29: 101–103.

F. GENETICS AND CYTOLOGY OF RICE

29. *Polygenic Nature of the "Gametic-Development Genes" in Rice*

(By Hiko-Ichi OKA)

The sterility found in hybrids between cultivated varieties of rice can be explained by assuming duplicate genes for gametic-development which, if both are recessive, cause deterioration of the gametes carrying them. The results of analyses for these genes carried out by the writer have been published in the Annual Report of 1954 (p. 40) and in the Japanese Journal of Breeding (2:217–224 & 3:23–30). The experiments consisted of a crossing of an "$(A \times B) \times C$"* design, where a clear segregation of fertile and semi-sterile plants takes place. By postulating one or two sets of duplicate Gametic-development (G. D.) genes, the behavior of progeny with respect to sterility can be successfully accounted for. In some ex-

* Varieties A and B are closely related, but differ in fertility in the hybrids with the third variety C.

periments of this type, however, cases have been found in which the distribution of the percentage of good pollen has several modes. In these cases an exact analysis of the responsible genes is rather difficult. Nevertheless, by assuming several sets of G. D. genes, the observed frequency distribution of good pollen percentages can be fitted to the expected distribution.

In the F_2, the percentage of good pollen, as well as of seed setting, varies from plant to plant in a continuous series. Exceptional cases may be found, as shown in Table 1. Here plants with pollen fertility of 95–90%, 75–70%, 55%, 40% and 30% appeared in an apparently discontinuous series. These values seem to correspond to the theoretical values: 1, 0.75, $0.75^2 \cdots 0.75^k$, which are to be expected when k sets of G. D. genes segregate independently. In the present case, the observed frequency distribution may be fitted to the expected distribution by assuming five sets of G. D. genes.

Table 1. An example of discontinuous distribution of pollen fertility in the F_2 (from a cross between two Philippine varieties belonging to the "Tropical-Insular group", 219×218).

	% of good pollen																Num. of
	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	plants
Obs.	2	6		2	4	6	1	1	6	3	2	5	1	2	1		1	43
		8				13				10			8		3			$X_2=3.19$
Exp.		5.68				14.10				14.15			7.05		1.81		0.22	$P>0.30$

The results of the observations given above seem to indicate that distantly related varieties of rice generally differ from one another with respect to several G. D. gene sets. Since these sets exist in multiplicity and control fertility, which is a quantitative character, they may be considered polygenes. The variation in fertility due to the G. D. genes may be computed by the formulas given in the Annual Report of 1954 (p. 42).

30. *Restriction of Gene Recombination in Hybrid Populations of Rice*

(By Hiko-Ichi OKA)

In hybrids between distantly related varieties of rice it is often found that the recombination of indepehdent genes is restricted to some extent. It was stated in the Annual Report of 1954 (p. 47) that this tendency, found in the F_2, could be explained by the effect of a set of G. D. genes. For a similar tendency appearing in later generations, however, a geno-

typic difference in propagation rate and other causes may be responsible in addition to the effects of G. D. genes, in the same manner as in the case of the change of gene frequency in hybrid populations (Jap. Jour. Breed. 5:207–212 and Ann. Rpt. Nat. Inst. Genet. 5:45–47.

Concerning the G. D. genes, the frequencies of different genotypes in the F_n of a hybrid, $\frac{X_1}{x_1}\frac{x_2}{X_2}$, may be found from the following formulas:

$\frac{X_1}{X_1}\frac{X_2}{X_2}$	$\frac{1}{4}-\frac{1}{2^{n-1}}+\frac{1}{4(3^{n-2})}=u_1$
$\frac{X_1}{X_1}\frac{x_2}{x_2}, \frac{x_1}{x_1}\frac{X_2}{X_2}$	$\frac{3}{8}-\frac{1}{2^n}+\frac{1}{8(3^{n-1})}=u_2$
$\frac{X_1}{x_1}\frac{X_2}{X_2}, \frac{X_1}{X_1}\frac{X_2}{x_2}$	$\frac{1}{2^{n-1}}-\frac{1}{3^{n-1}}=u_3$
$\frac{X_1}{x_1}\frac{x_2}{X_2}$ (Semi-sterile)	$\frac{1}{3^{n-1}}=u_4$

By using these formulas it will be shown that with repeated inbreeding u_3 and u_4 approach zero, while u_1 and u_2 come near 1/4 and 3/8 respectively. Thus, when two pairs of genes, A-a and B-b, are linked with X_1-x_1 and x_2-X_2 respectively, recombination between A-a and B-b, is restricted to some extent, although these genes are independent. In this case, recombination between X_1-x_1 and A-a, and between x_2-X_2 and B-b, is not disturbed by gametic selection. The frequencies of different combinations between A-a and B-b may then be computed from the values of u_1 to u_4 and from the frequency of recombination of two linked genes as shown by the formula of Nelder (Heredity 6: 387–397, 1952).

Next, we may consider a case in which different genotypes for two independent genes, A-a and B-b, differ in propagation rate. As mentioned in the Annual Report of 1954 (p.45), the propagation rate of the heterozygotes will not greatly influence the frequencies of those genotypes. If recessive homozygotes AAbb, aaBB and aabb have propagation rates p, q and r respectivety, the relative frequencies of different genotypes in the F_n will be shown by the following formulas:

AaBb	$\frac{1}{4^{n-1}}$	
AABb, AaBB	$\frac{1}{2^n}-\frac{1}{2^{2n-1}}$	(AB) $\frac{1}{4}+\frac{1}{4^n}+\frac{1}{2^n}$
AABB	$\frac{1}{4}-\frac{1}{2^n}+\frac{1}{4^n}$	

Aabb	$\frac{1-(2p)^{n-1}}{2^{2n-1}(1-2p)}$	(Ab)	$\frac{(2^{n-1}+1)\{1-(2p)^{n-1}\}}{4^n(1-2p)}$
AAbb	$\frac{(2^{n-1}-1)\{1-(2p)^{n-1}\}}{4^n(1-2p)}$		
aaBb	$\frac{1-(2q)^{n-1}}{2^{2n-1}(1-2q)}$	(aB)	$\frac{(2^{n-1}+1)\{1-(2q)^{n-1}\}}{4^n(1-2q)}$
aaBB	$\frac{(2^{n-1}-1)\{1-(2q)^{n-1}\}}{4^n(1-2q)}$		
aabb(ab)	$\frac{1-(4r)^{n-1}}{4^n(1-4r)}+\frac{2p\{1-(2p)^{n-2}\}}{4^n(1-4r)(1-2p)}-\frac{2p\cdot 4r\{(4r)^{n-2}-(2p)^{n-2}\}}{4^n(1-4r)(4r-2p)}$		
	$+\frac{2q\{1-(2q)^{n-2}\}}{4^n(1-4r)(1-2q)}-\frac{2q\cdot 4r\{(4r)^{n-2}-(2q)^{n-2}\}}{4^n(1-4r)(4r-2q)}$		

Two varieties of rice, 414 (a "Continental" variety, non-glutinous, with red seed coat) and 563 (a "Temperate-Insular" variety, glutinous, with colorless seed coat) were crossed, and the hybrid was propagated in bulk without selection. In the seeds of the generations from F_3 to F_7, the frequencies of different combinations of the glutinous gene (+: gl) and the seed coat color gene (Rc: rc) were observed. It has been found that the parental combination groups (+Rc and gl rc) tend to increase in successive generations at the expense of the recombination groups. By computing the values of several parameters for the G. D. genes and the propagation rate by using the above formulas, it has been found that the observed frequencies of different combination groups comform well to the expected frequencies.

In populations of hybrids between distantly related varieties of rice, the tendency to restrict gene recombination is observed not only for the major genes, but also for genes controlling various physiological characters. This tendency seems to be largely attributable to such genetical mechanisms as mentioned above.

31. *Inheritance of Several Agronomic Characters in a Varietal Hybrid of Rice*

(By Hiko-Ichi OKA)

Two distantly related rice varieties, Pei-ku and Taichung no. 65, were crossed in Taichung, Formosa, and the inheritance of heading date, panicle number per plant, height, panicle length and grain number per panicle were investigated by statistical-genetic methods. "Pei-ku" is a

Formosan native variety of the first-crop nature, belonging to the so-called *Indica* or "Continental" group; "Taichung no. 65" is a representative of the "Horai" variety belonging to the *Japonica* or "Temperate-Insular" group. The outline of the experiment is as follows: F_2 to F_5 bulk populations, propagated without selection, were grown in the second crop of 1953, and about 40 plants were randomly selected from each population. In the first crop of 1954, families raised from these seeds, together with the families belonging to the F_2 and parental varieties, were observed in an experiment of randomized blocks design. Measurements were taken on a single plant basis for each character.

For each character, the partitioning of variance components (D, H and E items) was conducted first by Mather's method (1949) using the data for F_2 and F_3. The covariances between characters were also partitioned in the same way. The principal conclusions reached are as follows:

(*1*) *Scale*: Both the convenient and logarithmic (starting from the lowest limit of variation) scales were used for all of the characters, and the scale with the smaller mean square of deviations of observed from expected values was chosen. It was found that the convenient scale was better for dealing with heading date and panicle number, while the logarithmic scale was more suitable for height, pancile length and grain number. The last three characters seem to be subject to a strong influence of gene interaction.

(*2*) *Linkage*: For each character, the significance of the effect of linkage was tested by Mather's method (comparison of the sums of squares of deviations of observed from expected values, calculated including and excluding $\overline{V}_{F_3}$). Linkage was not apparent in heading date and panicle number, but it was highly significant in height and grain number, and seemed to affect panicle length, to some extent. Since in these characters the heritable portion of $\overline{V}_{F_3}$ was larger than half of that of V_{F_2}, the linkages seem to be mainly of the repulsion phase.

(*3*) *The effect of competition*: It was stated in the Annual Report of 1954 (p. 54) that the effect of intergenotypic competition should be considered in partitioning variance components for panicle number. The same conclusion was reached in the present experiment.

(*4*) *Heritability*: Considering the problems of scale, linkage and competition as mentioned above, the values of D, H and E components were calculated for each character, and the values of heritability (for F_2 individual selection) were estimated. The results are as follows:

Heading date	0.823
Panicle numbler per plant.....	0.090

Height	0.317
Panicle length	0.237
Grain number per panicle	0.302

(5) *Genetic correlation*: The values of D, H and E components were estimated by covariance between characters, excluding the mean covariance of F_3 families in the same manner as in the linkage test. The correlation coefficients due to D. H and E components, i.e. correlations due to fixable heritable variation, to non-fixable heritable variation and to environmental variation respectively, were then computed. The results are as follows:

		rD	rH	rE
Heading Date	— Height	0.613	0.870	−0.187
〃	— Panicle Number	0.405	−0.706	−0.391
Height	— Panicle Number	−0.492	−0.340	0.409
〃	— Panicle Length	0.568	0.627	0.288
〃	— Grain Number	0.621	0.223	0.406
Panicle Length	— Grain Number	0.346	0.208	0.565

32. *Observations on Natural Populations of Formosan Wild Rice*

(By Hiko-Ichi OKA)

In Formosa, wild rice is found only in one locality, Patu, Tao-yuan Prefecture (northern part of the island). It has $n=12$ chromosomes, and can be hybridized easily with the cultivated rice; the F_1 shows no disturbance in chromosome pairing. This wild rice has thus been identified to be a variety of *Oryza sative spontanea*. It has, however, many features sharply distinct from the ordinary cultivated varieties of rice: It is a perennial weed, growing in the water of natural streams about one meter in depth. Important morphological characteristics are: very long anther and awn, and anthocyanian pigmentation in various parts of the plant. The seeds drop before maturation, and germinate after a dormant period of more than one year. The population of this plant continues flowering from spring to autumn. The flowers are open for several hours, and the emission of pollen occurs 3.9 ± 2.5 minutes after the opening of glumes (in October at Taichung).

To find the frequency of natural hybridization, several plants of the wild rice were planted in the center of eight surrounding plants of a glutinous variety. Seeds were gathered from the panicles of the wild plant which flowered at the same time as the surrounding plants, and the frequency of glutinous heterozygotes in the offspring was determined. The frequency of natural hybrids was found to be 30.7%. The wild rice

is by no means a self-fertilized plant.

Next, descendents of some twenty plants selected at random from the wild populations were examined. Most of them were found to be heterozygous for leaf sheath coloration, apiculus coloration, phenol reaction and other monogenic characters. Regarding quantitative characters such as heading date, height, and awn length, a wide range of variation was found within lines as well as between lines. The wild rice thus seems to be heterozygous for numerous genes. This heterozygosity is possibly due to the accumulation of mutations and to hybridization with the cultivated rice. The population of wild rice seems to have a great store of mutant genes. It is possible that if these mutants were properly accumulated and combined, genotypes similar to those of cultivated varieties would be produced.

Table 1. Individual variation among populations of wild rice in length-width ratio of grain.

Populations	Ratio of length to width 2.70	2.85	3.00	3.15	3.30	3.45	3.60	Num. of plants	Mean
A	1	2	3	3	2	1	1	13	3.12
B	4	7	4	1				16	2.87
C		1	2	2	3	11	2	21	3.34

Differences among natural populations of the wild rice were examined. Comparison of three populations, located about two kilometers apart and seemingly isolated from one another, yielded significant differences with respect to awn length, panicle shape, grain shape and other characters. As an example, the data for the length-width ratio of the grain are presented in Table I. Since these three populations are under comparable conditions and the shape of the grain is a character relatively immune to environmental conditions, the differences found among the populations indicate the presence of genotypic differences. The distribution of the wild rice in Formosa is now limited to a small area. If its range covered a wider area, greater difference among populations might have occurred.

33. *Karyology of Oryza sativa L, I. Karyological studies on haploid rice*

(By Yô TAKENAKA, Chao-Hwa HU, and Tuguo TATEOKA)

Meiotic studies of haploid rice have been reported by MORINAGA and FUKUSHIMA (1932, '34), NAKAMURA (1933) and RAMIAH (1934). MORINAGA and FUKUSHIMA gave no account of the presence of bivalents or other

chromosome associations in their first report. In their second report, however, they mentioned the presence of one or two chromosome pairs in some pollen-mother cells, and assumed them to be artifacts.

In four varieties of cultivated rice, we found in many pollen-mother cells in diakinesis or first anaphase, the so-called secondary association among the univalents as well as among a few bivalents. Aside from secondary associations, we have found 64% pollen-mother cells with univalents only, 33% with one bivalent and 2% with two bivalents; rarely, we even found cells with three bivalents or one or two trivalents.

MORINAGA and FUKUSHIMA observed loose pairing of meiotic chromosomes in 27.4% of the pollen mother cells. Observations on the frequency of bivalents in our material are in good agreement with these authors. When the types of chromosome association are classified without discriminating between true pairing and secondary association, the chromosome configurations found varied from 12(1) to 5(2)+2(1) with the mode at 2(2)+8(1) during diakinesis, from 12(1) to 2(3)+3(2) with the mode at 1(3)+3(2)+3(1) during dia-metaphase, and from 12(1) to 2(3)+3(2) with the mode at 3(2)+6(1) during meta-anaphase. The highest association found was 5(2)+2(1) at diakinesis, while that at dia-metaphase or meta-anaphase was 2(3)+3(2). The chromosome affinity appeared to increase from diakinesis to first metaphase and then to decrease from first metaphase to anaphase.

In the meiosis of diploid rice, SAKAI (1935) found secondary associations between bivalent chromosomes, ranging from 1(2)+10(1) to 2(3)+3(2) with the mode at 1(3)+3(2)+3(1). NANDI (1936) and OKUNO (1944) also obtained results which agreed fairly well with SAKAI'S.

Our results with respect to secondary associations are in full agreement with those of the above three authors. From their findings, these authors assume that the basic chromosome number of rice is five. We share this opinion and assume the secondary association to be due to residual homology. Thus the chromosome set of rice may be shown to be abcde a′b′c′d′e′ a″b″.

