

Bull. Univ. Osaka Pref., Ser. B, Vol. 26,
大阪府立大学紀要, 農学・生物学, Mar., 1974.

Inheritance of Radioresistance in *Drosophila*. II. Radioresistance and Maternal Effect in the Early Embryonic Stage

Eiji NAKASHIMA-TANAKA and Masahiro OGAKI

Department of Biology, University of Osaka Prefecture

Introduction

A comparison of radiation effect among strains having different sensitivity to irradiation, and a study of vicissitude of the strain difference in the course of developmental process, may help to bring about a better understanding of the mechanism of radiation effect on living organisms. In the previous papers^{7,10)} we reported about the inheritance of radiation resistance and sensitivity to ⁶⁰Co- γ -rays in adult *Drosophila melanogaster*. We found that the resistance to ⁶⁰Co- γ -rays was dominant over sensitivity and it was mainly due to the third chromosomal factor. Besides this major factor, another one(s) on the second chromosome may be concerned with the radiation resistance and sensitivity, but the effect of X-chromosome and the cytoplasm was negligible.

An experiment of strain difference in sensitivity due to UV-irradiation in early embryonic stage of *Drosophila melanogaster* has been carried out by Ghelelovitch.^{1,2)} According to Ghelelovitch, the UV-sensitivity in the early embryonic stage was hereditary and transmitted mainly by the second chromosomal factor, and such a characteristic in the early embryonic stage depended on the origin of the cytoplasm. Murakami and Tazima⁶⁾ studied the strain difference in the susceptibility to X-rays of the eggs of *Bombyx mori*. Murakami⁶⁾ found that the radiosensitivity and the maternal effect in the early embryonic stages of *Bombyx mori* were quite similar to those of *Drosophila*. Genetic analysis of radioresistance and sensitivity to X-rays in adult stage of Australian wild stocks of *Drosophila* was reported by Parsons *et al.*^{14,15)}

The correlation of radiosensitivity in embryonic stage with that in adult stage is, however, little known. In this paper we intend to show the correlation of radiosensitivity and resistance between adult flies and their embryos, and to discuss the cytoplasmic effect or maternal influence on the early developmental stages of *Drosophila melanogaster*. We also intend to discuss whether or not the gene(s) controls radiation resistance throughout the whole life from egg to adult. Preliminary data of this work were presented in previous papers.^{8,9)}

Materials and Methods

Newly hatched adult flies were kept for two days in a vial containing fresh media (raw sugar 15%, dry yeast 2%, agar-agar 2%, rice bran 5% and water) to which some amount of yeast paste was added. The well-fed flies were permitted to lay eggs for 2 hours and then they were transferred as softly as possible to an

egg collecting vial. On the bottom of the egg collecting vial a filter paper, spread with a thin film of food and moistened with 2 per cent acetic acid, was placed. The period of egg collection did not exceed 30 minutes, and the age of the eggs was calculated from the mid point of this period, the maximum error introduced thereby being ± 15 minutes. The filter paper, on which the eggs were deposited, was transferred to a petri dish without eggs being touched and was kept at $24.5 \pm 1^\circ\text{C}$ till irradiation by X-rays. The relative humidity was 40–60 per cent in the course of the experiments. The X-ray machine was run at 230 kVp, 20 mA, with 0.5 mm Al and 0.5 mm Cu filters and 500 R/min dose rate.

Two wild strains (Hikone-H and Mino-H) and one synthetic strain (1, 3-Hikone; *bw*) which are resistant to ^{60}Co - γ -rays, and one mutant strain (*bw*; *st ss*) which is sensitive, were used in this experiment. F_1 hybrid eggs obtained by crosses between the resistant Hikone-H strain and the sensitive *bw*; *st ss* strain was also used. As the evidence for radiation effect the embryonic mortality and survival ratios were calculated on the basis of the number of unhatched and hatched eggs 48 hours after irradiation, and the LD_{50} value was estimated from the dosage-mortality curves. The eggs from which larvae emerged could easily be distinguished, for the egg membranes collapsed and looked like flattened and wrinkled bags.

Results and Discussion

It has been reported by several investigators that radiosensitivity of *Drosophila* varies with each developmental stage of embryos.^{3,4,11-13} Ghelelovitch¹ also reported that the UV-sensitivity of *Drosophila* embryos varied with the increase of developmental time. In an experiment on the strain difference in radiosensitivity, it seems necessary to determine whether the difference is due to their own intrinsic nature or to a relative difference in the growth rate among strains. In order to examine this point the eggs of four strains were collected for 30 minutes and observed at hourly intervals from 12 to 27 hours after oviposition, then the observation was interrupted till 46 hours after egg laying. The results are presented graphically in Fig. 1.

No egg hatched before 12 hours and only a few eggs hatched later than 27 hours after oviposition. The maximum point of hatching was 22 hours after oviposition in the Hikone-H, 1, 3-Hikone; *bw* resistant and *bw*; *st ss* sensitive strains. But in the Mino-H resistant strain hatched one hour later than the other strains, nevertheless this strain was resistant. From the results presented in Fig. 1 it seems that the developmental age of the eggs collected for 30 minutes was nearly the same and there was no fundamental difference in the developmental stage among the strains. This may indicate that the strain difference in radiosensitivity is not due to a relative difference in the growth rate among the strains, but possibly to the intrinsic nature of the strains themselves.

Based on this supposition, we examined the radiation effect to the embryos of one, three, five and nine hours old, and compared the difference in radiosensitivity among above four strains. The experimental results in 1-hr-old eggs were shown in Fig. 2, which illustrated graphically the relation between dosage and mortality.

As shown in Fig. 2, sensitivity to radiation of the *bw*; *st ss* strain was higher than that of the other strains such as the Hikone-H and the 1, 3-Hikone; *bw* strains. The LD_{50} values were 230 R in the *bw*; *st ss* and 300 R in the other three Hikone-H, Mino-H and 1, 3-Hikone; *bw* strains. As was reported in the

previous paper,⁷⁾ in the adult stage the *bw ; st ss* strain was sensitive and the Hikone-H, Mino-H and 1, 3-Hikone ; *bw* strains were resistant. It is interesting to note that such a strain difference in the sensitivity to radiation was already observed in such an early embryonic stage, though it was very slight.

