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Takahashi, Masateru
Kyushu University Graduate School of Bioresource and Bioenvironmental Sciences

Lee, Jae Man
Kyushu University Graduate School of Bioresource and Bioenvironmental Sciences

Mon, Hiroaki
Kyushu University Graduate School of Bioresource and Bioenvironmental Sciences

Yoshida, Hiroko
Kyushu University Graduate School of Bioresource and Bioenvironmental Sciences

他

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Radiation Resistance and its Inheritance in the Silkworm, *Bombyx mori*

**Masateru TAKAHASHI[1], Jae Man LEE[1], Hiroaki MON[1], Hiroko YOSHIDA[1],
Yutaka KAWAGUCHI[1], Hideaki MAEKAWA[2], Katsumi KOGA[1‡]
and Takahiro KUSAKABE[1*]**

Laboratory of Silkworm Science, Kyushu University Graduate School of Bioresource and
Bioenvironmental Sciences, 6–10–1 Hakozaki, Fukuoka 812–8581, Japan

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Exposure to radiation causes various biological effects, which can be detected both in the exposed animals and their offspring. In the silkworm, *Bombyx mori*, many studies in somatic and genetic effects of radiation have been conducted using various doses and qualities of radiation sources, because of their usability for mutation induction. However, differences in sensitivity to radiation among silkworm strains remain unknown. In the present study, we have identified two resistant strains (p50 and m042) and two sensitive strains (f12 and m91) for γ–irradiation by screening genetic stocks of *B. mori* maintained in Kyushu University Graduate School. In reciprocal mating experiments, there is liability that F_1 progeny became more sensitive to γ–irradiation when the susceptible strains were used as the female parent. Even though details remain to be determined, it appears from the current studies that radiation sensitivity in the silkworm is controlled, at least in part, by maternal cytoplasmic inheritance.

Additional Key word: Silkworm strains, Radiation resistance, Maternal inheritance, γ–irradiation, reciprocal mating

INTRODUCTION

In the silkworm, *Bombyx mori*, radiation has been used as a tool for chromosomal cleavage to generate mutations, such as the sex–linked sable and sex–linked zebra (Tazima, 1941; Hashimoto, 1948). These were created by the methods in which chromosomal double–strand breaks (DSBs) by ionizing radiation (IR) induced marker gene translocation from an autosome into the sex–chromosome, female specific W. Similarly, recessive mutations were frequently induced by IR in the oocyte nuclei during meiosis (Murakami, 1966, 1971a, b). In these experiments was irradiated 2,000 to 15,000 rad (20 to 150 Gy) of X–ray, which must have inevitably broken DNA double strands causing chromosomal arrangements, but, in many cases, was not lethal to the silkworm. The robust resistance of this species to γ–irradiation has also been reported (Miki, 1986). We observed previously that γ–irradiation at a larval stage led to the abnormally immature wing but not to any other features of pupae (Takahashi *et al.*, 2006). And it is also reported that the degree of the abnormal wing formation can be used as an indicator of radiation resistance (Takada *et al.*, manuscript in preparation). In this study, we performed a screening for radiation sensitivity using twelve silkworm strains by this morphological aberration of the pupal wing as an indicator of radiation sensitivity, and then analyzed the mode of inheritance of the sensitive trait.

MATERIAL AND METHODS

Experimental animals

The silkworm stock strains used were b50, f39, f12, m042, m90, m91, p50, r06, u70, w061, w051 and yd202 maintained in the Institute of Genetic Resources, Faculty of Agriculture, Kyushu University Graduate School. Genetic backgrounds of b50, f12, m042, p50, r06, u70 and w061 strains were listed in the Silkworm Genetic Resource Database by National BioResource Project (NBRP) (http://www.nbrp.jp/index.jsp). The m90 strain is known for spontaneous parthenogenesis and the m91 strain is reported to undergo parthenogenesis development at a very low rate compared with standard types. Larvae were raised on mulberry leaves at 25 °C.

The evaluation of sensitivity against γ–irradiation

Fifth instar larvae raised on mulberry leaves were exposed to γ–ray using an external gamma ray emission facility in the ^{60}Co Irradiation Laboratory of Kyushu University Graduate School. After additional raising on mulberry leaves, the irradiated larvae enter into the larval–pupal ecdysis. Five to six days after the ecdysis, pupae were taken out of cocoons and photographed. We evaluated the radiation sensitivity by the abnormality of wing regions.

RESULTS

Screening of susceptible silkworm strains against exposure to γ–irradiation

In order to identify the silkworm strains sensitive to

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[1] Laboratory of Silkworm Science, Kyushu University Graduate School of Bioresource and Bioenvironmental Sciences, 6–10–1 Hakozaki, Fukuoka 812–8581, Japan,

[2] Division of Radiological Protection and Biology, National Institute of Infectious Diseases, Tokyo 162–8640, Japan

* Corresponding author (E–mail: kusakabe@agr.kyushu–u.ac.jp)

‡ Present address: Department of Biological Substances and Life Science, Kyushu Kyoritsu University, Kitakyushu 807-8585, Japan.