G. GENETICS AND CYTOLOGY OF BARLEY, WHEAT AND *AGROPYRON*

34. *Nullisomic Dwarfs and Tetrasomic Giants in Hexaploid Wheat*

(By Seiji MATSUMURA)

Dwarf plants possessing 40 chromosomes (20_{II}), found among the off-

spring of the pentaploid hybrid, *Triticum polonicum*×*T. Spelta*, are nullisomics, deficient in a chromosome pair of the D-genome. As it is the a~g-chromosome in the D-genome which is missing, they are called a~g-dwarfs. From crossing experiments it has been determined that SEARS' Nulli-XVI and XVII correspond to the f- and c-dwarfs, respectively.

From the results of a nullisomic analysis in the F_2 of hybrids between a~g-dwarfs and *T. compactum*, it has been concluded that the gene for *compactum*, *C*, is located on the e-chromosome of the D-genome.

The gigas-plants with 42 chromosomes ($1_{IV}+19_{II}$) found among the offspring of nullisomic dwarfs are called a~g-gigas, according to the original 7 different a~g-dwarfs. They may be called D-nulli- and AB tetrasomics. It has been assumed that the suppernumerary A-genome chromosomes, a_A in a-gigas and c_A in c-gigas, are the same as SEARS'VII and III respectively, while the additional B-genome chromosome, f_B, in f-gigas corresponds to SEARS' I.

35. *Karyotypes of Diploid Agropyron Species*

(By S. MATSUMURA and S. SAKAMOTO)

It has been inferred from previous result obtained by the senior author that the genome possessed by *Agropyron glaucum* ($2n=42$) and *A.*

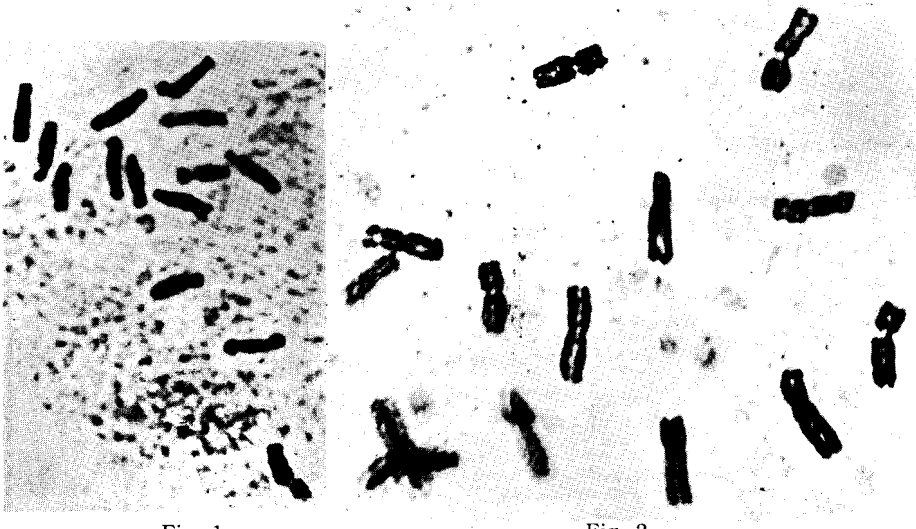


Fig. 1 Fig. 2

Fig. 1: Somatic chromosomes of *A. triticeum*.
Fig. 2: Somatic chromosomes of *A. elongatum*.

elongatum ($2n=70$) which is homologous with one of the genomes in *Triticum*, is the B-genome (MATSUMURA 1949). According to the hypothesis of MCFADDEN and SEARS (1946), *A. triticeum*, an annual plant having 14 somatic chromosomes, should have the B-genome. We are sceptical about this hypothesis.

It is established beyond doubt that all chromosomes of the B-genome in wheat have median or submedian constrictions. But all the 7 chromosomes of *A. triticeum* show subterminal centromeres, as shown in Fig. 1. The diploid *A. elongatum* ($2n=14$) is an interesting plant in having chromosomes with median or submedian constrictions (Fig. 2).

36. *Agropyron and Related Genera Collected in Nepal*

(By S. MATSUMURA and S. SAKAMOTO)

During the Japanese Expedition to the Himalayas, 1952–53, three species of *Agropyron*, two of *Elymus* and one of *Brachypodium* were collected.

Agropyron semicostatum NEES. seems to have the widest distribution of all the three species of the genus. It is tetraploid ($2n=28$), and is quite different from *A. tsukushiense* (HONDA) OHWI which was formerly included in *A. semicostatum* NEES. The latter species, which is of the widest occurrence in Japan, is a hexaploid ($2n=42$), while *A. ciliare* (TRIN.) FRANCH. which is distributed throughout Japan, is a tetraploid ($2n=28$). In general, the ear morphology of *A. semicostatum* resembles that of *A. ciliare*, but the single spikelets of the former species are rather similar to those of *A. tsukushiense*. Our cytological and morphological observations sustains OHWI's classification of these species. *Agropyron Gmelini* (LEDEB.) SCRIBN. et SMITH is also a tetraploid ($2n=28$), and its morphology is similar to that of *A. semicostatum*. Both species belong to Section *Roegneria*. *Agropyron striatum* NEES. shows distinction in the shape of its spikes from the species mentioned above.

Elymus dahuricus TURCZ. and *E. sibiricus* L. are hexaploids ($2n=42$). They are similar in external morphology, and are widely distributed in the Far East including Manchuria and Korea.

Brachypodium sylvaticum BEAUV. var. *lozoniense* HARA is diploid ($2n=18$). Its somatic chromosomes are very small compared with those of *Agropyron* and *Elymus*. There is a distinct difference between *Brachypodium* and *Agropyron* or *Elymus* in the basic chromosome number, which is 9 in the first genus and 7 in the other two genera.

37. *Off-type Plants Observed in a Wheat Variety, "Saitama No. 27"*

(By Kanji GOTOH)

It is well known that various kinds of off-type plants or rogues occur in some varieties of wheat and oats. In few cases, however, the cause of the appearance of such abnormalities is fully understood, and they are often considered uncontrollable genetic changes. Rogues are known among our wheat breeders to occur occasionally in field populations of the wheat strain, "Saitama No. 27".

My preliminary experiments on this variety disclosed that except for the *D* strain of the variety, which contained 88% of tall-type plants of winter habit, no difference could be found in all the agronomic characters examined among the 4 strains used in this experiment. In the strains other than *D*, tall and low off-type plants were rarely found.

The experiments performed in 1953 and 1954 on *D* showed that there was a wide range of variation in plant height, ranging from 84 cm to 135 cm, and also a striking variability in the date of heading, ranging from April 14 to 28 in 1954. The data obtained in these two years suggest that these variations are probably governed by polygenes.

The response of the winter-habit lines of the *D* strain to a long-day condition was examined in plants kept in a phytotron maintained at 20°C. Under these conditions the earliest line attained the booting stage in 61 days after seeding, and the other lines followed, their development extending over more than 1 month. The normal plants of this variety were of a spring habit, and they began to produce the uppermost leaves 27–30 days after seeding. According to the number of days from seeding to the booting stage, all the winter-habit lines were classified into 4 groups. The means for each group were calculated for culm length in the 1953 crop and for heading in the 1954 crop under field conditions. Thus, it was found that the later groups among the winter-type strains tended to exhibit relatively longer culms and to produce ears later than the earlier groups. The differences in both characters among the 4 groups were statistically significant at the 1% level. The presence of such variability suggests the complexity of the mechanism controlling this abnormality.

GOTOH, K. Jap. Jour. Genet. 31., (in press).

38. *Polygenic Differences in Agronomic Characters among Some Local Strains of a Barley Variety, "Hakata No. 2"*

(By Kanji GOTOH)

Polygenic segregation in agronomic characters during the period of multiplication may be of minor importance from the practical view-point. However, the problem as to how natural selection affects the polygenic characters of cultivated varieties may be of some interest from a theoretical viewpoint. In order to obtain some information on this problem and to throw some light on the effect of natural selection upon a cross-bred variety, five local strains of a two-rowed beer barley variety, "Hakata No. 2" were examined comparatively.

On visual inspection, the botanical features of all the five strains appear similar, and all differences found among them are of a statistical nature. Among the local strains the differences in culm length, ear length, number of spikelets on one side and weight of 1000 grains have proved to be statistically significant; it is suggested that these differences are governed by polygenes.

According to the frequency distribution of ear length and number of spikelets, the ear length is shorter and the number of spikelets smaller in local strains derived from northern localities than in southern strains. This relation largely represents a latitudinal cline.

The results of these comparative experiments indicate that the original population of "Hakata No. 2" was probably heterozygous with respect to several polygenic characters, especially ear length, and that variations were released through polygenic segregation in the progeny. It is assumed that the variation found in these characters among the local populations is due in large measure to natural selection under different environmental conditions.

GOTOH, K. Jap. Jour. Genet. 31., (in press).

39. *Population Structure of the Barley Variety, "Iwate Mensury No. 2"*

(By Kanji GOTOH)

In order to confirm the conclusions derived from the data for "Hosogara No. 2" (GOTOH, '55-a, -b), the population structure of the local strains of another mixed barley variety, "Iwate Mensury No. 2", was analyzed in the same manner as for the "Hosogara No. 2".

Five population samples obtained from three northern localities (*A*, *B*, *C*) and two from southern localities (*D*, *F*) were compared for culm length, ear length and grain yield. The differences found among these samples are statistically significant. It has been found that these local strains have distinct genetic constitutions in regard to 3 marker genes: *S s*, governing long or short rachilla hairs; *Hs hs*, governing presence or absence of hairs on the basal leaf-sheath and *Wh wh*, governing winter or spring habit. 7 of the 8 genotypes expected from all the possible combinations of these 3 sets of marker genes were actually found in the 5 local strains examined.

Furthermore, 6 genotypes were discriminated in the *A* population which is apparently closely related to the original population, in regard to the date of heading (ranging from April 16 to May 8), as well as in morphological features.

The frequency of the genotype (*S hs wh*), the commonest of the genotypes in all the 5 strains, was 41 %, 72 %, 47 %, 100 % and 100 %, in *A*, *B*, *C*, *D* and *E*, respectively. The frequency of spring-habit plants increases from the northern habitat to the southern habitat, indicating that this type has a higher adaptability to the warmer climate. The genotype with the combination, *S hs wh*, of "Iwate Mensury No. 2" corresponds to that of the Z-type of "Hosogara No. 2". In the latter variety, most of the population samples obtained from southern localities belong to the Z type, and they are practically homogeneous in respect to spring habit. Thus, the results obtained from both varieties are similar in many points, and the conclusions derived from the data obtained for "Hosogara No. 2" are supported by the results of the experiment with "Iwate Mensury No. 2".

GOTOH, K. 1955–a. Jap. Jour. Genet. 30 (3): 95–106.
——— 1955–b. Ibid. 30 (5): 197–205.
——— Ibid. 31. (in press).

H. RADIATION GENETICS OF WHEAT AND BARLEY

40. *Effect of X-rays on Fertility and Mutation Rate in Cereals*

(By S. MATSUMURA and T. FUJII)

A. Einkorn wheat

Dormant seeds of *Triticum monococcum* were exposed to various kinds of X-rays with different dosages or wave lengths, without a filter. The

frequencies of ears with chromosome aberrations in X_1-plants and of head progenies with gene mutations in X_2 increased with X-ray dosage (5,400–13,500 *r*). It was also fairly certain that both the chromosome aberrations and gene mutations increased in inverse proportion to the wave length (c.f. Ann. Rep. Nos. 3 & 5). The frequencies of both these changes were invariably higher in var. *vulgare* than in var. *flavescens*. Thus, var. *flavescens* shows a higher tolerance to X-rays than var. *vulgare* (Table 1).

There were some quantitative differences among the various kinds of X-rays in their effect upon the single-spike fertility of X_1-plants. The mean single-spike fertility in untreated plants was 82.67 % in var. *flavescens* and 53.32 % var. *vulgare*; that of X-rayed plants generally decreased in direct proportion to the increase of dosage. This relation is in good accord with that between the frequency of chromosome aberrations or gene mutations and X-ray dosage. Also, there is a marked difference in fertility between the 80 and 180 KVP lots. The relation between the rate of induced sterility to wave length coincides roughly with the relation between the frequency of chromosome aberrations or gene mutations to wave length (Table 1). In all cases fertility was markedly lower in var. *vulgare* than in var. *flavescens*. The former variety matures later than the latter, and its fertility often becomes low and variable under the influence of high summer temperatures. Even if this seasonal factor is taken into consideration, it is certain that the fertility of var. *vulgare* is more severely affected by X-rays than that of var. *flavescens*. This might be due to the difference in tolerance mentioned above.

Table 1. Relation between the frequency (%) of induced chromosome aberrations or gene mutations and the fertility of spikes of X_1-plants in *Triticum monococcum*

Dosage (*r*)	Voltage (KVP)	var. *flavescens*			var. *vulgare*		
		Chromosome aberration in X_1	Gene mutation in X_2	Fertility of spike in X_1	Chromosome aberration in X_1	Gene mutation in X_2	Fertility of spike in X_1
Control	—	0.00	0.00	82.67	0.00	0.00	53.22
5,400	180	5.77	6.67	76.50	5.88	11.36	43.28
8,100	180	12.50	12.73	69.57	15.09	19.23	32.67
13,500	180	28.95	37.04	57.90	38.08	40.00	17.70
8,100	130	12.50	3.64	67.82	14.49	9.31	41.47
8,100	80	7.50	5.56	75.23	3.95	8.69	41.06

B. Two-rowed barley

Dormant seeds of several varieties of *Hordeum distichum* for malting were subjected to X-ray treatment, which was applied without filter, at 180 KVP, 3 mA, 13 cm, 384.5 *r*/min and 8,100 & 13,500 *r*. There was no difference in the germination rate between untreated and treated seeds, but the effect of X-rays upon the fertility of X_1-plants was in proportion to the dosage. The fertility was reduced to 68.21–99.95 % of that of untreated plants at 8,100 *r* and further to 44.02–76.27 % at 13,500 *r*. Various kinds of chlorophyll mutants were also found among the seedings of the X_2 generation. The frequency of head progeny with mutations was 4.22 % at 8,100 *r* and increased to 11.50 % at 13,500 *r*. This is in accord with the relation of sterility to dosage.

41. *Chromosome Aberrations in Einkorn Wheat Induced by Irradiation*

(By Seiji MATSUMURA)

To examine the relation between the frequency of chromosome aberrations and the wave length of X-rays in the PMC's of *Triticum monococcum*, dormant seeds were exposed to X-rays of different wave lengths (80, 130 and 180 KVP), and different intensities (*r*/min) at the same dose (8,100 *r*), without filter, as recorded in the foregoing report. In the present experiment X-rays of different wave lengths at the same dosage (8,100 *r*) and intensity (95 *r*/min) were used with different filters; also the effect of γ-radiations by Co^{60} was examined for comparison. The thickness of the filter was adjusted in inverse proportion to the wave length; that is, at 80 KVP no filter was inserted into Matsuda's Type KXC-17 apparatus, while at 130 KVP a filter of 0.3 Cu+0.5 Al, and at 180 KVP one of 0.8 Cu +1.5 Al was used. At 50 KVP and 20 KVP, irradiation was applied by another apparatus, a Modified Type KR-75 and Type TX-20 (Grenz-rays) without filter, respectively. The data are shown in Table 1.

At 80–180 KVP, the results recorded in the foregoing report were confirmed, but at 50 KVP the aberration frequency was unexpectedly high (c.f. Ann. Rep. No. 4). This raised a question as to whether or not the dosages were correctly measured with Matsuda's dosimeter or the "*r*"-meter. Therefore, measurements were checked with Siemen's Universal Dosimeter and it has become clear that the dosages at 80–180 KVP produced by the Type KXC-17 were decreased from to 8,100 *r* to 6,190–6,580 *r*, while the dosage at 50 KVP were increased to 8,350 *r*.