The susceptibility of embryos to radiation was rapidly decreased as the embryos grew, and the difference between the resistant and the sensitive strain became more pronounced in 3-hr-old embryos than the 1-hr-old ones. The experimental results of the four strains in 3-hr-old embryos, 48 hours after X-ray irradiation were given in Table 1 and illustrated graphically in Fig. 3, which shows the dosage-mortality curves of the four strains. As can be seen in Fig. 3, the LD₅₀ value of the 3-hr-old embryos was about four to five times as large as that of the 1-hr-old embryos, being 1,500 R in the three resistant strains and 950 R in the sensitive strain.

In Fig. 3 it was found that the eggs of the *bw ; st ss* sensitive strain were killed approximately 100 per cent by the dose of 1,700 R, while the embryos of the three resistant strains were killed only about 60 per cent by the same dose.

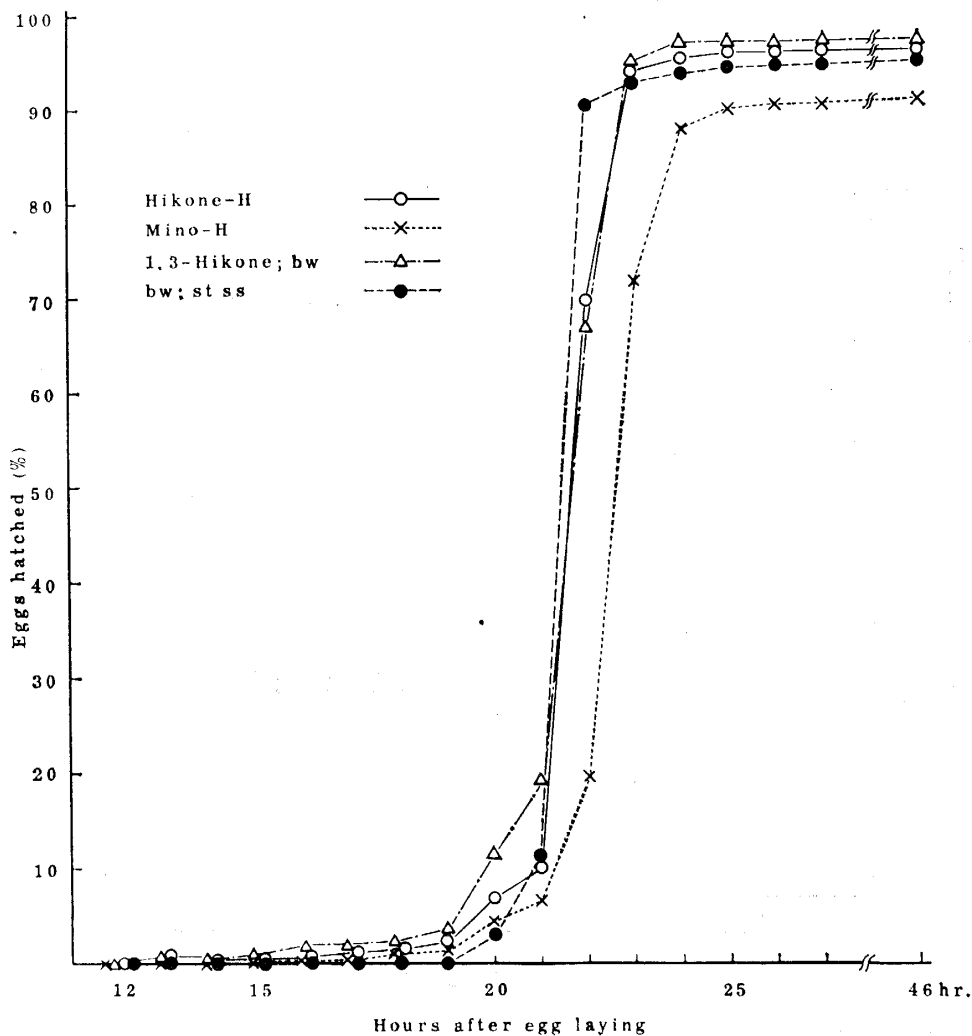


Fig. 1. Hatching time of the embryos of two wild strains (Hikone-H and Mino-H), one synthetic strain (1,3-Hikone; *bw*) and one mutant strain (*bw ; st ss*) under the normal condition.

In order to kill approximately 100 per cent of the embryos of the resistant strains, the eggs had to be irradiated by the dose of about 3,000 R. From these results it is clear that the strains of Hikone-H, Mino-H and 1,3-Hikone; *bw* are resistant and the *bw ; st ss* strain is sensitive, even at the early embryonic stage. Therefore, it can be concluded that there is a striking parallelism in the radiation effect between the adult and early embryonic stages.

In the study on 5-hr-old embryos the LD_{50} value was 900 R in the three resistant strains and 650 R in the sensitive strain, sensitivity being slightly higher than the 3-hr-old embryos. Henshaw and Henshaw,³⁾ Hildreth⁴⁾ and Packard¹¹⁻¹³⁾ reported that the radiosensitivity of embryos changed definitely with the stage of development. The eggs grew more sensitive during cleavage and blastulation, rather resistant at the time of gastrulation, and more sensitive again after gastrulation. From the view point of the LD_{50} value, it may appear that the radiosensitivity in the 5-hr-old embryos is higher than in the 3-hr-old embryos. So far as this fact is concerned, our results are very similar with theirs. In our experi-