γ–radiation, irradiation at a dose of 100 Gy was performed to larvae on day 3 of the fifth larval stage. All strains exposed to γ–radiation exhibited no obvious growth inhibition or abnormal appearance during larval. After pupation, we could detect the abnormal wing formation in some strains, but not in others (Fig. 1). On the basis of this visual diagnostic criterion, it was inferred that the m91 and f12 strains were the most susceptible, and the u70, m90, b50, f39, w051, w061 and d202 strains were intermediate types. On the other hand, p50 and m042 were categorized as resistant strains. The two resistant and the two susceptible strains were subjected to further characterization. As shown in Figure 2A, the p50 and m042 strains had less distinct effect on the wing formation compared with the m91 and f12 strains, when irradiated on day 2 of the fifth instar at a dose of 50 Gy. These resistant and susceptible strains showed little difference upon exposure to 100 and 150 Gy. Although a severe damage on wing formation was observed at a dose of 150 Gy in all the four strains, there were no obvious effects on the fertility even in the next generation. These results confirmed that the silkworm is highly resistant to γ–irradiation, but showed that there is a definite difference in the sensitivity among strains when the pupal wing is taken into consideration.

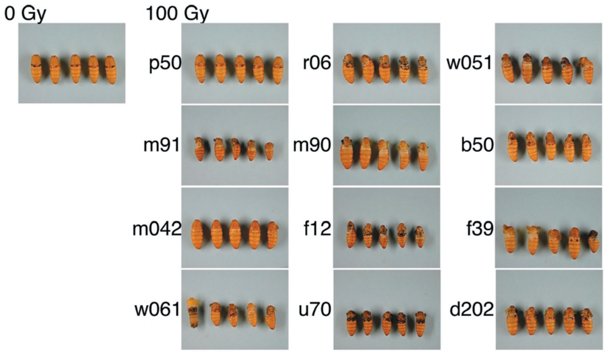

Fig. 1. Screening for radiation sensitivity using twelve silkworm strains based on the morphological variation of the wing. The irradiation was carried out on day 3 of the fifth instar. See the MATERIALS AND METHODS for other details.

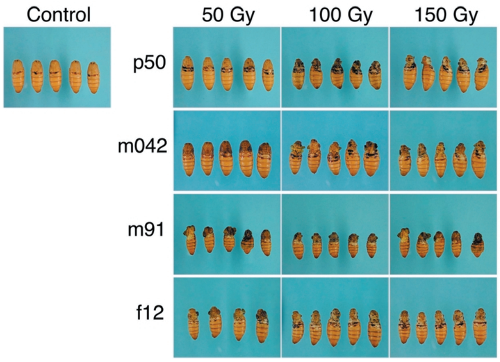

Fig. 2A. Comparison of susceptibility against γ–irradiation between resistant and sensitive silkworm strains.
The irradiation was carried out on day 2 of the fifth instar. The radiation sensitivity was evaluated by observing the morphological variation of the wing. See MATERIALS AND METHODS for further details. Resistant strains, m91 and f12; sensitive strains, p50 and m042.

Heredity of sensitivity against γ–irradiation in the silkworm

The γ–ray susceptibility of the m91 strain was previously pointed out and, moreover, this character was considered to be transported maternally to progeny (Murakami, personal communication). Therefore, we have tried to pursue whether this was the case or not. Reciprocal crosses between m91 and p50 and between m91 and m042 were performed, and then each F_1 was exposed to γ–irradiation on day 2 of the fifth instar (Fig. 2B). Differences in susceptibility of pupal wings between reciprocal crosses were detected in the combi-

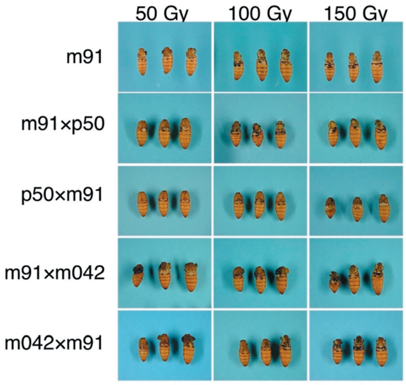

Fig. 2B. Comparison of susceptibility against γ–irradiation between F_1 generations from reciprocal crosses using the strains m91/p50 and m91/m042.
Each of F_1 from the indicated reciprocal crosses was irradiated on day 2 of the fifth instar. The radiation sensitivity was evaluated by observing the morphological variation of the wing. See MATERIALS AND METHODS for further details.