There were marked differences between the X-rays and γ-rays in their effect upon germination rate and aberration frequency. The γ-rays were

more productive of abnormalities than the X-rays; at 16,200 *r* of γ-rays, all the seedings were uniformly small and died about one month after germination, while with comparable X-ray treatment the disturbances were apparently less.

Table 1. Relation between wave length of X- or γ-rays and frequency of chromosome aberrations in *Triticum monococcum*

Dosage (*r*)	Voltage (KVP)	Filter	Current (mA)	No. of observed ears	Aberrations	
					No.	%
Control	—	—	—	18	0	0.00
8,100(6,190)**	180	0.8Cu+1.5Al	3	62	7	11.29
8,100(6,190)	130	0.3Cu+0.5Al	3	45	3	6.67
8,100(6,580)	80	—	4	67*	2	3.03
8,100(8,350)	50	—	10	57	6	5.26
—(8,100)	20	—	10	44	1	2.27
8,100	γ-ray(Co^{60})	—	—	86	12	13.95
12,150	〃 (〃)	—	—	62	21	33.87

* One was haploid.

** ( ) measured by Siemen's Universal Dosimeter.

42. "*Virido-albina*" *Mutant in Einkorn Wheat Induced by X-rays*

(By Tarô FUJII)

Chlorophyll mutants in Einkorn wheat (*Triticum monococcum flavescens*) which appear spontaneously are not uncommon; such mutants may also be easily induced by ionizing radiations. Most of them are Mendelian recessives. MATSUMURA and FUJII (1955) found several chlorophyll mutants of the types "albina", "chlorina", "virido-albina", "basi-viridis" and "striata", etc., induced by X-irradiation. Some "virido-albina" mutants appeared among the X_2-generation progeny of seeds treated with 8,100 *r* dose, at 180 KVP. In this mutant about 3/4 of the base of each leaf is white, while the rest is light green. This characteristic is already apparent in the seedling stage and the mutant invariably dies in the field in winter time.

When some of such mutant plants were placed in the phytotron (20°C) and exposed to fluorescent light (about 4,000 lux), the leaves gradually became green, the change starting from the veins, and in about 15 days the plant assumed the appearance of a "basi-viridis" mutant with a light-

green leaf base. It required 30 more days of such treatment for the plants to gain a normal chlorophyll content. Similar results were obtained with "basi-viridis" mutants kept in the phytotron; a significant increase of chlorophyll content was observed. On the other hand, "albina", "chlorina" and "striata" mutants did not gain in chlorophyll content under the same treatment. The chlorophyll content of normal plants and several chlorophyll mutants are shown in Table 1, measured in optical density $\left(T=-\log\frac{I}{I_0}\right)$ of a 1/10 dilution of fresh leaves.

The "virido-albina" mutants reached the heading stage in the phytotron, while they were lethal in the field. The time of heading and ripening in these plants was delayed about 15 days as compared with their normal mates, and they were significantly reduced in height, number of tillers, and fertility. Seeds of the recovered "virido-albina" showed a relatively high germination frequency, about 80%. The seedlings were of course "virido-albina". When the recovered "virido-albina" plants were returned to the field, the successive leaves showed the "virido-albina" characteristic and the mutants died in the winter.

The oxygen consumption or respiration rate was measured by a Warburg respirometer. In the "virido-albina" mutant this rate was about half that of the normals, and in the recovered "virido-albina" it was somewhat higher than in the normals. But the activity of cytochrome oxidase in the mutants grown in the field and in the phytotron was similar to that of the normals. FUJII (1955) has reported a similar tendency toward a decrease of the chlorophyll content and the activity of cytochrome oxidase in the "chlorina" mutant. From these results it has been surmised that a recovery of the chlorophyll content without a normal activity of cytochrome oxidase can not be expected. The relation between the increase of chlorophyll content and the activity of enzymes awaits further investigation.

Table 1. Relative chlorophyll content of several chlorophyll mutants examined with a spectrophotometer (1/10 dilution of fresh weight)

Mutants	Optical density in 4230 Å	
	plants in field	plants in phytotron
Normal	0.47	0.51
Chlorina	0.35	0.41
Virido-albina	0.29	0.51
Basi-viridis I	0.36	0.50
Basi-viridis II	0.39	0.49

I. CYTOLOGY AND GENETICS OF *NICOTIANA*

43. *Cytogenetic Studies on the Genus Nicotiana, VII*

(By Yô TAKENAKA, Chao-Hwa HU, and Katsuhisa SHIMOYAMA)

The reduction divisions in PMC's were studied in 9 interspecific hybrids: *N. tabacum*×*N. solanifolia*, *N. tabacum*×*N. Langsdorffii*, *N. Debneyi*×*N. tabacum*, *N. Debneyi*×*N. trigonophylla*, *N. sylvestris*×*N. tomentosa*, *N. alata*×*N. Langsdorffii* and its reciprocal, *N. rustica*×*N. Langsdorffii* and *N. longiflora*×*N. Langsdorffii*.

(1) F_1 of *N. tabacum* ($n=24$)×*N. solanifolia* ($n=12$)

At MI in PMC's of the F_1 of *N. tabacum*×*N. solanifolia*, from 1 to 5 chromosome pairs were found, with the mode at 1 pair. In the same hybrid, GOODSPEED (1954) found 0–7 bivalents with the mode at 2. Accordingly, the conjugational affinity between the *N. tabacum* and *N. solanifolia* genomes is assumed to be similiar to that in the intragenomatic pairing within *N. tabacum*.

(2) F_1 of *N. tabacum* ($n=24$)×*N. Langsdorffii* ($n=9$)

In the hybrid between *N. tabacum* and *N. Langsdorffii*, which seems to have never been investigated cytologically, the number of bivalents in PMC's varied from 5 to 12, with the mode at 11. It is assumed that most of the chromosomes of *N. Langsdorffii* have a similarity in structure to the corresponding chromosomes of *N. tabacum*.

(3) F_1 of *N. Debneyi* ($n=24$)×*N. tabacum* ($n=24$)

At MI of PMC's of this hybrid, 0–6 bivalents were found with the mode at 2. Kostoff (1943) also observed univalents or 1–3 bivalents in the majority of the hybrids of this combination. From these results it appears that there is no pairing between the *N. tabacum* and *N. Debneyi* genomes, since it is known that there is a slight degree of intragenomatic affinity in *N. tabacum*.

(4) F_1 of *N. Debneyi* ($n=24$)×*N. trigonophylla* ($n=12$)

At MI in PMC's of this hybrid, mostly univalents, and a few—one or two, rarely 4–5—bivalents were found. GOODSPEED (1954) also observed mostly univalents, occasionally one or two and rarely three bivalents, in the same hybrid. According to KOSTOFF (1943), this hybrid usually undergoes an asyndetic meiosis, with one or two bivalents, cells with more than two bivalents being exceedingly rare. From these observations, it appears that the syndetic relationship between the *N. Debneyi* and *N. trigonophylla* genomes is low; it is even possible that the few bivalents may

have resulted from intragenomatic syndesis within the parental genomes.

(5) F_1 of *N. sylvestris* ($n=12$)×*N. tomentosa* ($n=12$)

At MI in PMC's of this hybrid, 0–7 bivalents were found with the mode at 3. GOODSPEED (1954) found pairings ranging from 0 to 7 with the mode at 3 in the same hybrid, from 0 to 7 with the mode at 2–3 in the F_1 of *N. sylvestris*×*N. Setchelli* and F_1 of *N. sylvestris*×*N. otophora*. KOSTOFF (1943) found in the F_1 of *N. sylvestris*×*N. tomentosa* principally univalents, together with 1 to 4 bivalents and rarely more than 4 bivalents. He also found in the F_1 of *N. sylvestris*×*N. tomentosiformis* even fewer bivalents than in the former combination. TAKENAKA (1955) found 1–9 bivalents with the mode at 4 in the F_1 of *N. sylvestris*×*N. tomentosiformis*, and also 0–5 bivalents with the mode at 2–3 in the F_1 of *N. sylvestris* ×*N. otophora*. From these results we can assume the presence of 2–4 homologous chromosomes, or large homologous sections, in the *N. sylvestris* genome and the genomes of the *tomentosa* group.

(6) F_1 of *N. alata* ($n=9$)×*N. Langsdorffii* ($n=9$) and its reciprocal

The meiosis of PMC's of the F_1 of *N. alata*×*N. Langsdorffii* was generally normal, with 9 bivalents in most cases. Sometimes 7 bivalents and one quadrivalent occurred, and also some other chromosome configurations, such as $7_{II}+1_{III}+1_I$, $8_{II}+2_I$, $6_{II}+1_{IV}+2_I$, $5_{II}+2_{IV}$ and $6_{II}+2_{III}$ in a few cases. In the same hybrid, AVERY (1938) found most frequently $6_{II}+1_V+1_I$, occasionally $7_{II}+1_{III}+1_I$, rarely 9_{II} and a few others, while KOSTOFF (1943) observed most frequently 9_{II} and sometimes $8_{II}+2_I$. Our observations essentially agree with those of the above two authors. KOSTOFF, however, has found no polyvalent, while AVERY observed a quinquevalent in many mother cells, and rarely other polyvants; we also occasionally found quadrivalents and rarely trivalents.

AVERY assumed that such extensive multivalency between the *alata* and the *Langsdorffii* genomes was due to the establishment of numerous reciprocal translocations affecting three to five or more chromosomes; the formation of a quinquevalent in the modal class in this hybrid was also interpreted as due to the two reciprocal translocation—an interpretation which also accounted for the distinctions in chromosome morphology in these two species. He also stated that the larger chromosomes with a median or an approximately median centromere constituted multivalents. TAKENAKA (1955) has reported on a quadrivalent which commonly occurs in the meiosis of *N. Langsdorffii*; the two middle elements of this quadrivalent are large and have a median spindle-attachment while the two terminal elements are small and have a subterminal attachment.

Two chromosomes of the quadrivalent occurring in the F_1 of *N. alata* ×*N. Langsdorffii* are assumed to be derivatives of the quadrivalent found

in *N. Langsdorffii* by TAKENAKA, one large and with a median attachment and the other small and with a subterminal attachment. The large chromosome observed by AVERY among multivalents in the same hybrid is probably identical with the large chromosome with a median attachment found in *N. Langsdorffii*.

(7) F_1 of *N. rustica* ($n=24$)×*N. Langsdorffii* ($n=9$)

At MI in PMC's of this hybrid, 0–8 bivalents were found with the mode at 4. Besides the bivalents and univalents, rarely polyvalents occurred. In the same hybrid, KOSTOFF also observed 5–7 bivalents together with some polyvalents.

In the meiosis of F_1 of *N. paniculata*×*N. Langsdorffii*, KOSTOFF (1943) found 3–8 bivalents, and largely confirmed DREMLUG'S view (1936). In the meiosis of F_1 of *N. undulata*×*N. Langsdorffii* studied by GOODSPEED, (1954) there were 0–4 bivalents with the mode at zero. Of the strikingly rare chromosome pairings occurring in three hybrids between *N. undulata* and three species of *alata* group, namely *N. Langsdorffii*, *N. longiflora* and *N. attenuata*, GOODSPEED states, "Such extremely limited pairing as occurs may be attributed to residual homologies from one postulated 6-paired ancester in the PETUNIOID complex in each of the two species concerned in each hybrid".

From the observations of many pairings in the F_1 of *N. paniculata* ×*N. Langsdorffii* and of extremely rare pairings in the F_1 of *N. undulata* ×*N. Langsdorffii* the strong chromosomal affinity found in the F_1 of *N. rustica*×*N. Langsdorffii* may be ascribed to the homological relation between the *Langsdorffii* genome and the *paniculata* genome, which is assumed to be one within two subgenomes composing the *rustica* genome, rather than that between the *Langsdorffii* genome and the *undulata* genome which is assumed to be the other subgenome within the *rustica* genome.

(8) F_1 of *N. longiflora* ($n=10$)×*N. Langsdorffii* ($n=9$)

At MI in PMC's of this hybrid, many univalents and 2–8 bivalents occurred, with the mode at 4, although rarely trivalents and still more rarely quadrivalents were observed. One large chromosome was always seen as an element of the polyvalents, resembling the large chromosome with the median attachment of *N. Langsdorffii*.

KOSTOFF (1943) has found that the chromosomes in the *N. Langsdorffii* genome usually conjugate with 9 chromosomes of *N. longiflora*, one chromosome remaining as a univalent. Besides this type of configuration, some others, $8_{II}+1_{III}$, $8_{II}+3_{I}$, $7_{II}+5_{I}$, $7_{II}+1_{III}+2_{I}$, were observed. AVERY (1938) also found in the same hybrid 6–9 bivalents, with the mode at 9.

The marked difference between our findings on the meiotic conjugation

of this hybrid and those of AVERY and KOSTOFF is hard to ascribe to the difference in culture conditions or to genetic differences in the parents used for crossing.

AVERY found one large pair among the bivalents which was composed of one large and one small chromosome held together by two chiasmata to produce a "frying pan" bivalent. He identified the two chromosomes concerned as a large median chromosome of *N. Langsdorffii* and one of the short subterminal chromosomes of *N. longiflora*, and he showed, on the basis of the number and position of chiasmata, that the latter chromosome is homologous in the major part of its length with one arm of the former chromosome. In the same hybrid, he also assumed that the trivalent, which is rather frequent, was a product of conjugation of a large chromosome of *N. Langsdorffii* with two smaller chromosomes, presumably of *N. longiflora*. TAKENAKA (1955) found a quadrivalent appearing usually at MI of *N. Langsdorffii*, with its two middle large elements having median attachments and the two small terminal ones having subterminal attachments. Accordingly, it is very difficult to determine whether the two small chromosomes of the trivalent found in the F_1 of *N. Langsdorffii* × *N. longiflora* are two *N. longiflora* chromosomes.

44. Cytogenetic Studies on the Genus Nicotiana, VIII. A Haploid Plant of Nicotiana tabacum

(By Yô TAKENAKA and Masao TANAKA)

A variety of *Nicotiana tabacum*, "Bright Yellow", was pollinated with pollen of *N. alata* which had been treated with X-rays (4,800r). From the seeds obtained, 8,730 were sown, 150 germinated, but only two plants reached maturity. One of them was a hybrid, and the other was a haploid plant which was a little smaller than the parental diploid Bright Yellow plant.

At MI in PMC's of the haploid plant, 24 univalents were most frequent, and in about 30% of the PMC's one to three bivalents were observed; there were 0.39 bivalents per PMC on the average.

In their study on meiotic behavior in a haploid plant of *N. tabacum* "*purpurea*" conducted by CLAUSEN and MANN as early as 1924, complete lack of pairing at MI was reported, while CHIPMAN and GOODSPEED (1927) saw in the same material an occasional bivalent, and interpreted it as being due to the adherence of two chromosomes closely associated on the MI spindle rather than a reflection of a pachytene pairing, since no association was observed at diakinesis or in earlier stages. KOSTOFF found

in a haplont of *N.* "triplex"—*N. tabacum*×(*N. sylvestris*×*N. tomentosiformis*)—and in a haplont of the amphidiploid of *N. sylvestris*×*N. tomentosiformis*, both of which he considered equivalent to a haploid *N. tabacum*, chromosome associations ranging from zoro to three pairs. The former had 0.35 and the latter 0.34 bivalents per PMC. Our observations on the haploid tobacco reported above agree with these recorded by the previous authors.

On the contrary, GOODSPEED (1934, 1954), in the three hybrids, *otophora*×*N. sylvestris*, *N. sylvestris*×*N. Setchellii*, and *N. sylvestris*×*N. tomentosa*, found zero to seven bivalents with the mode at two to three, while TAKENAKA (1954, 55) counted, in the hybrids *N. sylvestris*×*N. tcmentosiformis*, *N. sylvestris*×*N. tomentosa* and *N. sylvestris*×*N. otophora*, one to nine bivalents with the mode at four, zero to seven with the mode at three, and zero to five with the mode at two, respectively. TAKENAKA's observations largely agree with GOODSPEED's findings concerning chromosome affinity between the *sylvestris* genome and those of the *tomentosa* group.