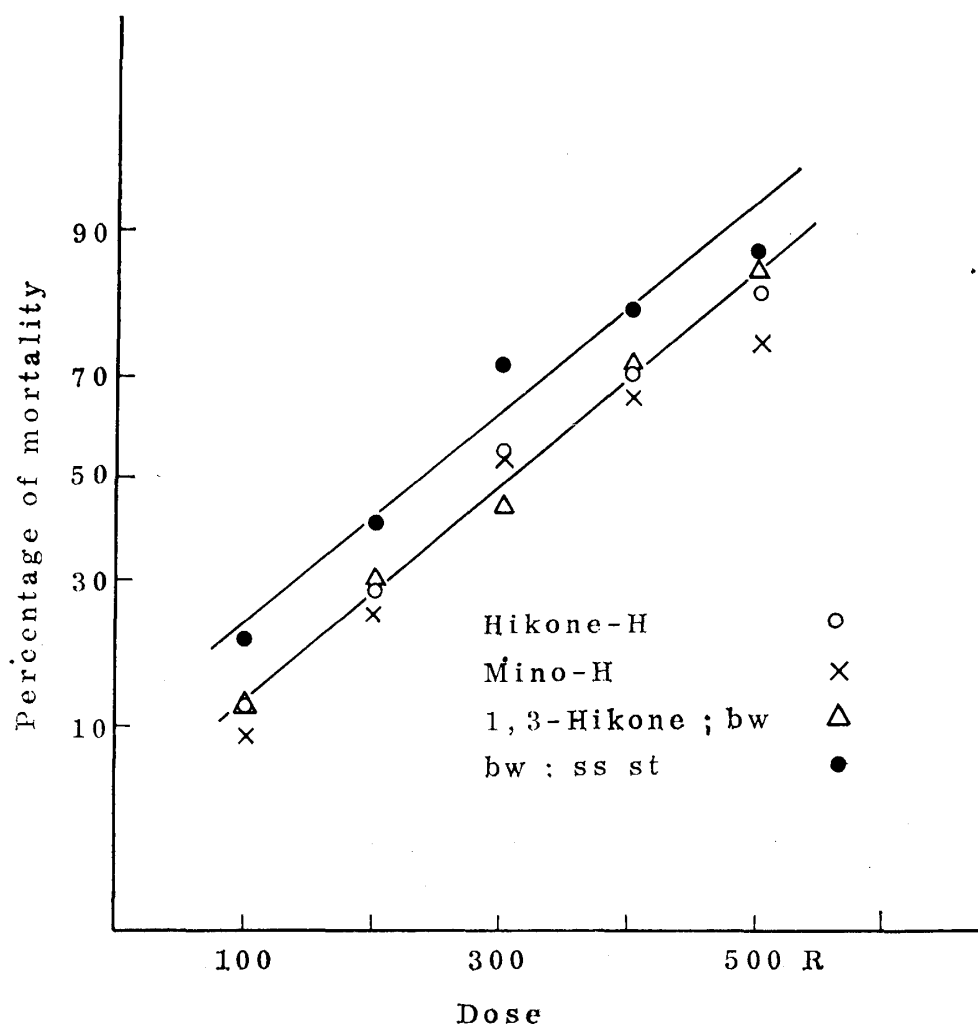


Fig. 2. Dosage-mortality curves for 1-hr-old eggs of two wild strains (Hikone-H and Mino-H), one synthetic strain (1,3-Hikone; *bw*) and one mutant strain (*bw ; st ss*), 48 hours following X-ray irradiation with various doses.

Table 1. Effect of X-rays on mortality of 3-hr-old eggs of four strains, Hikone-H, Mino-H, 1, 3-Hikone; *bw* and *st ss*, at 48 hours after irradiation.

Dose	Hikone-H	*	Mino-H	*	1, 3-Hikone; <i>bw</i>	*	<i>bw</i> ; <i>st ss</i>	*
Control	8/375 = 2.13		90/ 851 = 10.57		15/534 = 2.80		75/ 623 = 12.03	
500	27/663 = 4.07	1.88	138/1039 = 13.28	3.03	34/507 = 6.70	4.01	253/1354 = 18.68	7.55
750	—	—	—	—	—	—	163/ 594 = 27.44	17.51
900	42/331 = 12.68	10.77	159/ 918 = 17.32	7.54	110/570 = 19.29	16.96	246/ 506 = 48.61	41.58
1100	—	—	—	—	—	—	412/ 571 = 72.15	68.34
1300	271/773 = 35.05	33.53	508/1194 = 42.54	35.74	268/727 = 36.86	35.04	542/ 634 = 85.48	83.49
1700	431/714 = 60.36	58.47	705/1127 = 62.55	58.12	558/889 = 62.76	61.68	786/ 792 = 99.24	99.13
2100	171/192 = 89.06	88.71	333/ 397 = 83.87	81.96	633/668 = 94.76	94.60	—	—
2500	383/396 = 96.71	96.45	803/ 863 = 93.04	92.20	432/438 = 98.63	98.59	—	—

*, Per cent mortality adjusted by the following formula: $\frac{n-n_c}{1-n_c}$. *n*, mortality in treatment. *n_c*, mortality in control.

ments, however, a prominent feature of killing effect was observed in the 5-hr-old embryos. As was indicated in Fig. 4 the mortality curves did not show the sigmoid shape as observed in the 1- and 3-hr-old embryos. The probit treatment of the data did not straighten the curve out. The curves apparently had a point of inflection. With the doses below 800 R in the sensitive strain and 1,000 R in the resistant strains, the higher the dose, the greater the effect. On the other hand, with doses above those the effect of radiation did not grow larger, although a marked difference in the susceptibility to radiation between the resistant and sensitive strains still remained.

In another experiment to confirm this prominent feature of the presence of the dose-independent range, it was demonstrated again (Fig. 5). By referring to these results we may suppose that concerning the LD_{50} value, it is not appropriate to compare the susceptibility of 3-hr-old embryos with that of 5-hr-old ones, because of the presence of dose-independent range. Thus the radiation sensitivity may be said to have undergone a qualitative change around five hours after

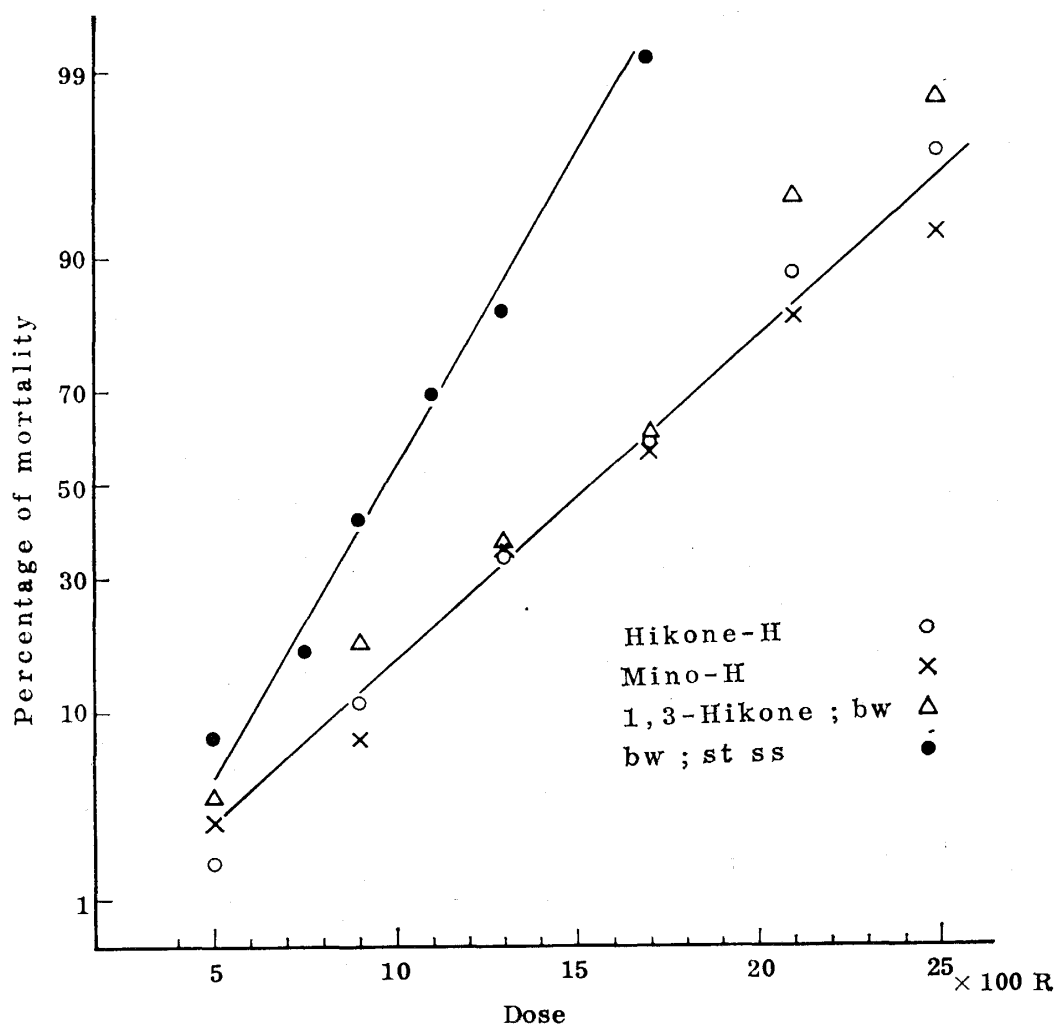


Fig. 3. Dosage-mortality curves for 3-hr-old eggs of two wild strains (Hikone-H and Mino-H), one synthetic strain (1,3-Hikone; *bw*) and one mutan tstrain (*bw*; *st ss*), 48 hours following X-ray irradiation with various doses.

oviposition.

In a study on 9-hr-old embryos another conspicuous feature was observed (Fig. 6). The resistance was markedly strengthened and the killing effect was not found with dose range from 3,000 R to 10,500 R in both the resistant and sensitive strains. It is very interesting to note that in this stage the strain difference observed in the earlier developmental stages disappeared.

In previous papers ^{7,8)} it was reported that the maternal or cytoplasmic influence on the radiosensitivity was negligible in the adult stage, judging from the results obtained in the irradiation of F₁ progenies from the reciprocal crosses between the resistant and the sensitive strain. They also reported that the resistance to radiation in the adult stage was dominant over sensitivity and it was mainly due to a factor on the third chromosome. On the other hand, in the F₁