Accordingly, the number of bivalents at MI of the hybrid between *N. sylvestris* and species of the *tomentosa* group, is always higher than that of a haploid tobacco. Concerning the decrease of intragenomatic affinity in the haploid tobacco, CLAUSEN (1941) makes the assumption that, "the alterations which have diminished its duplicational completeness must have arisen largely since it became established as an amphidiploid".

KOSTOFF (1943), on the contrary, found in the hybrid *N. sylvestris*×*N. tomentosa*, univalent chromosomes mostly with 1 to 4 bivalents, rarely more than 4, and in the F_1 of *N. sylvestris*×*N. tomentosiformis* somewhat fewer bivalents than in the former cross. He stated that meiosis of the *N. "triplex"* haploid and that of the haploid of amphidiploid *N. sylvestris*×*N. tomentosiformis* could not be distinguished from the meiosis in the F_1 of *N. sylvestrix*×*N. tomentosiformis*.

The differences among KOSTOFF's, GOODSPEED's, and TAKENAKA's findings, in hybrids between *N. sylvestris* and the *tomentosa* group, may be ascribed to the different methods of cultivation of their materials. Nevertheless, it is certain that the number of bivalents in the haploid tobacco is smaller than that in the hybrids between *N. sylvestris* and species of the *tomentosa* group.

45. *Genetical Studies on the Cherry-red Leaf Color in Tobacco*

(By Kan-Ichi SAKAI, Sinya IYAMA and Kenjiro SAIO)

In 1954, it was found that strains within a variety differed signi-

ficantly with respect to the degree to which leaf color turned cherry-red after harvest. In the same year, we selected three strains from each of the high and low groups with regard to the degree of cherry-redness, and selfed seeds were obtained from four to six plants in each of these strains. The total number of lines thus obtained was 30. Plants were grown from the seeds and planted in the experimental plots by the complete randomized block method, with three replications. Seven leaves from each of 15 plants per plot were collected, and after drying they were arbitrarily assigned to five classes with scores from 0 to 4 according to the degree of cherry-redness. The class values arbitrarily given to seven leaves of any single individual were combined by multiplication by appropriate weighting factors to construct a score, X. Then an analysis of variance of the X's, as well as a calculation of the correlation between years with regard to the degree of cherry-redness, was made.

1) *Finding appropriate weighting factors for classifying each leaf with regard to cherry-redness*

If we assume scores for the cherry-redness of a single leaf and a single individual to be X_l and X_i, respectively, the score will be given by

$$X=a_0x_0+a_1x_1+a_2x_2+a_3x_3+a_4x_4$$

where x_0, x_1, x_2, ... and x_4 stand for the number of leaves belonging to the arbitrary classes, 0, 1, ... and 4. Then, for the score of a single plant, X_i, $\sum_{k=0}^{4} x_k$ equals seven, while for the X_l of a single leaf, $\sum_{k=0}^{4} x_k$ equals one, in which $x_k \geqq 0 (k=0, 1, \ldots, 4)$. Weighting factors should be such as to make the ratio of variance of X_i to that of X_l, (V_{Xi}/V_{Xl}), take the maximum value. It was found that a formula having the following respective weighting factors would be most appropriate for the construction of the score.

$$X=0x_0+0.035x_1+0.110x_2+0.194x_3+1.000x_4$$

2) *Variance analysis*

Results of analysis of variance of X values obtained in the manner just described are presented in the following table.

Many more cherry-red leaves appeared in 1955 than in 1954. It is suggested that environmental conditions may affect their appearance, even though genes are certainly also responsible. Correlation between the incidence in parent and offspring was high ($r=0.670$, significant at the 1% level) and it was found that strains which showed a high incidence in

the previous year scored high in the next year again.

Source	d.f.	Mean square
Between strains	5	4.8727**
Between lines within strains	24	0.4952
Replication	2	1.1308*
Error	58	0.3253

**, *) Exceeds the 1% and 5% point, respectively.

J. GENETICS AND CYTOLOGY OF SOME FLOWERING PLANTS

46. Further Investigations of Sex Chromosomes in Rumex acetosa L.

(By Yô Takenaka)

Many papers have been published on polyploid and aneuploid plants of *Rumex acetosa* by Ono (1930–35), Yamamoto (1932–38) and the present writer (1931–41). These studies have demonstrated that the sex of this plant is determined according to the so-called *Drosophila* scheme. The following is a preliminary account of the result obtained by the writer's further study.

(1) Frequency of triploid intersex plants found in the field.

$3x$♀, plants with the chromosome complement $18a+2X+2Y$, are rarely found in the field. They are assumed to be the product of conjugation between an unreduced gamete with a reduced gamete, namely, $(12a+2X)+(6a+2Y)$ or $(6a+X)+(12a+X+2Y)$. If the possibility of certation in fertilization between diploid and haploid pollen is taken in account, the first combination is more likely than the second.

The writer found 3328 diploid females, 1960 diploid males and 7 triploid intersexes in a field near Misima. Because of their chromosome patterns the intersexes are grouped with males, not with females. Accordingly, the frequency of $3x$♀ is 7/1967, or about 1/300. Triploid females must be more frequent than $3x$♀ individuals, since it is assumed that female-producing pollen $(6a+X)$ prevails over male-producing pollen $(6a+2Y)$ on certation in fertilization. But it is difficult to distinguish $3x$♀ from $2x$♀ in the field.

(2) Offspring of $3x$♀

$3x$♀ plants are rarely recognized in the field when we register plants

based on size and fertility. The chromosome complement of $3x$♀ is $18a+3X$. Seeds obtained from these plants are few and show poor germination.

The sex ratio among the 40 individuals of the nature offspring of these plants was 23♀ : 12♂ : 5♀. The chromosome complements of 28 individuals observed are as follows:

	Chromosome complement	Number of plants	Sex-index*
♀	14=12a+2X	9	1:1
	21=18a+3X	1	1:1
	14+1f**=12a+2X+1f	1	1:1
	16=12a+2X+2Y	1	1:1
	15=13a+2X	3	1.08:1
	16=14a+2X	1	1.17:1
♀	19=15a+2X+2Y	1	1.25:1
	19=17a+2X	3	1.42:1
	20=18a+2X	1	1. 5:1
♂	14=11a+X+2Y	1	1.83:1
	15=12a+X+2Y	6	2:1

* Sex index is calculated according to the formula 6a:1X=1:1

** f—chromosome fragment

The plants examined were generally $2x$ and $3x$, or had chromosome numbers of approximately $2x$ or $3x$. The sex-index was (1–1.17) : 1 in the female, (1.83–2) : 1 in the male and (1.25–1.5) : 1 in the intersex, which is intermediate between the two.

(3) Offspring of $4x$♀

Among the intersexes assumed to be $3x$♀, one was found to be $4x$♀. The chromosome complement was $24a+3X+2Y$. This plant is possibly a product of fertilization of an unreduced egg by an unreduced pollen grain rather than being a descendant of a triploid plant. Among the offspring of this tetraploid intersex were only females and intersexes no male plants. Among the 10♀ and 34♀ plants, there were 34 plants having the following chromosome complements.

	Chromosome complement	Number of plants	Sex-index
♀	21=16a+4X+Y	1	0.67:1
	14=12a+2X	2	1:1
	21=18a+3X	1	1:1
	22=18a+3X+Y	3	1:1
	23=18a+3X+2Y	1	1:1
♀	19=16a+2X+Y	1	1.33:1

21=17a+2X+2Y	7	1.42:1
20=18a+2X	2	1. 5:1
21=18a+2X+Y	7	1. 5:1
22=18a+2X+2Y	5	1. 5:1
21=19a+2X	1	1.58:1
22=19a+2X+Y	2	1.58:1
22=20a+2X	1	1.67:1

These plants were $3x$, or had chromosome numbers not exactly but approximately $3x$, and only a few $2x$'s were found. The sex-indexes of these plants were (1.33–16.7) : 1 in the intersexes and 1:1 or smaller in the females.

47. Cytogenetic Studies on the Hybrid between Lilium tigrinum and L. Maximowiczii

(By Yô TAKENAKA and Setsuji KATAOKA)

TAKENAKA and NAGAMATSU (1930) found 36 chromosomes in root-tip cells of *Lilium tigrinum* and supposed this species to be an auto-triploid. *L. Maximowiczii*, which is similar in appearance to *L. tigrinum*, has 24 chromosomes (MORINAGA and FUKUSHIMA 1931, NODA 1955). SATO (1936) reported various heteroploid chromosome numbers for the F_1 of the hybrid, *L. tigrinum* × *L. Maximowiczii*. TAKENAKA (1950) demonstrated sterility in intraspecific crosses of *L. tigrinum*, but hybrid fertility in combination with *L. Maximowiczii*. In the same paper, he reported the variation in chromosome number in the F_1 hybrids, and confirmed SATO's record.

Species belonging to the genus *Lilium* have polyploid chromosome sets with an apparent basic number of 12. Many other species of the family *Liliaceae* also possess multiples of 12 chromosomes. It is doubtful, however, whether 12 is really the basic chromosome number in the genus *Lilium*, in view of the secondary associations found at meiosis in some species.

We are studying the morphology and number of chromosomes in relation to the external plant morphology of the F_1 of *L. tigrinum* × *L. Maximowiczii*, and also the details of chromosome variation, including chromosome pairing and secondary associations in the F_1 hybrids.

The chromosome numbers in F_1 hybrids which have been clarified up to the present are listed below.

It is interesting that the number of individuals having 2n=28 is relatively very large. Presumably, a great majority of the egg nuclei of *L.*

Somatic chromosome number	24	25	26	27	28	29	30	31	32	33	34	35	36	25+f*	28+f	32+f	Total
Frequency	8	2	6	2	11	1	3	1	5	0	0	3	3	1	1	1	47

* f—chromosome fragment

tigrinum have 18 chromosomes, and the microspores of *L. Maximowiczii* have 12 chromosomes. It is expected, therefore, that the chromosome number of the F_1 plants will be mostly 30. The fact that the majority have 2n=28, contrary to the expectation, suggests that this number represents a genetic equilibrium.

48. The Degree of Heritability of Five Characters in Eggplant

(By Kanji GOTOH)

Examination of 45 lines of F_3 and F_4 plants derived from the cross, Kikunaga × Sendai-naga No. 1, was made in a randomized block arrangement with two replications. The number of days from seeding to flowering, plant height, diameter of stem, index of fruit shape and number of fruits per plant were determined on an individual basis, and the means of 7 plants per line in each block were used for the calculation. Based on the data of variance analysis, genetic variance and the degree of heritability in per cent were estimated for each character.

Table 1. Heritability in per cent.

Character	F_3	F_4
Number of days from seeding to flowering	64.9	72.6
Plant height	52.0	69.6
Diameter of stem	55.0	58.3
Number of fruits per plant	34.5	48.6
Index of fruit shape	88.5	90.6

As seen in Table 1, the degree of heritability was higher in the F_4 than in the F_3 generation in all comparisons, and the index of fruit shape showed the highest degree of heritability. This was followed by the number of days from seeding to flowering, the plant height and diameter of stem, with the number of fruits per plant presenting the lowest heritability.

For the number of days from seeding to flowering and for the index of fruit shape, similar degrees of heritability were found by the different estimation methods and in the different materials (GOTOH, '53 and '54).

GOTOH, K. 1953. Genetica 26(5/6):453-467.
——, 1954. Jap. Jour. Genet. 29(3):89-97.

49. *Genetics of the Photoperiodic Behavior of Japanese Morning Glory, Pharbitis Nil* CHOIS., *a Short-day Plant*

(By Sadao SAKAMOTO)

In order to study the difference in photoperiodic behavior according to the habitat of this plant and to investigate its genetics, six strains of the Japanese morning glory—four wild strains derived from Nepal (ca. 28°N), a strain from Tendan (Tentan) in a suburb of Peking, North China (40° N), and a strain named Violet, cultivated in Japan—were used. For measuring the photoperiodic behavior, the position of the node on the main axis carrying the first flower bud in relation to the natural day-length in Misima (35°N) was examined. To secure a uniform external condition, the plants were kept in a phytotron (air-controlled greenhouse) regulated at 30°C and 80% relative humidity from December 1954 to November 1955.

The average positions of the node in these plants under the natural day-length in Misima are shown in Table 1.

Table 1. Variation in the average position of the node on the main axis carrying the first flower bud under natural day-length in Misima (35°N).

No. of experiment	I	II	III	IV	V	VI	VII	VIII	IX
Date of seeding	22, Dec. '54	3, Feb. '55	22, Apr. '55	1, June '55	1, July '55	12, Aug. '55	1, Sept. '55	1, Oct. '55	1, Nov. '55
Nepal strains									
849	2.0	—	∞	—	18.5	5.0	5.3	3.1	2.3
850	2.7	4.2	∞	∞(21.3)*	23.5	5.7	5.5	3.3	2.8
852	2.4	—	∞	—	—	4.9	6.0	3.6	3.0
856	2.2	2.7	∞	—	19.0	3.0	3.2	3.0	2.2
Tendan	1.0	2.1	3.4	4.6	5.2	3.7	3.2	2.6	1.5
Violet	1.7	2.1	7.5	∞(39.0)*	23.4	6.6	5.1	3.0	1.9

∞: did not initiate flower bud up to 20th-40th node.
*: only 3 plants of 850 initiated flower buds on the 18th, 21st and 25th node, respectively, and only 3 plants of Violet initiated flower buds around the 40th node.

In December, the month with shortest day-length, all plants of all strains initiated flower buds at lower nodes. Under longer day-lengths, only Tendan initiated flower buds regularly, while in other strains the flower initiation was scarce; the Nepal strains particularly did not initate flower buds on the main axis up to the 20th–40th node.

Generally, with an increase of the natural day-length, (1) the position of the node having the first flower bud ascended, (2) the individual fluctuation of this position increased, and (3) the difference among the strains became larger. Regarding the differences among the six strains, (1) all the Nepal strains showed similar photoperiodic behavior and (2) the sensitivity to the dark period decreased in the order of Tendan>Violet> Nepal.

From these results it may be inferred that the critical dark period for the Nepal strains is longer than 9.5 hours, for the Tendan it is shorter than 8.5 hours, and for the Violet, it is about 9.5 hours. Judging from the natural day-length of habitats to which the strains are adjusted, it is clear that there is a certain parallelism between their photoperiodic behavior and the day-length of their habitats.

Some plants of Nepal strain No. 850, Tendan and Violet were intercrossed and the photoperiodic behavior of the F_1 plants was examined by the method mentioned above. All the F_1 plants showed a behavior approximately intermediate to the parents and there was no difference between the reciprocal crosses.

50. Polyembryony in Citrus

(By Kazuo FURUSATO, Yasuo OHTA and Kenji ISHIBASHI)

Investigations on polyembryony in *Citrus*, mainly *C. Natsudaidai*, *C. sulcata* and *C. Unshiu*, were carried out from 1952 through 1955, and yielded the following results:

(1) No correlation was found between the number of seeds contained in a fruit and the mean embryo number per seed. The correlation coefficient fluctuated irregularly between $+0.84$ and -0.84, from year to year, from tree to tree, and even from branch to branch.

(2) The mean embryo number in adult plants (about 30 years old) was higher than that in young plants (about 5 years old). This difference was found in all species investigated, and was statistically significant in all cases.

(3) The mean embryo number varied according to the direction in which the fruiting branches were growing; it was higher on a north-side

branch than on a south-side branch of a tree. This was found in all investigated species and the difference was statistically significant. But there was no significant difference between the top and the base of a tree.

(4) Adjacent fruits resemble each other both in seed number and in the variation of embryo number per seed.

It is concluded that the mean embryo number per seed is considerably influenced by the physiological condition of the tree.

51. *Parthenocarpy in Citrus Natsudaidai*

(By Kazuo FURUSATO and Eijiro SUZUKI*)

For breeding a seedless *C. Natsudaidai*, it was thought important to find out whether or not parthenocarpy occurs. For this purpose, hormone treatment was applied.