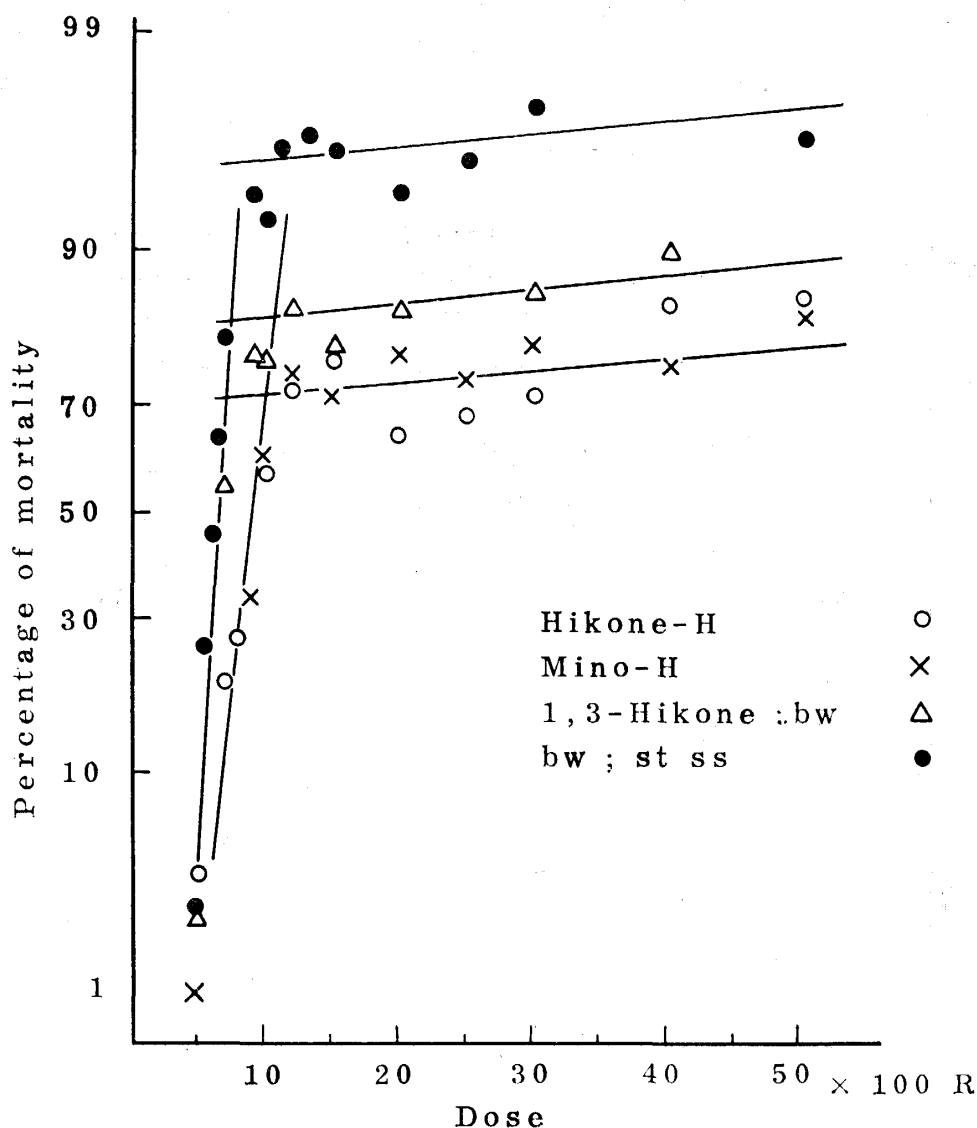


Fig. 4. Dosage-mortality curves for 5-hr-old eggs of two wild strains (Hikone-H and Mino-H), one synthetic strain (1,3-Hikone; *bw*) and one mutant strain (*bw*; *st ss*), 48 hours following X-ray irradiation with various doses.

embryos from the reciprocal crosses between the resistant and the sensitive strain, Hikone-H ♀ × *bw*; *st ss* ♂ and *bw*; *st ss* ♀ × Hikone-H ♂, the tendency of the susceptibility to radiation was markedly different from that in the adult flies.

The experimental results of 3-hr-old embryos obtained from the *bw*; *st ss* sib and the Hikone-H sib matings together with their hybrids from the reciprocal crosses are given in Fig. 7. The LD₅₀ values of the *bw*; *st ss* sib and the Hikone-H sib mating strains were 920 R and 1,450 R respectively. The LD₅₀ value of the F₁ hybrid embryos between the *bw*; *st ss* females and the Hikone-H males was 920 R, which was identical with the degree of radiosensitivity of the sensitive *bw*; *st ss* sib mating strain. On the other hand, the LD₅₀ value of the F₁ hybrid embryos between the Hikone-H females and the *bw*; *st ss* males was 1,450 R, which was identical with that of the resistant sib mating Hikone-H strain.

In Fig. 8 the hatchability in the 5-hr-old eggs of the *bw*; *st ss* sib mating, Hikone-H sib mating and their reciprocal hybrids is presented. As shown in Fig. 8 the shape of curves was the same as that presented in Fig. 5, the dose-independent range being seen with the doses from 900 R to 3,000 R for the sensitive group of *bw*; *st ss*/*bw*; *st ss* and *bw*; *st ss*/Hikone-H; from 1,300 R to 3,000 R for the resistant group of Hikone-H/Hikone-H and Hikone-H/*bw*; *st ss*.

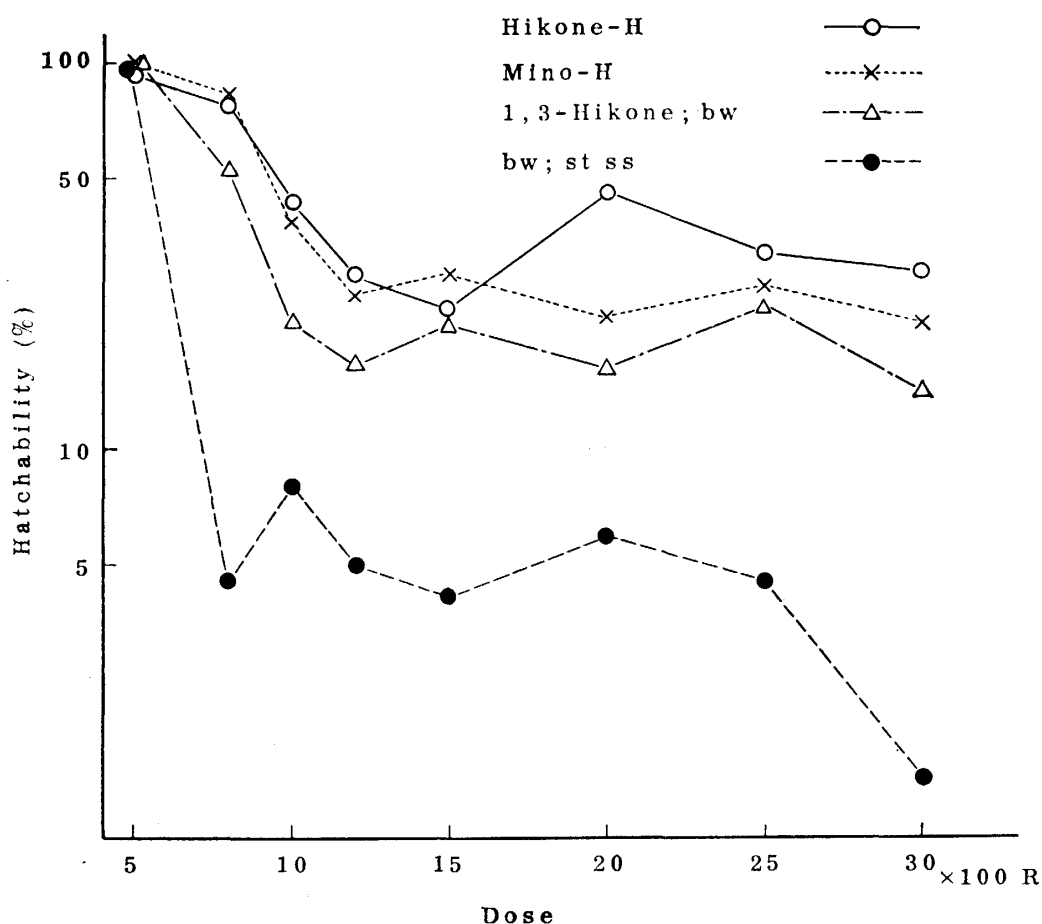


Fig. 5. Hatchability curves for 5-hr-old eggs of two wild strains (Hikone-H and Mino-H), one synthetic strain (1,3-Hikone; *bw*) and one mutant strain (*bw*; *st ss*), 48 hours following X-ray irradiation with various doses.

It is very clear from Figs. 7 and 8 that in the F_1 zygotes from such reciprocal crosses as Hikone-H ♀ × *bw*; *st ss* ♂ and *bw*; *st ss* ♀ × Hikone-H ♂, the degree of susceptibility to radiation was markedly different in each zygote; it is mainly due to the direction of the crosses. Thus, the F_1 progenies showed the same type of radiation sensitivity as did their mothers. Furthermore, the radiosensitivity markedly decreased with the development of the embryos and the difference in susceptibility between the resistant and the sensitive strains disappeared and no maternal effect was seen in the 9-hr-old embryos. These findings seem to suggest that the susceptibility to radiation in early embryonic stages of both inbred and hybrid lines might be due to the influence of cytoplasm transmitted from the mother to eggs, regardless of the control of the paternal strains.

According to the study by Ghelelovitch¹⁾ on a comparison of the UV-sensitivities in the two strains of *Drosophila* in their various early embryonic stages, sensitivity differed by the strains and stages, but this difference disappeared in later stages. From the results of reciprocal crosses between the resistant and sensitive strains, he also indicated that the sensitivity in early embryonic stage was due to the cytoplasm derived from the maternal parent and supposed that the cytoplasmic factor was not self-replicating but oogenetic one determined by the genotype of the mother.²⁾ Recently, it was demonstrated by Murakami⁵⁾ in a study of X-ray sensitivity of silkworm eggs that in early embryonic stages the

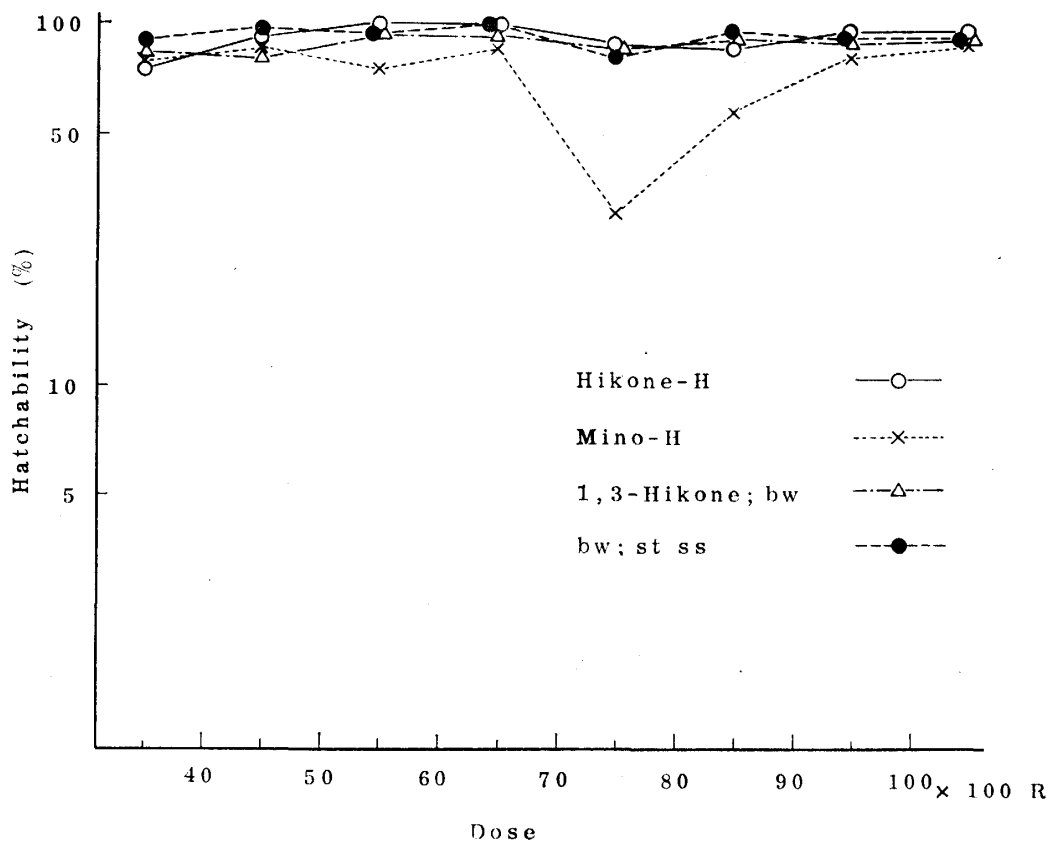


Fig. 6. Hatchability curves for 9-hr-old eggs of two wild strains (Hikone-H and Mino-H), one synthetic strain (1,3-Hikone; *bw*) and one mutant strain (*bw*; *st ss*), 48 hours following X-ray irradiation with various doses.

cytoplasmic effect might be causing the differential sensitivity in different strains but disappeared at later embryonic stage.