The experiment was carried out in the flowering period by spraying with various concentrations of phytohormone such as Naphthalene Acetic Acid, Tomatotone, 2.4.D, 2.4.5.T, Flutone and Tomatohooks.

Only one seedless fruit was produced by the phytohormone treatment, that one with 2.4.5.T. solution (42 ppm). This fruit differed in no way in size, taste and other characters from ordinary fruits containing seeds. This seems to show that *C. Natsudaidai* has a tendency to parthenocarpy.

Another experiment, namely injection of N.A.A. into young fruits, yielded one almost seedless fruit having only small immature seeds. However, the fruit did not grow much and had a solid texture, and it was found unsuitable for human consumption.

52. *Polyploid Grape Varieties*

(By Kazuo FURUSATO, Kenji ISHIBASHI and Yasuo OHTA)

A few cultivated grape varieties which appeared to be polyploid were examined for their chromosome complex.

The results are as follows:

Variety	n	2n
Centennial	38	76
Muscat Canon Hall		76
Kyohō		76

* Shizuoka Univ.

Kyohō bud-variant	76
Kyohō × Kyogei	76
Ishihara-wase	76
Delaware, having gigantic fruit	38

Thus, Delaware, in spite of having very large fruit, was diploid, and its offspring, obtained by self-pollination, were all diploid; no triploid was found. This variety may be a chimera with a tetraploid epidermis. This point will be elucidated shortly by further examination.

53. *F_2 Offspring of the Hybrid between Watermelon and Colocynthe*

(By Kazuo FURUSATO and Akira MIYAZAWA)

The hybrids (F_1) between *Citrullus vulgaris* (n=11) and *Citrullus colocynthis* (n=11) showed normal chromosome pairing with 11_{II} at meiosis, and their seed yield was high.

The B_1 from the back-cross to the F_1, and the F_2 showed a segregation of characters as indicated in the following table.

Character	Water melon	Colocynthe	B_1 and F_2
Colour of endocarp	pink	white	pink, white, orange, yellow, yellowish white, yellowish green, greenish white
Taste of endocarp	sweet	bitter	acid, not acid, sweet, bitter,
Texture of endocarp	soft	hard	soft, hard, intermediate
Colour of endocarp	green	dark green striped	green, dark green, striped

Thus, the segregation proved to be very complicated.

54. *Heading and Flowering of Sugar Cane*

(By Kazuo FURUSATO)

Some sugar canes (Co. no. 285 and C. no. unknown) were planted to obtain flowering panicles. During the first year, 1953, the plants were kept in a phytotron, at 30°C; they were transplanted the following year, 1954, to the field. After the middle of September of that year, they

were illuminated by artificial light for a month and half. As they did not bloom, they were again transplanted in the late fall into a green house. Panicles appeared in the latter part of July 1955, only on three-year old stems.

The heading of sugar cane usually takes place in the autumn in plantations in the Northern Hemisphere. But in the above plants, the formation of flower buds apparently took place in the spring. The light period that induces differentiation of flower buds in sugar cane is known to be $12^h 30^m$. It is highly probable that the differentiation of flower buds in the above plants took place in April.

55. Production of Triploid Sugar Beet Seeds

(By Seiji MATSUMURA)

Sugar beet $2x$ and $4x$ seeds mixed in the ratio 1:3 were sown, and "$3x$" seeds[1] were collected from both $2x$ and $4x$ cuttings (mother beets) ("original mixed seeds"). In the combination No. 4398 (398-$4x$)$\times$162 ($2x$), the frequency of $3x$ seedlings was the highest, while the combination No. 4398$\times$399 ($2x$) showed the lowest frequency of $3x$ and the highest of $2x$ (Table 1). It is supposed that the predominance of $2x$ in the latter case was due to the difference in the flowering period between the parental strains, because No. 399 is one of the earliest varieties and No. 4398, being an autotetraploid, belongs to the late varieties. Thus, the synchronisation of the flowering period of the $2x$ and $4x$ plants seems to be of great importance for the production of "$3x$" seeds on a large scale by farmers. Moreover, to increase of the percentage of $3x$, the numerical

Table 1. Frequencies of $2x$, $3x$ and $4x$ seedlings in three triploid combinations, obtained from mixed seeds of $2x$ and $4x$ in the ratio of 1:3

Combinations	$2x$	$3x$	$4x$	Total
4398$\times$162	63	57	5	125
(%)	(50.4)	(45.6)	(4.0)	(100)
4398$\times$401	27	19	7	53
(%)	(50.9)	(35.9)	(13.2)	(100)
4398$\times$399	47	10	1	58
(%)	(81.0)	(17.3)	(1.7)	(100)

1) To be exact, mixed seeds of $2x$, $3x$ and $4x$.

ratio of $4x$ to $2x$ seeds should be higher than 1:3 in mixed sowing.

In the second series of experiments "$3x$" seeds were obtained by planting $2x$ and $4x$ beets in mixed arrangement in the ratio 1:3. Mixed seeds of $3x$-A (progeny of $4x$) and $3x$-B (progeny of $2x$) were sown in the ratio 2:1 ("AB mixed seeds"). In the third experiment, the "$3x$" seeds were obtained from mixed planting of $2x$ and $4x$ beets in the ratio 1:3 ("mixed beet").

Comparative studies of yield and sugar content were carried out for the three different sowings mentioned above (original mixed seed, AB mixed seed, and mixed beet lots) in the experimental fields of the Nippon Beet-Sugar Manufacturing Company in Hokkaido. There was no significant difference among them. But the sugar yield in these three sowings of "$3x$" was rather better than that of No. 192 ($2x$) which is the most widely grown variety.

56. Karyotaxonomic studies in Poaceae, III

(By Tuguo TATEOKA)

(1). Morphological convergence between *Brachypodium sylvaticum* and *Agropyron yezoense*—*Brachypodium sylvaticum* closely resembles in appearance to *Agropyron yezoense*. In the structure of spikelet to which a great taxonomic significance is given, the two species have a greater resemblance than that between *A. yezoense* and certain other species of *Agropyron*, for example, *A. ciliare*. The species belonging to *Agropyron* have large somatic chromosomes of which the basic number is 7, and the species of the genus *Brachypodium* are characterized by small chromosomes of which the basic number is not clear owing to the diversity of the numbers. Viewed from this point, the two genera must be assumed to be phylogenically distinct. *Agropyron yezoense* has $2n=28$ large chromosomes, while *Brachypodium sylvaticum* has $2n=18$ small chromosomes. From these observations, the systematic distinction of the two plants is quite evident. Then, the striking resemblance of spikelet structures between *Agropyron yezoense* and *Brachypodium sylvaticm* should be looked upon as due to convergence which has developed in the course of evolution.

(2). Cytological survey of various species. The survey of chromosome constitution of various groups in Poaceae has been continued. In 1955, somatic chromosome numbers in the 20 species listed below were confirmed.

Species	2n		
Phyllorachis sagittata	24	*Poa nemoralis*	35 (5X)
Bromus tectorum	14	*Melica ciliata*	18
Lolium rigidum	14	*Asperella longe-aristata*	28
L. italicum	14	*Uniola latifolia*	48
Briza media	14	*Sieglingia decumbens*	36
Cynosurus cristatus	14	*Lophatherum sinense*	48
Festuca ciliata	14	*Chikusichloa aquatica*	24
Phragmites Karka	48	*Setaria palmifolia*	54
Panicum repens	45 (5X)	*S. excurrens* var. *pauciseta*	72
Oplismenus compositus	72	*Paspalum orbiculare*	60

From the results thus obtained, some informations concerning the systematic positions of the genera *Phyllorachis*, *Uniola*, *Lophatherum*, etc. were available. These cytological observations were supplemented by the knowledge of anatomical structures of leaves, especially in the genera *Chikusichloa*, *Sieglingia*, *Uniola*, etc.

K. GENETICS AND BIOCHEMISTRY OF PLNT PIGMENTS

57. Studies on Natural Anthocyanins

(By Kôzô HAYASHI and Yukihide ABE)

a) ***Crystallization of blue anthocyanin, and its chemical properties.*** Since the hypothesis that certain flower colors, especially blue colors, are connected with the presence of metal complex salts of anthocyanins was proposed in 1949 by K. Shibata and K. Hayashi, no extensive investigation or criticism of this problem has been published. Very recently, H. Kikkawa and his co-workers (Science, 121:43, 1955, and other publications in Japanese) have suggested that certain pigments occurring in animals, especially the eye pigment of *Drosophila*, are due likewise to the formation of metal complex salts of pigment molecules. This has induced us to undertake a renewed chemical study of blue anthocyanins. We have carried out a series of experiments aiming at a complete purification of the blue pigment in some plants. We have succeeded in obtaining the pigment substance found in *Commelina communis* in pure crystalline form. Beautiful blue needle crystals obtained from the petals of this weed showed several outstanding characteristics, such as: unusual stability against strong hydrochloric acid, non-permeability through a cellophane membrane, reversion of electrophoretic behavior in contrast to that of the

ordinary red form of anthocyanins, and so on. In addition, it has been confirmed that the blue pigment crystals contain a considerable amount of metallic components, probably only Mg and K. Other metallic elements, such as Mo, Fe, Co and Ni, do not seem to take part in the molecular constitution of the crystals. These findings strongly support our previous view that the principle of flower color variation consists not in the acidity of the cell sap but chiefly in the formation of metal-anthocyanin-complexes. Further precise studies on the chemical structure of this interesting substance are in progress. Thus, our metal complex theory of color variation may provide a sound basis for the biochemical genetics of flower colors.

b) *Survey of anthocyanins in some alpine plants.* An extensive investigation has been made to obtain a comprehensive knowledge of the distribution of anthocyanin pigments in alpine plants growing in Japan. Samples were taken from flowers and fruits of various plants found in mountainous districts of Middle Japan. Althogether about a hundred species from 34 families were examined by paper-chromatographic methods. Primary emphasis was laid upon the identification of the anthocyanin components, because the glycosidic combination appears to be appreciably complex, and, accordingly, its determination may be more or less tedious and difficult. Full accounts of the experimental results will be published in the Botanical Magazine (Tokyo) shortly.

58. *Genetic Control of Acylation of Anthocyanin Pigment in Eggplant*

(By Yukihide ABE and Kanji GOTOH)

The anthocyanin pigments appearing in the fruit-coat, stem and flower of eggplant were analysed by the paper-chromatographic method. It has been shown that in general the anthocyanins found in one variety are the same in all kinds of organs. Each of the 6 horticultural varieties of eggplant, Burma, Sendai-naga No. 1, Heta-murasaki, Shinkuro, Taiwan-naga and Emerald, possesses a characteristic acylated glycoside beside a small amount of other constituents. Only the variety Black Beauty has a different glycoside which is entirely free of organic acid. The main anthocyanin spot appearing in paperchromatograms of the variety Burma, seems to be identical with that of nasunin (*p*-hydroxycinnamoyldelphinidin 3-diglucoside), judging from the Rf values found by irrigation with several solvent mixtures. However, careful examination of the products by saponification and partial hydrolysis has revealed

that the main glycoside in Burma is not nasunin but its derivative, associated with an unknown substance. On the other hand, the main pigment of Black Beauty has been proved by similar tests to be delphinidin 3-glucorhamnoside.

The F_1 plant from the cross, Burma × Black Beauty, has an anthocyanin identical with that of Burma (acylated), and the F_2 plants have anthocyanins either of the Burma type (76 individuals) or of the Black Beauty type (non-acylated, 22 individuals), corresponding to the monogenic segregation ratio, 3:1, ($x^2=0.3401$), (based on examinations of fruit-coats and stems). Thus, the acylation in anthocyanin molecule seems to be controlled by a single gene, and its effect appears both in fruit-coat and stem.

59. *Further Analytic Studies of Flower Pigments of Japanese Morning Glory in Relation to Flower Color Inheritance*

(By Yukihide ABE)

In *Pharbitis Nil*, the production of anthocyanins in the corolla is controlled by at least four complementary genes, $+^{ca}$, $+^{c}$, $+^{r}$ and $+^{a}$ (Hagiwara, T., 1930, 1931). In order to obtain more substantial information about this matter, some preliminary experiments were made by paper-chromatography in 4 white-flower strains, 1 light yellow-flower strain, several full-colored flower strains, and also in various F_1 hybrids between them. These experiments have shown certain interrelationships between the genotype and the pigment composition as shown in the following table:

Genotype of plant	Flower color	Constituents found: G1	G2	G3	G4	G5	B1	B2	B3	Y1	Y2	Anthocyanin
c_a $+^{c}$ $+^{r}$ $+^{a1)}$	White	+	±									
$+^{ca}$ c $+^{r}$ $+^{a}$	White	‡	+									
$+^{ca}$ $+^{c}$ r $+^{a}$	White	+	+	+	‡	‡	+	+				+ probably as leucobas
$+^{ca}$ $+^{c}$ $+^{r}$ a(?)	White	+				+	+	‡	+			+ 〃
Unknown	Light yellow	+	+			+		+		‡	+	+ 〃
$+^{ca}$ $+^{c}$ $+^{r}$ $+^{a}$	Colored (red~blue)	+		±			+	+		+		++
		≀		≀			≀	≀		≀		
		±		±			±	±		±		

G: Probably flavonoid pigments B: Substances showing blue flucrescence in U.V. Y: Pale yellow substances. 1) all homozygous

In F_1 plants obtained from the cross between white-flower strains as well as from white × light yellow, and white × full colored, the production of anthocyanins is found to occur in the corolla, and the non-anthocyanin constituents characteristic of the parent plants could be detected in small amount in F_1 plants. On the whole, the pigment composition of F_1 plants resembles that of the full colored flowers. Accordingly, it may be surmised that these non-anthocyanin constituents take part in the biosynthesis of anthocyanin pigment in the corolla. The identification of these substances is in progress.

60. *Separation of Natural Anthocyanins by Column Chromatography*

(By Tôru ENDO)

In the course of investigations of the biochemical genetics of flower color, it became necessary to find a more simplified technique for the separation and quantitative estimation of anthocyanin components. Consequently many partition or adsorption systems made up of various media, organic solvents and aqueous acids, have been tested. In preliminary experiments it was shown that a mixture of anthocyanins was distinctly separated into its pigment components by partition chromatography using a cellulose column.

The procedure is as follows; cellulose powder (Whatman, B Quality) is put with about ten times as much water into a glass tube (35 cm. in length and 4 cm. in inside diameter, equipped with a glass filter at the bottom) up to the 30 cm. level, and a small volume of Hyflo Super Cel is added together with water. For the purification of cellulose, about 50 cc. of concentrated nitric acid is run slowly from the top to the bottom of the column. After washing with about one liter of water, the column is irrigated with about 500 cc. of the aqueous phase of the solvent mixture, *n*-butanol–concentrated hydrochloric-acid/water (5:1:4, *V*/*V*). Just before the irrigation has been through, an aqueous solution of the anthocyanin mixture, (*e.g.* from *Viola tricolor.*) is pipetted into the top of the column, and developed with about 500 cc. of the organic phase of the above solvent mixture. By this treatment, the separation of anthocyanin components is sufficiently good to give a well-developed chromatogram corresponding to the *Rf* values in paper chromatography. On continuous irrigation with the same solvent, the effluent solution is separated into 40 cc. portions by a fraction collector. Each fraction is subjected to a paper chromatographic test for anthocyanin components. It has thus become clear that flavonol pigments can be easily separated from the

anthocyanin group, and they are usually found in the earlier fractions. Quantitative estimation and crystallization of each anthocyanin component, after the acid fractionation of flower extracts of *Viola tricolor*, are now in progress.

L. STUDIES ON COMPETITION

61. *Chromosome Number, Hybridity and Competitive Ability in Rice.*

(By Kan-Ichi SAKAI and Hirosi UTIYAMADA)

It has been demonstrated by a series of experiments that chromosome doubling in pure-line varieties of plants brings about a decrease of competitive ability, while an amphidiploid species is superior in competitive ability to one or both parental species. It was also found in a barley experiment that competitive ability was independent of vigor in the growth of F_1 hybrids.

Thus, the mechanism that makes allopolyploidy advantageous and autopolyploidy disadvantageous in terms of competition still remains to be investigated. This report deals with results of an experiment comparing the competitive ability of parents and their F_1 hybrids in rice in either the diploid or the tetraploid condition. The species of rice, *Oryze sativa* L., includes two subgroups, *Indica* and *Japonica*, which differ from each other in so many respects that one might even consider them different species.

Diploid and tetraploid chromosome races of four *Japonica*, and diploid races of two *Indica* varieties, together with four kinds of diploid and tetraploid F_1 hybrids, were grown and tested for competitive ability in relation to the diploid test strain, Norin No. 29, one of the most popular commercial varieties in Japan. Plants of the test variety were grown alternately with plants of each of the races mentioned above. The experiment was conducted by the split-plot method with four replications, but the data were analyzed according to the method of randomized blocks. Measurements were taken on the test variety, and top weight and number of panicles were recorded on an individual plant basis. Statistical analysis of the data thus obtained has shown that variation due either to chromosome number or to hybridity is highly significant. Mean values of the panicle number of the standard test variety mixplanted with $2X$ and $4X$ races of parental varieties and their hybrids are presented below.

		2X	4X		2X	4X
Top weight (gr.) of the standard test variety mix-planted with:	S_1*	38.45	42.41	S_3*	35.04	41.64
	S_2	36.31	39.70	K_1	27.91	—
	F_1	28.93	36.59	F_1	17.34	34.13
	S_3	37.79	43.74	S_1	42.15	46.06
	S_4	33.71	51.35	K_2	34.47	—
	F_1	33.19	46.06	F_1	26.68	41.63

* S_i stands for the i-th variety of Japonica subgroup, and K_j the j-th variety of Indica subgroup.

It is to be concluded from this data that strains which allow the test variety to have larger values are weaker competitors than those allowing the test strain to have smaller values. It is apparent from the table that the $4X$ plants are always weak in competition with the $2X$ plants, both in parental varieties and in the hybrids. It is shown further that in some cross combinations the competitive ability of the F_1 hybrids is approximately equal or inferior to that of the stronger parent, while in others it is far superior to both parents. As a result of interaction between these two opposed tendencies, the $4X$ plants of F_1 hybrids may be stronger or weaker competitors than either parent, or they may be somewhat stronger than the weaker parent. Thus, it is quite probable that some hybrids, very strong in the diploid condition, may be, in the tetraploid condition, stronger than the weak diploid parent, or even stronger than the strong parent. The strong competitive ability of the F_1 hybrids is probably due to an over-dominance effect of the genes governing competitive ability.

Sakai, K. I. and H. Utiyamada (1956) Studies on Competition in Plants. VII. Jour. Genet. (In the press)

62. *Further note on the Effect on Competition of a Varying Number of Competing and Non-competing Individuals*

(By Kan-Ichi SAKAI)

In Annual Report No. 5 (for 1954), I have described results of my experiments on the same problem using barley and rice varieties. This paper is to supplement these results with some information obtained from two further experiments. The plants used in the present experiments were a variety of upland rice and the so-called red rice in one combination, and two varieties of barley in the other. The scheme of planting

is identical with that in the previous experiments except that the competing plants are arranged among the six surrounding plants at random. The analyses of variance of the data have shown that in these experiments the effect of the varying number of competing individual is always statistically significant. Mean values of a few characters in seven kinds of treatments in the upland rice and the red rice are presented below.

Character	Number of competing individuals among six surrounding plants						
	0	1	2	3	4	5	6
			Upland rice				
Top weight (gr.)	9.41	7.05	6.92	6.42	5.95	5.26	4.89
Culm number	3.39	2.75	2.74	2.49	2.48	2.44	2.37
			Red rice				
Top weight (gr.)	7.72	8.65	7.55	9.05	9.45	11.92	13.52
Culm number	4.50	5.11	4.50	5.16	5.27	6.52	6.78
Kernel number	169.3	178.2	170.0	208.9	208.6	266.6	304.7

The findings in these two experiments agree with those of the previous ones, and a general conclusion which may be drawn from the past and present series of experiments is that the increment or decrement in quantitative characters of a plant exerted by the surrounding competitors is directly proportional to the number of competing individuals.

Sakai, K. I. 1956. Studies on Competition in Plants VIII. Jour. Genet. (In the press)

63. *Possible Establishment of an Equilibrial State due to Competition in Mixed Populations of Autogamous Plants*

(By Kan-Ichi SAKAI and Yuichiro HIRAIZUMI)

This report deals with a theoretical approach to the problem of the relation between competitive ability and propagating capacity of two genotypes in a mixed population of an autogamous plant species, eventually leading to an equilibrium. Let us assume the propagating capacities of the two genotypes, A and B, to be 1 and $1-s$, and the competitive abilities to be c_2 and c_1, respectively. If the frequency of A and B in the t-th generation in the mixture be $1-P_t$ and P_t, then their rates of propagation become $1-P_tc_2$ and $(1-s)+(1-P_t)c_1$, respectively. The frequency of B in the $t+1$-th generation becomes

$$P_{t+1}=\frac{P_t\{1-s+(1-P_t)c_1\}}{(1-P_t)(1-P_tc_2)+P_t\{1-s+(1-P_t)c_1\}}$$

and

$$\frac{dP_t}{dt}=\frac{P_t^3(c_1-c_2)+P_t^2(c_2+s-2c_1)+P_t(c_1-s)}{P_t^2(c_2-c_1)+P_t(c_1-c_2-s)+1}$$

Solving the equation, we get

$$t=\frac{1}{c_1-s}\log P_t-\frac{1-s}{c_2-s}\log(1-P_t)-\left\{\frac{1}{c_1-s}-\frac{1-c_2}{c_2-s}\right\}\cdot\log\{(c_1-c_2)P_t-(c_1-s)\}+\log K$$

or

$$e^{(c_1-s)(c_2-s)t}=\frac{KP_t^{(c_2-s)}}{(1-P_t)^{(1-s)(c_1-s)}\{(c_1-c_2)P_t-(c_1-s)\}^{(c_2-c_1-c_2s+c_1c_2)}}$$

Of the above two formulas, it is to be noticed that in the case of $\{(c_1-c_2)P_t-(c_1-s)\}<0$, $\{(c_1-s)-(c_1-c_2)P_t\}$ would be more suitable. An equilibrium between two genotypes will be reached when

$$1>\frac{c_1-s}{c_1-c_2}>0$$

1. $c_1>s>c_2$
2. $c_1<s<c_2$
3. $c_1=s=c_2$

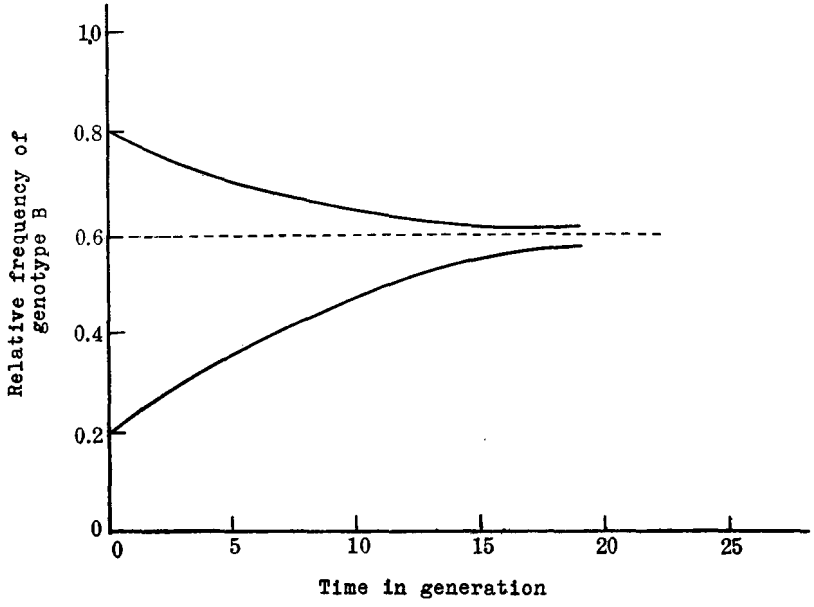


Fig. 1.

Thus, among these three conditions, a stable equilibrium will be established only when $c_1 > s > c_2$, with the frequency of B, $P_B = (c_1 - s)/(c_1 - c_2)$. A graphical example, with $s=0.5$, $c_1=0.8$ and $c_2=0.3$, is presented in Figure 1.

64. *Competition between Autotetraploids and their Diploid Prototypes in Nicotiana tabacum L.*

(By Kan-Ichi SAKAI)

An experiment comparing the competitive ability of diploid and autotetraploid races of two varieties of *Nicotiana Tabacum L.*, White Burley and Bright Yellow was conducted. Individual plants of the two chromosome races of each variety were space-planted in rows, either in pure-stand plots or alternately in mixed plots. The experiment was conducted by the complete randomized block method with three replications. Data were taken on top weight, plant height, leaf- and flower number on an individual plant basis, together with the flowering date. The analysis of variance of the data obtained has shown that the difference between the two chromosome races with regard to the five characters examined was statistically highly significant. The effect of interaction between chromosome races and competition was also statistically significant, except for the number of leaves and flowering date. The mean values of top weight, plant height, and number of flowers in pure-stand and in mixed plots of diploid and autotetraploid plants of the two varieties are presented below. *P* and *M* in the table stand for pure-stand and mixed plots, respectively.

	White Burley				Bright Yellow			
	2X		4X		2X		4X	
	P	M	P	M	P	M	P	M
Top weight (gr.)	452.5	701.9	419.7	206.2	773.0	1112.7	494.4	239.0
Plant height (cm)	110.4	113.5	89.8	76.0	161.9	161.8	131.0	114.1
Number of flowers	103.7	177.9	43.3	22.1	250.0	353.7	91.4	39.4

It is apparent from the table that these three characters of the diploid plants significantly increase when surrounded by autotetraploids instead of diploids, while those of the tetraploids decrease by being grown in mixture with diploid plants. It is concluded accordingly that the doubling of homologous sets of chromosomes decreases the competitive ability of tobacco plants as has been found for rice and barley in experiments reported before.

SAKAI, K. I. 1956. Studies on Competition in Plants. VI. Cytologia Vol. 21 (In the press).

65. *Variation in Competitive Ability among Varieties and Hybrid Strains of Rice*

(By Hiko-Ichi OKA and Kan-Ichi SAKAI)

The experiments on competitive ability in rice so far reported suggest that the varieties belonging to the so-called *Indica* or "Continental" group are superior in competition to those of the so-called *Japonica* or "Temperate-Insular" group. However, intervarietal variation in competitive ability has not been sufficiently investigated. In order to study this variation among cultivated varieties of different phylogenetic groups, and among strains derived from hybrids, experiments were conducted at Taichung, Formosa (in the second crop of 1955), with 53 varieties from various Asian countries and 63 F_{10} strains which had been obtained by propagating the hybrids between distant varieties in bulk or in pedigree culture without selection. For measuring the competitive ability, Taichung no. 65, the famous "Horai" (improved Japanese) variety extensively grown in Formosa, was used as the test strain. Fifteen test plants were grown among about 100 plants of each variety or strain so as to be surrounded by them. The panicle number per plant of the test strain was compared with that in single stand plots. The significance of differences was estimated from the results of variance analysis for single stand plots, distributed over the experimental field and 36 in total number.

The variation in competitive ability thus found among the varieties and hybrid strains are set out in Table 1.

Table 1. Variation in competitive ability among cultivated varieties and strains derived from hybrids (Increase or decrease of panicle number due to competition with Taichung no. 65)

Group	Weak						Strong			Num. of var.'s
	−3	−2	−1	0	1	2	3	4	5	
Cultivated varieties:										
"Continental"				1	2	5	11	10	4	33
"Tropical-Insular"	2		4	2	3	1				12
"Temperate-Insular"			3		2	2		1		8
F_{10} strains from:										
Bulk propagation			2	3	9	16	9	1		40
Pedigree culture			1	5	11	4	1	1		23

The table shows that the "Continental" (*Indica*) varieties generally have high competitive ability, while the Insular (*Japonica*) varieties vary in a wider range in this character. It may also be seen that among the

F_{10} strains, those from bulk populations have higher competitive abilities than those from pedigree culture. The same tendency has been recognized in F_8 strains of another cross, Pei-ku×Taichung no. 65 (unpublished data). This may be due to the fact that growth in bulk population encourages selection favoring higher competitive ability.

66. *Variation in Competitive Ability of Rice Varieties Appearing in Response to the Method of Growing.*

(By Hiko-Ichi OKA and Kan-Ichi SAKAI)

As described in the Annual Report of 1954 (p. 56), the change in the proportion of genotypes in a mixed population of autogamous plants can be expressed as a function of competitive ability (p) and relative propagation rate in the single stand ($1-q$). In order to get an example of the change of competitive ability in response to growth conditions, a mixed population (50:50) of two rice varieties, Taichung no. 65 (a representative "Horai" variety in Formosa) and Pei-ku (a Formosan native variety of first-crop nature), were grown successively for three generations at Taichung, Formosa (from 1954 to 1955), under four different conditions: (1) no-fertilizer, single plant per hill, (2) no-fertilizer, 5 plants per hill, (3) no-fertilizer, single plant per hill, the period in the nursery bed being twice as long as usual, and (4) a large quantity of fertilizer, single plant per hill. The randomized block design with two replications was employed and the proportion of Taichung no. 65 in plant number and in seed number (mix-harvested) were examined in each generation.

It was found that the proportion of Taichung no. 65 decreased under all four conditions, the rate of decrease differing for each. The relative propagation rates ($1-q$) under different conditions were estimated from the data of single stand plots. Using those ($1-q$) values, the value of p in each generation was computed by the following formula (derived from that given in the Annual Report of 1954), in which a_0 and a_1 stand for the proportions of plant number and seed number, respectively.

$$p=\frac{a_1-a_0+(a_0+a_1-2a_0a_1)q}{a_0(1-a_0)}$$

The competitive ability of Taichung no. 65 compared with Pei-ku is shown in Table 1.

As the data show, the competitive ability of Taichung no. 65 decreased in highly fertilized plots and increased when the period in the nursery bed was prolonged. It is known, however, that in order to get better crops, Taichung no. 65, rather than Pei-ku, is fitted to utilize a large

Table 1. Competitive ability (p) and relative propagation rate (1-q) of Taichung no. 65 to Pei-ku under different conditions.

Growing condition	First crop 1-q	First crop p	Second crop 1-q	Second crop p
(1) No fertilizer, single plant	0.872	−0.398	0.925	−0.122
(2) 〃 , five plants	0.815	−0.536	0.912	−0.129
(3) 〃 , single plant, long nursery bed period	0.807	−0.203	0.938	0.038
(4) High dosage of fertilizer, single plant	0.949	−0.603	1.368	−0.597

quantity of fertilizer, but that often yields a poor crop when the nursery bed period is prolonged. It is interesting to find a variety which apparently shows a higher competitive ability under conditions relatively unfavorable to the plant.