The same situation was also observed, as mentioned above, in this study on the X-ray resistant and sensitive strains of *Drosophila*. It is very significant to note, however, that the strain difference, which was evident in the early and disappeared in the late embryonic stage, reappeared at the adult stage. Such a vicissitude of the strain difference in the course of developmental process up to the adult stage may be explained by the following assumption: (1) The radiosensitivity of an embryo at early developmental stage is determined, not by the genotype of that individual, but by that of its mother. (2) The cytoplasmic factor determined by mother is not self-replicating, since the strain difference disappeared in later embryonic stage. (3) Major resistant gene(s) located on the right arm of

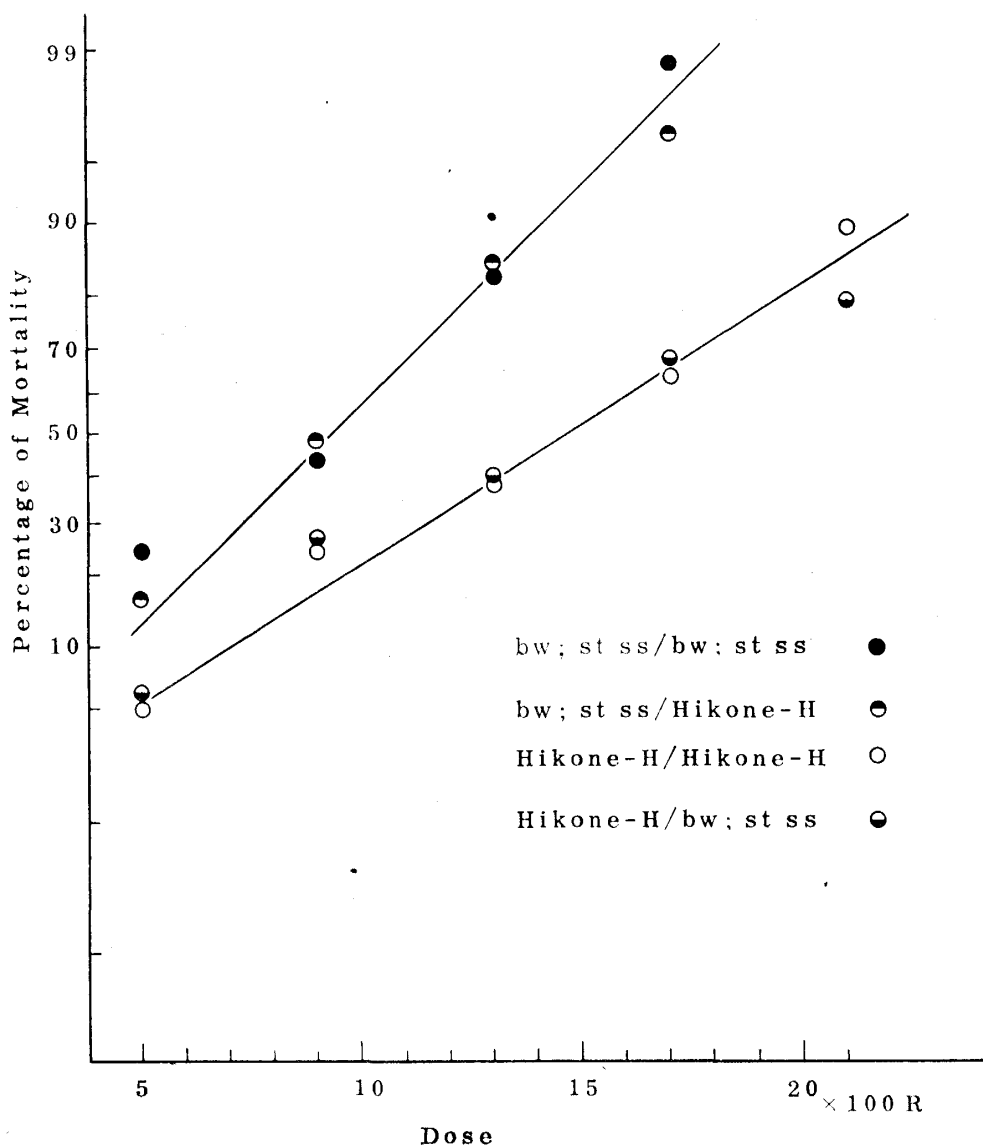


Fig. 7. Dosage-mortality curves for 3-hr-old eggs of two inbred strains (Hikone-H/Hikone-H and *bw; st ss/bw; st ss*) and F_1 hybrid eggs from their reciprocal crosses (Hikone-H/*bw; st ss* and *bw; st ss/Hikone-H*), 48 hours following X-ray irradiation with various doses.

the third chromosome should begin to exert its specific function at some stage in the developmental process, when differentiation toward whichever of radiosensitivity should take place.

Summary

Susceptibility to X-rays in early embryos of the *bw; st ss* strain in *Drosophila melanogaster* was higher than that of the Hikone-H, Mino-H and 1, 3-Hikone; *bw* strains, which shows a striking parallelism in the radiation effect exists between the embryonic stage and adult stages. The susceptibility in egg stages varied as the embryonic development advanced. The difference of the susceptibility to X-rays between the radioresistant and the radiosensitive strains was seen in the embryos of one, three and five hours old, but not seen in nine hours old. What is meant by the dose effect curves indicative of embryonal mortality seem to differ not only quantitatively but also qualitatively after five and nine hours after egg laying. Reciprocal crosses between the sensitive and the resistant strains showed that the radiosensitivity in early embryonic stages of *Drosophila* seemed