M. GENETICS AND CYTOLOGY OF MICROORGANISMS AND VIRUSES

67. *Electron-microscopical Studies of Ultra-thin Sections of Penicillium chrysogenum. II. The Fine Structure of Mitochondria-like Cytoplasmic Granules.*

(By Seizo TSUDA)

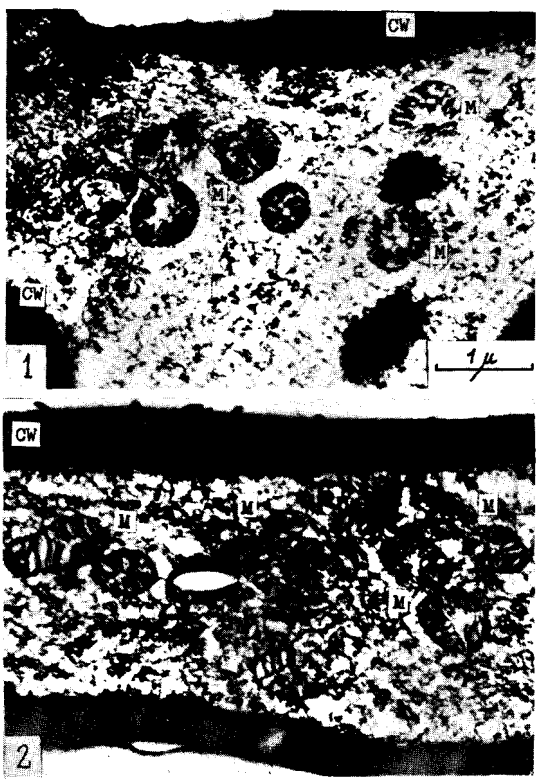
The ultra-thin sectioning technique, developed since the invention of the electron microscope, has made it possible to penetrate deeper into the structure of microorganisms. Cytological studies of this kind have been carried out by many workers, CHAMPAN and HILLIER (1953), (1956), SJÖSTRAND (1954), AGAR *et al.* (1955), and others.

The results of the present author's investigations in fungi have previously been reported in part (1955, 1956). They show that the cytoplasm and nucleus of these organisms have a more or less reticular structure. The present paper offers some additional information on the fine structure of the mitochondria-like cytoplasmic granules of *Penicillium chrysogenum*. The materials and the methods have been described in a previous report (1955).

The cell wall (CW) is thick and the cytoplasm shows a loose filamentous structure; a great number of cytoplasmic mitochondria-like granules are

scattered through the cytoplasm, as seen in the figures. The cytoplasmic granules (M) are mostly spherical or oval. They are surrounded by a limiting membrane, and there is seen a system of internal ridges within each granule.

Figure 1 shows transverse sections of mitochondria-like granules (M), each having a system of radial ridges. Notice the considerable variation in the number of the granules contained in the cells shown in Figs. 1 and 2. The granules vary in size, ranging from 0.61 to 0.91 μ.



CW: cell wall; M: cytoplasmic granule.

Figure 2 shows longitudinal and transverse sections of the cytoplasmic granules. At the left end of the micrograph is a longitudinally sectioned granule showing a number of internal ridges. The cytoplasmic granules are grouped in the middle of this cell.

TSUDA, S. 1955. Electron microscopical studies of ultra-thin sections in *Aspergillus*, *Penicillium* and *Neurospora*. Indian Phytopathology. 8: 83-93.

TSUDA, S. 1956. Electron microscopical studies of ultra-thin sections of *Penicillium chrysogenum* (II) Journal of Bacteriology. 71 (4): 450-453.

68. *Studies on a Doubly Lysogenic Strain of Pseudomonas solanacearum*

(By Mitsuo TSUJITA and Chiaki MATSUI)

The two lysogenic strains of *Pseudomonas solanacearum*, T-c 200 and S-9, produce phages of different types but serologically related, which may be called T-c 200 and S-9 phages. The liberation of these phages from bacteria occurs spontaneously, and cannot be induced by ultra-violet irradiation.

A doubly lysogenic strain which harbors the two prophages T-c 200 and S-9, within the same cell, is produced by superinfection of T-c 200 phage with S-9 bacteria. From this doubly lysogenic strain a new type phage which has characters of both parents in host range specificity is produced.

The non-lysogenic S-10 bacteria can be artificially changed into single lysogenic S-10 (S-9) bacteria by infection with S-9 phage. Before this treatment S-10 bacteria have no affinity for Sp_1 phage (they do not adsorb the phage). Through this treatment, however, the lysogenic S-10 (S-9) bacteria acquire an affinity for Sp_1 phage. A similar modification of the affinity for Sp_1 virulent phage was observed in E-79 and E-3 bacterial strains when these were lysogenized with the S-9 phage. This phenomenon may be considered a kind of transformation due to phage infection.

By superinfection of T-c 200 phage with the singly lysogenic S-10 (S-9) strain, a doubly lysogenic S-10 (S-9, T-c 200) strain can be obtained, and a new type phage is also produced.

The following four possible causes, may be responsible for the production of this new type phage, i) simultaneous maturation of both the parent phages in the same lysogenic cell, ii) host induced modification, iii) spontaneous mutation, or iv) genetic recombination. Of these, genetic recombination is apparently the most plausible explanation of our experimental results. To determine the types of recombination, we have carried on analytical experiments, and found that the new type phage originated through recombination in the prophage stage. This may represent a new type of recombination.

69. *Studies on the Lysogenicity of Pseudomonas solanacearum: Removal of Lysogenicity of the Bacterial Strain T-c 200 by Ultra-violet Irradiation.*

(By M. TSUJITA and C. MATSUI)

Among various specificities characteristic of the T-c 200 of *Pseudomonas*

solanacearum the removal of lysogenicity by irradiation with ultra-violet light and the inactivation of free virus are the subjects of this report.

Experimental method: T-c 200 bacteria, cultured for 24 hours in potato dextrose extract (slant), were washed in distilled water, and one ml of the bacterial suspension was exposed to UV light (relative energy 2537Å: 3129–5730Å 100:17; irradiation distance 45 cm). The irradiated suspension was diluted with potato dextrose solution and plated on agar. After 48 hours at 34°C, the number of surviving bacteria in the suspension was determined by making colony counts. About 150–200 colonies among the

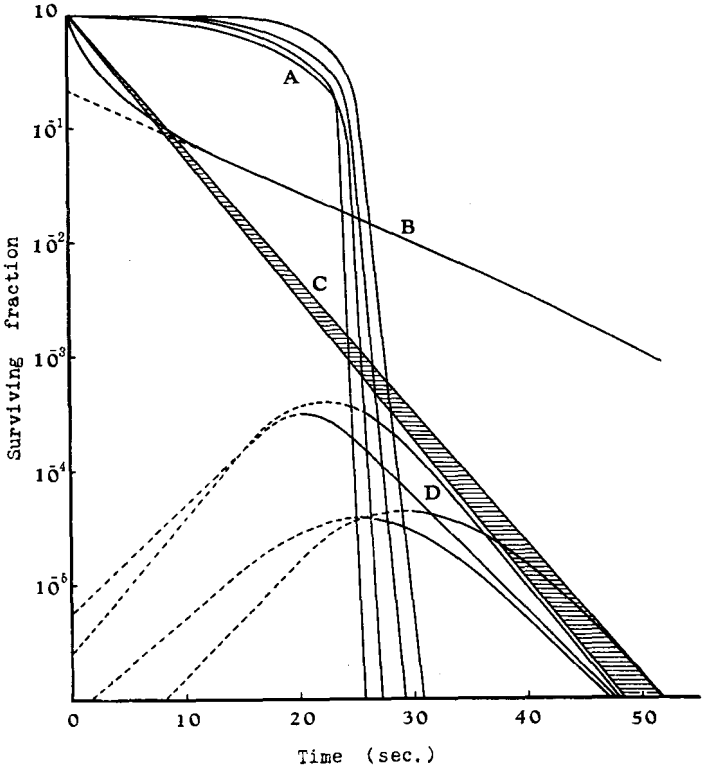


Fig. 1. Logarithmic plot of the UV survivors of T-c200 strain in *Pseudomonas solanacearum*.

A. Lysogenic strain T-c200 as a plaque former on S IX.
B. Free virus liberated from lysogenic strain T-c200.
C. Lysogenic strain T-c200 as a colony former. The results of experiments repeated four times are included in the shaded area.
D. Non-lysogenic T-c200 as a colony former.

300 to 500 colonies on a plate were picked at random and cultured in 3 ml of potato dextrose solution. Then the lysogenic character of each colony was examined by the following method.

The T-c 200 virus was spread on UV-irradiated bacterial cells of the indicator strain, and if plaques were formed, the lysogenicity of the bacterial cells was assumed to have been removed. On the other hand, in order to count the number of surviving viruses in the irradiated suspension, the non-lysogenic T-c 200 was used as an indicator strain.

Experimental results and consideration: The experimental results are presented in Figure 1. Curve B, drawn by plotting the concentration of the UV survivors of free virus seems to indicate that T-c 200 phages could be inactivated by 1/2 to 1/3 hit at the initial stage of UV irradiation. This may be a kind of multiple hit curve, and may be accounted for by assuming two types of phages present in mixture, one resistant and the other sensitive to UV. The curves D, representing the production of non-lysogenic bacteria, could not be plotted until about 20 seconds after the beginning of UV-irradiation, because of technical difficulty. The results of the 4 experiments are rather variable. This may be due to the difference in the physiological condition of the bacteria at the time of irradiation. The broken lines of D are made to fit parabolic curves. However, if this were the case, non-lysogenic bacteria should have appeared at the start of the irradiation; the experimental results do not agree with this presumption. It is likely that non-lysogenic bacteria had rapidly increased in an early stage of irradiation.

70. *Studies on the Multiplication of Bacterial Virus Affecting Streptomyces griseus (II).*

(By Seizo TSUDA)

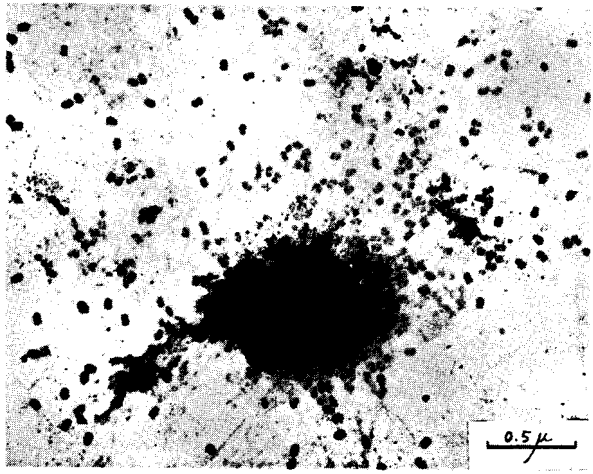
Although fundamental problems relating to phage multiplication have been attacked with various genetic, biochemical and morphological methods, important questions still remain to be solved, especially concerning gene and chromosome replication, and DNA synthesis in phage.

This investigation, carried out with the aid of an electron microscope, is concerned with the multiplication of the bacterial virus affecting *Streptomyces griseus* spores.

The material was the S-1 phage strain infecting the MA-14 strain of *Streptomyces griseus*, originally supplied in 1955 by Dr. H. B. Woodruff. The spores were obtained from a 6-day slant culture on potato-glucose agar medium.

The S-1 phage has a uniform, tadpole shape. Its head is an ellipsoid

measuring about 60 by 70 mμ, nearly as large as the head of T_2, T_4 and T_6 *Eschericia coli* phages; the tail is about 150 mμ long, and about 10 mμ wide. The head appears something like a coffee-bean and it has an internal structure, with two elongate granules lying side by side.



The cytoplasm of the spore cell infected by phages presents a very coarse texture, especially in the later period of multiplication of the phage. In this appearance the infected spore cell is in sharp contrast to a normal cell which shows no internal structure under observation by an electron microscope.

Mature phages are liberated from the host spore cell, together with the cytoplasmic inclusions of the cell. The cytoplasm of the spore cell eventually takes on a mesh-work structure when the multiplication of phage has ceased.

N. THEORETICAL GENETICS

71. *Index for Individual Selection in Autogamous Plants on the Basis of Individual Measurements, Line Means and Line-group Means.*

(By Kan-Ichi SAKAI)

This paper deals with the theoretical construction of a selection index for single individuals on the basis of individual measurements, the line means and the line-group means.

Let the genetic value of an individual be Y and its phenotypic value be x_1, the mean value of the line involving the individual be x_2, and the mean value of the group of the lines be x_3. The weighting factors to be given to x_1, x_2 and x_3 for constructing a selection index X are denoted as b_1, b_2 and b_3. Then,

$$X=b_1x_1+b_2x_2+b_3x_3\,.$$

Values of the three b's making the correlation between X and Y the maximum will be obtained by solving the following three simultaneous equations:

$$\begin{aligned}
b_1V_{x_1}+b_2W_{x_1x_2}+b_3W_{x_1x_3}&=W_{x_1Y}\\
b_1W_{x_1x_2}+b_2V_{x_2}+b_3W_{x_2x_3}&=W_{x_2Y}\\
b_1W_{x_1x_3}+b_2W_{x_2x_3}+b_3V_{x_3}&=W_{x_3Y}
\end{aligned}$$

V and W stand respectively for variance and covariance. Variances of x_i and covariances between x_i and x_j for the n-th hybrid population will be given as follows:

$$V_{x_1(F_n)}=\frac{2^{n-1}-1}{2^{n-1}}D+\frac{2^{n-1}-1}{4^{n-1}}H+E$$

$$V_{x_2(F_n)}=W_{x_1x_2(F_n)}=\frac{2^{n-2}-1}{2^{n-2}}D+\frac{2^{n-2}-1}{4^{n-1}}H+\frac{E}{N}$$

$$V_{x_3(F_n)}=W_{x_1x_3(F_n)}=W_{x_2x_3(F_n)}=\frac{2^{n-3}-1}{2^{n-3}}D+\frac{2^{n-3}-1}{4^{n-1}}H+\frac{E}{NN'}$$

where D, H and E are components of variation (see Mather, 1949), N and N' stand for the number of individuals per line and the number of lines per group, respectively. Covariances between x_i and Y will be given as follows:

$$W_{x_1Y(F_n)}=\frac{2^{n-1}-1}{2^{n-1}}D+\frac{2^{n-1}-1}{4^{n-1}}H$$

$$W_{x_2Y(F_n)}=\frac{2^{n-1}-1}{2^{n-2}}D+\frac{2^{n-2}-1}{4^{n-1}}H$$

$$W_{x_3Y(F_n)}=\frac{2^{n-3}-1}{2^{n-3}}D+\frac{2^{n-3}-1}{4^{n-1}}H$$

Thus, we are to solve the following simultaneous equations to find the value of the b's.

$$\begin{aligned}
b_1V_{x_1}+b_2V_{x_2}+b_3V_{x_3}&=V_{x_1}-E\\
b_1V_{x_2}+b_2V_{x_2}+b_3V_{x_3}&=V_{x_2}-E/N\\
b_1V_{x_3}+b_2V_{x_3}+b_3V_{x_3}&=V_{x_3}-E/NN'
\end{aligned}$$

A few numerical examples are given for an F_4 hybrid population for

any character having a heritability value of 0.1 or 0.8 in the F_2 generation. K stands for the H/D ratio.

Heritability (F_2)	K	Selection index
0.1	0	$X=x_1+18.6x_2+14.5x_3$
	1	$X=x_1+5.5x_2+15.1x_3$
	5	$X=x_1+4.9x_2+1.9x_3$
0.8	0	$X=x_1+0.9x_2+0.04x_3$

The heritability value of the selection index is given by

$$h_X^2=\frac{\left[(b_1+b_2+b_3)^2-\frac{(b_1+2b_2+2b_3)^2+4b_1b_3-2b_2^2}{2^{n-1}}\right]D}{b_1^2V_{x_1}+b_2(2b_1+b_2)V_{x_2}+b_3(2b_1+2b_2+b_3)V_{x_3}}$$

The correlation coefficient between X and Y is given by

$$r_{XY}=\frac{\left(b_1+b_2+b_3-\frac{b_1+2b_2+4b_3}{2^{n-1}}\right)D+\left(\frac{4b_1+2b_2+b_3}{2^{n+1}}-\frac{b_1+b_2+b_3}{4^{n-1}}\right)H}{\sqrt{(V_{x_1}-E)[b_1^2V_{x_1}+b_2(2b_1+b_2)V_{x_2}+b_3(2b_1+2b_2+b_3)V_{x3}]}}$$

SAKAI, K. 1956. Theoretical Studies in Plant Breeding Technique. III. Jap. Jour. Breed. (In the press)

72. *Formulas for Estimating the Effect of one Chromosome on a Quantitative Character by the So-called "Reciprocal-Translocation Method"*

(By Hiko-Ichi OKA)

When a gene A-a is located on one of two chromosomes involved in a reciprocal translocation, $\begin{matrix}1-2 & 3-4\\ 1-3 & 2-4\end{matrix}$, the frequencies of the three genotypes, AA Aa and aa, among fertile and semi-sterile plants in the F_2 may be shown by the following formulas, in which p represents the recombination value between A-a and the interchange point.