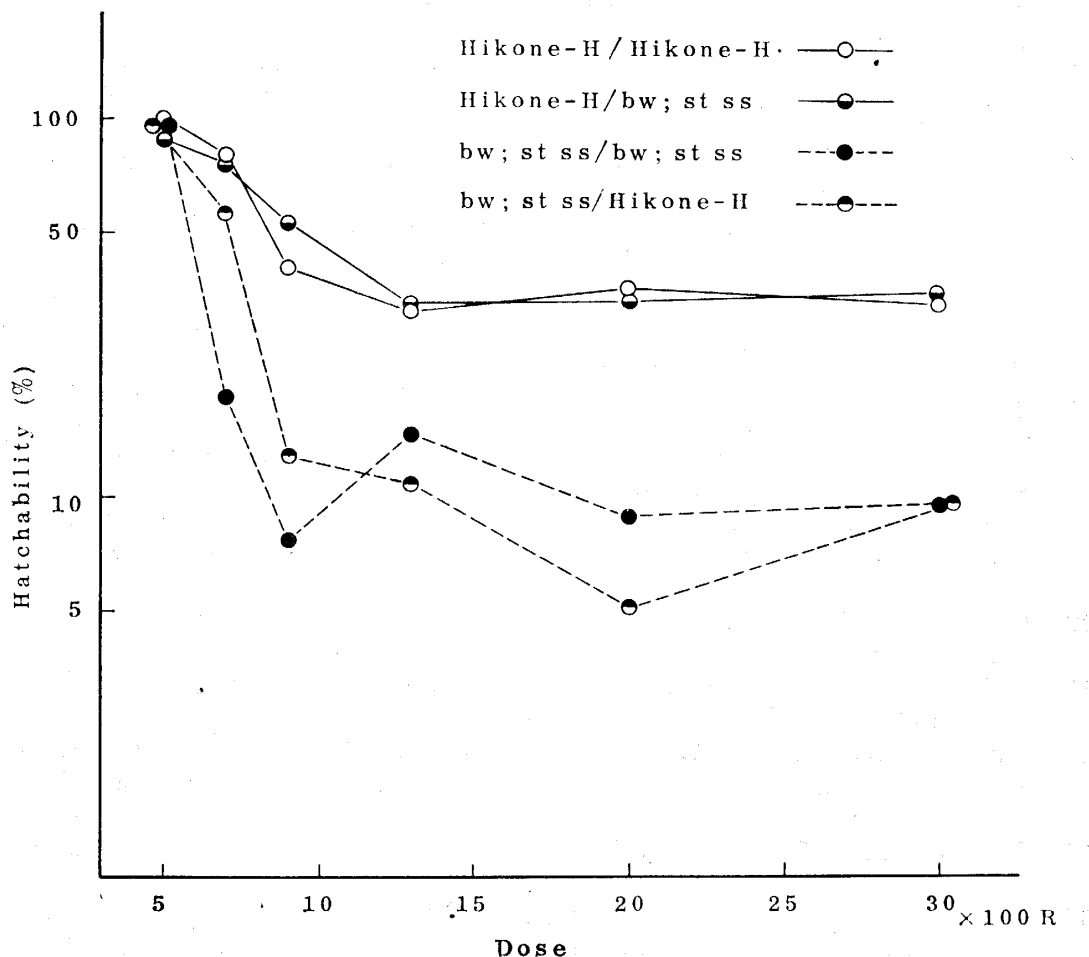


Fig. 8. Hatchability curves for 5-hr-old eggs of two inbred strains (Hikone-H/Hikone-H and *bw; st ss/bw; st ss*) and F₁ hybrid eggs from their reciprocal crosses (Hikone-H/*bw; st ss* and *bw; st ss*/Hikone-H), 48 hours following X-ray irradiation with various doses.

to depend largely upon the characteristics of the female parents. Thus, the maternal effect was observed in early embryonic stages but no cytoplasmic or maternal effect was seen in later embryonic stages.

Acknowledgments

The authors are very grateful to Drs. T. Asai, A. Takeda, Y. Hori and other members of the Radiation Center of Osaka Prefecture for their technical assistance in X-ray irradiation.

References

- 1) Ghelelovitch, S. 1966. La sensibilité des oeufs de la *Drosophila* (*Drosophila melanogaster*, Meig.) à l'action létale des rayons ultraviolets, I. Évolution de la sensibilité avec l'âge de l'embryon. *Int. J. Radiation Biol.* **11**: 255-271.
- 2) Ghelelovitch, S. 1968. La sensibilité des oeufs de la *Drosophila* (*Drosophila melanogaster*, Meig.) à l'action létale des rayons ultraviolets, II. Déterminisme génétique de la sensibilité des stades embryonnaires postérieurs à la gastrulation. *Mutation Res.* **5**: 117-131.
- 3) Henshaw, P. S., and C. T. Henshaw. 1933. Changes in susceptibility of *Drosophila* eggs to X-rays. I. A correlation of changes in radiosensitivity with stages in development. *Radiology.* **21**: 239-251.
- 4) Hildreth, P. E. 1967. Developmental effect of X-rays on embryos of *Drosophila melanogaster*. *Proc. Nat. Acad. Sci.* **58**: 1924-1929.
- 5) Murakami, A. 1969. Comparison of radiosensitivity among different silkworm strains with respect to killing effect on the embryos. *Mutation Res.* **8**: 343-352.
- 6) Murakami, A. and Y. Tazima. 1967. Studies on strain differences in radiosensitivity in the silkworm. I. Screening of sensitive and resistant strains to embryonic radiation killing. *Ann. Rept. Natl. Inst.* (Japan). **17**: 98-100.
- 7) Ogaki, M. and E. Nakashima-Tanaka. 1966. Inheritance of radioresistance in *Drosophila*. I. *Mutation Res.* **3**: 438-443.
- 8) Ogaki, M. and E. Nakashima-Tanaka. 1969. Genetic analysis of radiosensitivity in *Drosophila melanogaster*. *Japan. J. Genet.*, **44**, Suppl. **2**: 27-28.
- 9) Ogaki, M., E. Nakashima-Tanaka and S. Matsunaga. 1969. Genetic control of radiosensitivity in *Drosophila melanogaster*. *Gamma-Field Symposium No. 8 Genetic Control of Radiosensitivity 1969*: 33-50.
- 10) Ogaki, M. and E. Tanaka. 1963. Inheritance of tolerance to gamma radiation in *Drosophila melanogaster* (Abstr). *Genetics.* **48**: 904-905.
- 11) Packard, C. 1926. The measurement of quantitative biological effects of X-rays. *J. Cancer Res.* **10**: 319-339.
- 12) Packard, C. 1928. A biological measure of X-ray dosage. *J. Cancer Res.* **11**: 282-292.
- 13) Packard, C. 1935. The relation between age and radiosensitivity of *Drosophila* eggs. *Radiology.* **25**: 223-230.
- 14) Parsons, P. A., I. T. MacBean and B. T. O. Lee. 1969. Polymorphism in natural populations for genes controlling radioresistance in *Drosophila*. *Genetics.* **61**: 211-218.
- 15) Parsons, P. A., I. T. MacBean and B. T. O. Lee. 1969. Evidence for genes for radioresistance in natural populations of *Drosophila*. *Japan. J. Genet.*, **44**, Suppl. **2**: 29-31.