Genotype	*Increment*	*Frequency*	*Mean*
Fertile plants (F)			
AA	d	$\frac{1}{2}(1-2p+2p^2)$	
Aa	h	$\frac{1}{2}(4p-4p^2)$	$2p(1-2p)h$
aa	$-d$	$\frac{1}{2}(1-2p+2p^2)$	

Semi-sterile plants (S)

AA	d	$p-p^2$	
Aa	h	$1-2p+2p^2$	$(1-2p+2p^2)h$
aa	$-d$	$p-p^2$	

It will be found from these frequency formulas that the difference between fertile and semi-sterile plants in mean measurement is, putting $1-2p=P$,

$$M_{F_2}(S)-M_{F_2}(F)=(1-4p+4p^2)h=P^2h \quad \ldots\ldots\ldots\ldots (1)$$

The variance of F_2 fertile and semi-sterile plants, and the difference between them will also be found, respectively, to be as follows:

$$V_{F_2}(F)=\frac{1}{2}(1+P^2)d^2+\frac{1}{4}(1-P^4)h^2+E$$

(E: Non-heritable component)

$$V_{F_2}(S)=\frac{1}{2}(1-P^2)d^2+\frac{1}{4}(1-P^4)h^2+E$$

$$V_{F_2}(F)-V_{F_2}(S)=P^2d^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Formula (2) can be applied for the variance of F_3 family means and the F_2/F_3 covariance. The mean variance of F_3 families may be written as follows:

$$V_{F_3}(F)=\frac{1}{4}(1-P^2)d^2+\frac{1}{8}(1-P^2)h^2+E$$

$$V_{F_3}(S)=\frac{1}{4}(1+P^2)d^2+\frac{1}{8}(1+P^2)h^2+E$$

$$V_{F_3}(S)-V_{F_3}(F)=\frac{1}{2}P^2d^2+\frac{1}{4}P^2h^2 \quad \ldots\ldots\ldots\ldots\ldots (3)$$

The above formulas, (1), (2) and (3), may hold good irrespective of the effect of genes located on other chromosomes not involved in the translocation, and do not contain the environmental component of variance. If we can get data by a proper design of experiment, the values of d, h and p may be estimated by using these formulas.

73. *Solution of a Process of Random Genetic Drift with a Continuous Model*

(By Motoo KIMURA)

Random genetic drift is a process of change in gene frequency in a population due to random sampling of gametes in reproduction. Since FISHER (1922, 1930) and WRIGHT (1931) much theoretical work has been done on this subject but the results have been largely at the level of

asymptotic formulae. Recently I have obtained the complete solution of this process for the case of a pair of alleles using a continuous model. Since the details have been given in the accompanying reference (1), only the results will be presented here.

Consider a random mating population of N breeding individuals of a diploid organism and let A and A' be a pair of alleles whose frequencies in the population are x and $1-x$ respectively.

In order to describe the process adequately we have to specify the following three probabilities denoted by $f(1, p; t)$, $f(0, p; t)$ and $\phi(x, p; t)dx$, giving respectively the probabilities that A becomes fixed in the population by the t^{th} generation, that A is lost by the same generation and that the frequency of A lies between x and $x+dx(0<x<1)$ in the t^{th} generation, all starting from the gene frequency p at the 0^{th} generation:

$$f(1, p; t)=p+\sum_{i=1}^{\infty}(2i+1)pq(-1)^i F(1-i, i+2, 2, p)e^{-[i(i+1)/4N]t},$$

$$f(0, p; t)=f(1, q; t)$$

$$\phi(x, p; t)=\sum_{i=1}^{\infty}pqi(i+1)(2i+1)F(1-i, i+2, 2, p)$$
$$\times F(1-i, i+2, 2, x)e^{-[i(i+1)/4N]t},$$

where $q=1-p$. F in this expression represents the hypergeometric function which is defined by

$$F(1-i, i+2, 2, x)=1+\frac{(1-i)(i+2)}{1\cdot 2}x+\frac{(1-i)(2-i)\cdot(i+2)(i+3)}{1\cdot 2\cdot 2\cdot 3}x^2$$
$$+\cdots(i=1, 2, 3, \cdots)$$

For large t, we have the following asymptotic formula given previously (2);

$$\phi(x, p; t)\sim 6pqe^{-(1/2N)t}+30pq(1-2p)(1-2x)e^{-(3/2N)t}+\cdots$$

(1) KIMURA, M. 1955. Solution of a process of random genetic drift with a continuous model. Proc. Nat. Acad. Sci., 41:144–150.

(2) KIMURA, M. 1954. Process leading to quasi-fixation of genes in natural populations due to random fluctuation of selection intensities. Genetics, 39: 280–295.

74. *Stochastic Models for Senescence in Paramecium Based on Random Segregation of Macronuclear Elements*

(By Motoo KIMURA)

It has been known to biologists for a long time that if cultures of *Paramecium* are kept under conditions of exclusively asexual reproduction they lose vigor ("aging" or "senescence") and die out eventually.

Recently T. M. Sonneborn has made extensive studies of this phenomenon and conceived the hypothesis that the again is due to an accumulation of random chromosome segregations in the macronucleus. The following is a summary of a mathematical study of stochastic models suggested to me by Dr. J. Lederberg. The macronucleus is considered to be of very high ploidy, consisting of, say, m chromosome sets, each with n chromosomes scattered at random inside the nucleus. As the first model, we assume that at macronuclear division each chromosome reduplicates itself, followed by the random distribution of chromosomes into two groups in equal number to form the daughter macronuclei. As the second model, we assume that after the reduplication each chromosome has an equal chance of going to either side independently of the other chromosome. In both models death by aging is assumed to occur whenever all chromosome of any one type are lost from the nucleus by random segregation. According to Sonneborn the usual strains have $n \fallingdotseq 41$ including strains of $n=35$ and 50. Also, m is at least 100, so that mn would be of the order of 5000. The stochastic processes involved are complicated but it is possible to develop asymptotic theories to calculate the probability of survival (Ω_t) after the t^{th} generation of exclusivly asexual reproduction. For the first model,

$$\Omega_t \sim (1/\sqrt{2}\,\exp\,[(2\log_e 2-1)n-((n-1)/4m)t]$$

and for the second model,

$$\Omega_t \sim (1-e^{-4m/t+4})^n$$

In both cases Ω_t should be taken much smaller than unity.

For $m=100$, $n=41$, generations giving 99 % death, 99.9 % death and 99.99 % death are respectively about 202,225 and 248 generations for the first model and 175,210,246 generations for the second. These results agree reasonably well with the finding of Sonneborn that with exclusivly asexual reproduction many of the cultures die before 200 generations and almost all cultures die out before 324 generations.

The models allow us to calculate the proportion of individuals showing a recessive character due to segregation of factors in macronucleus. If we start from heterozygotes Aa, the proportion of recessives after t generations is

$$P_I(t) \sim \frac{1}{2}(1-e^{-t/2m})$$

for the first model and

$$P_{II}(t) \sim (e^{-2m/t+4}-e^{-4m/t+4})/(1-e^{-4m/t+4})$$

for the second. Numerical values of the proportion are given in the accompanying table for several values of $t(m=100)$.

In addition, we can calculate the allowable number of successive macronuclear regenerations. The present models seem to suggest that, if regeneration occurs from a random fragment of 1/33 of the macronucleus, at most only a few regenerations are possible.

t	P_I	P_{II}
50	—	0.024
100	0.197	0.127
200	0.316	0.273
300	0.389	0.341
∞	0.500	0.500

75. *Genic Selection in a Finite Population (Interaction between Genic Selection and Random Genetic Drift)*

(By Motoo KIMURA)

Consider a randomly mating population of effective size, N, and let x and $1-x$ be frequencies of gene A and its allele A'. If A has a selective advantage s over A' measured in Malthusian parameters, the probability density $\phi(x, p; t)$ that x lies between x and $x+dx(0<x<1)$ at the t^{th} generation given that $x=p$ at $t=0$ will satisfy the following singular partial differential equation;

$$\frac{\partial \phi}{\partial t}=\frac{1}{4N}\frac{\partial^2}{\partial x^2}[x(1-x)\phi]-s\frac{\partial}{\partial x}[x(1-x)\phi]\,,\quad (0<x<1)\,,$$

with initial condition

$$\phi(x, p; t)=\delta(x-p)\,.$$

Recently this equation was used by Wright and Kerr (1954) in connection with their selection experiment in very small populations, and the state of steady decay was successfully analysed by Wright.

I have succeeded in obtaining the complete solution in terms of the oblate spheroidal wave function studied by J. A. Stratton and others (1941),

$$\phi(x, p; t)=\sum_{k=0}^{\infty} C_k e^{-\lambda_k t+cx} V_{lk}(z)$$

where $c=Ns$ and $z=1-2x$. The spheroidal function $V_{lk}(z)$ is expressed as a series of the Gegenbauer polynomials

$$V_{lk}(z)=\sum_{n=0,\,1}{}' f_n^k T_n^{\nu}(z)\,,$$

where f_n^k's are constants and the primed summation is over even values of n if k is even, odd values of n if k is odd. The boundaries $x=0$ and $x=1$ act as absorbing barriers. The details, together with further dis-

cussion, are given in another publication (see reference below), in which the rate of decay due to fixation and loss of the gene has been tabulated for various selection intensities and effective population sizes. It has been shown that if Ns is less than about 1, there is a considerable chance for fixation of a nonadaptive gene.

KIMURA, M. Stochastic processes and distribution of gene frequencies nnder natural selection. Cold Spring Harbor Symposium on Quantitative Biology. Vol. 20, (in press).

76. *Rules for Testing Stability of Selective Polymorphism*

(By Motoo KIMURA)

Recently there has been increased interest in polymorphism and many new examples have been found in diverse organisms. This poses an interesting problem in the theory of population genetics: What are the conditions necessary and sufficient for the maintenance of stable genetic polymorphism by selection? I have obtained a solution subject to two restrictions: (1) random mating, and (2) constant genotypic selective values.

Suppose that a given locus has n alleles and let x_i be the frequency of the i^{th} allele, A_i. Let $a_{ij}(=a_{ji})$ be the fitness of individuals of genotype A_iA_j. The fitness may be expressed in Malthusian parameters in a continuous model or as the selective value in the discrete case.

The necessary and sufficient conditions for the maintenance of all the n alleles in stable equilibrium are: (i) the quadratic form

$$T=\sum_{i=1}^{n-1}\sum_{j=1}^{n-1} t_{ij}x_ix_j\,,\quad (t_{ij}=t_{ji})$$

where

$$t_{ij}=a_{ij}-a_{in}-a_{jn}+a_{nn}$$

be negative definite, i.e.

$$t_{11}<0\,,\quad \begin{vmatrix} t_{11} & t_{12} \\ t_{21} & t_{22} \end{vmatrix}>0\,,\quad \begin{vmatrix} t_{11} & t_{12} & t_{13} \\ t_{21} & t_{22} & t_{23} \\ t_{31} & t_{32} & t_{33} \end{vmatrix}<0\,,\ \text{etc. up to order } (n-1)\,.$$

and (ii) $(-1)^{n-1}\Delta_i>0$ for all $i=1, 2, \cdots, n$. Here Δ_i is the determinant made by substituting one's for all the element of i^{th} column in the matrix

$$A=[a_{ij}]\,.\quad (i, j=1, \cdots, n)\,.$$

If such a stable equilibrium exists, the equilibrium frequency of the i^{th} allele is given by

$$\hat{x}_i = \Delta_i / \sum_{i=1}^{n} \Delta_i$$

and the mean fitness of the population becomes

$$\bar{a} = \Delta / \sum_{i=1}^{n} \Delta_i ,$$

where $\Delta = |A|$. The result can be extended to cover the case of independent multiple loci. It is then possible to show that without dominance, epistasis alone can not maintain a selective polymorphism. For a detailed discussions, see the following report:

KIMURA, M. Rules for testing stability of selective polymorphism. (to be published)

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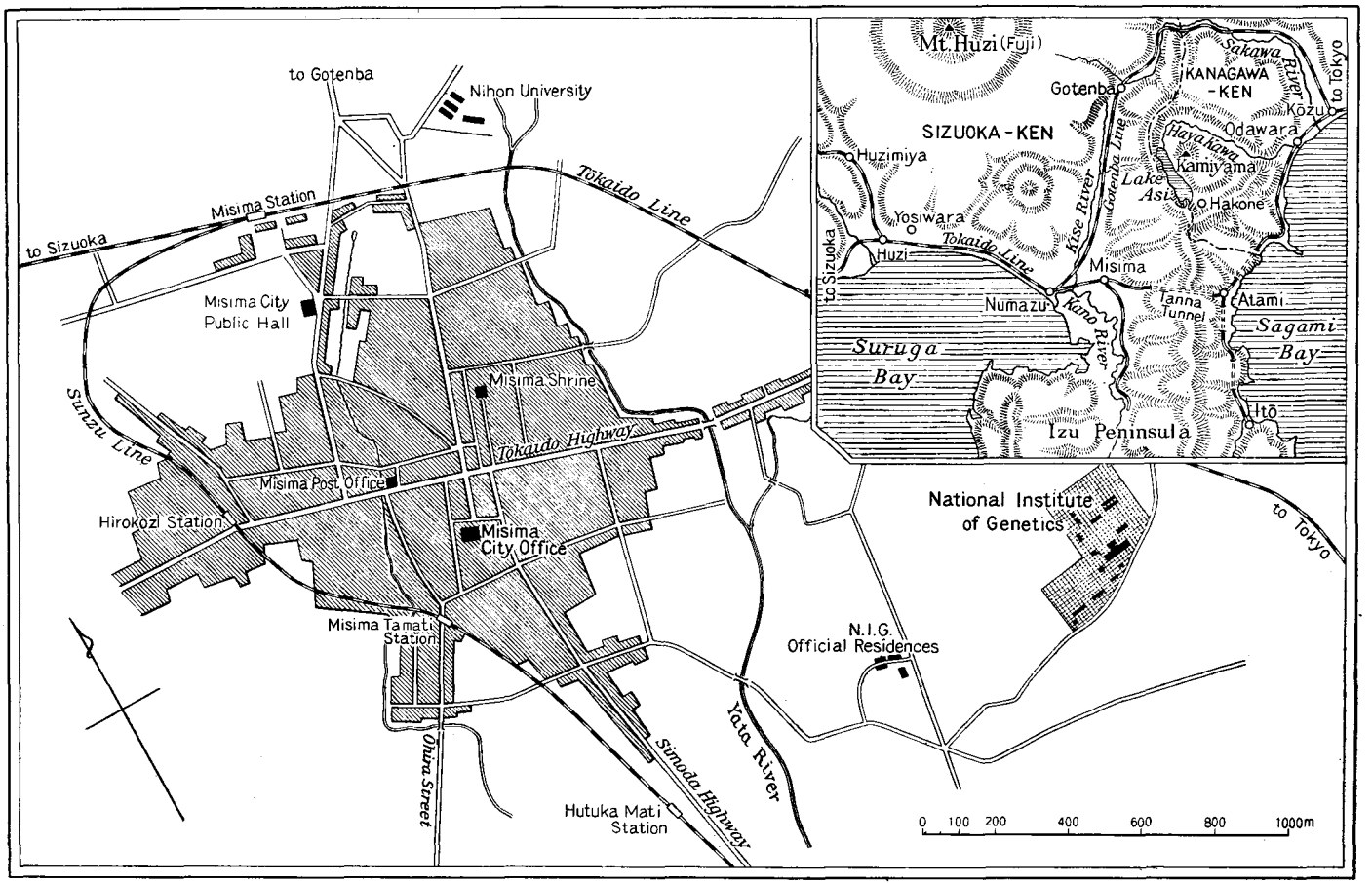
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