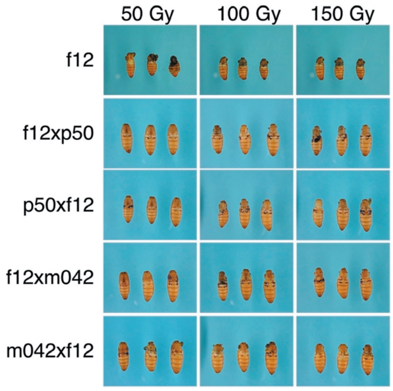

Fig. 2C. Comparison of susceptibility against γ–irradiation between F_1 generations from reciprocal crosses using the strains f12/p50 and f12/m042.
Each of F1 from the indicated reciprocal crosses was irradiated on day 2 of the fifth instar. The radiation sensitivity was evaluated by observing the morphological variation of the wing. See MATERIALS AND METHODS for further details.

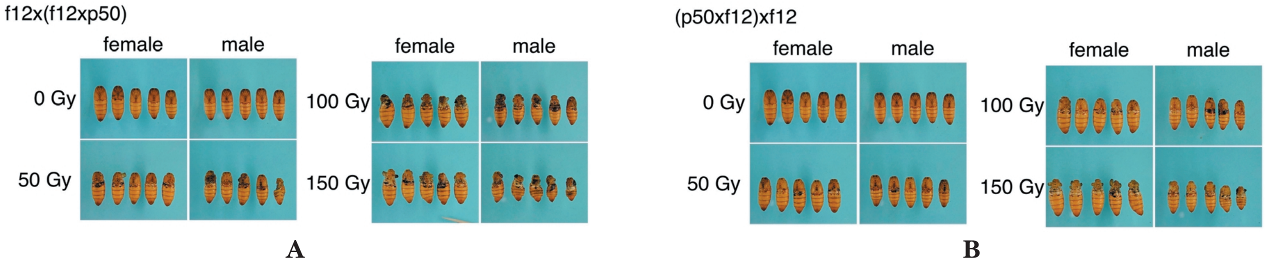

Fig. 3. Comparison of susceptibility against γ–irradiation between BF_1 generations of the f12 and p50 strains.
A The BF_1 [f12×(f12×p50)], i.e., the progeny from f12 female mated with F_1 male (from the cross f12 female×p50 male), and B its counterpart [(p50×f12) and f12], i.e., the progeny from F_1 female (from the cross p50 female×f12 male) mated with f12 male, were irradiated on day 2 of the fifth instar at the indicated doses (females and males separately). The radiation sensitivity was evaluated by observing the morphological variation of the wing. See MATERIALS AND METHODS for further details.

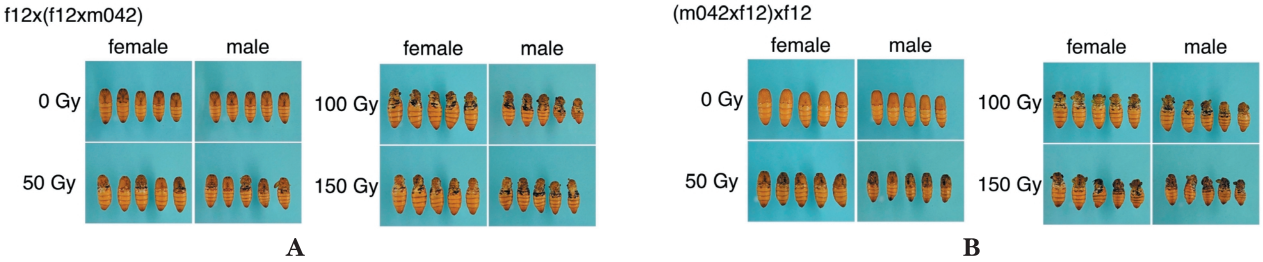

Fig. 4. Comparison of susceptibility against γ–irradiation between BF_1 generations of the f12 and m042 strains.
A The BF_1 [f12×(f12×m042)], i.e., the progeny from f12 female mated with F_1 male (from the cross f12 female×m042 male), and B its counterpart [(m042×f12) and f12], i.e., the progeny from F_1 female (from the cross m042 female×f12 male) mated with f12 male, were irradiated on day 2 of the fifth instar at the indicated doses (females and males separately). The radiation sensitivity was evaluated by observing the morphological variation of the wing. See MATERIALS AND METHODS for further details.

nation of m91 and p50, but were not obvious in the combination of m91 and m042. These results suggest that the heritable trait in concern exhibits a maternal effect, although depending on the mating partner. To confirm this, another sensitive strain, f12, was subjected to reciprocal cross, followed by irradiation and wing observation (Fig. 2C). There was no apparent abnormality on wing formation on the crosses between f12 females×p50 males and p50 females×f12 males. On the other hand, progeny from f12 females×m042 males displayed abnormality but that from m042 females×f12 males did not.

Next, we have investigated the susceptibility to irradiation using four BF1 crosses achieved in two combinations, f12×(f12×p50) and f12×(f12×m042) as a presumably sensitive combination, and (p50×f12)×f12 and (m042×f12)×f12 as a presumably resistant combination, where f12×(f12×p50) represents the progeny of f12 females mated with F_1 males from the cross f12 females×p50 males. Individuals of f12×(f12×p50) received severe damage at a dose of 50 Gy, whereas those of (p50×f12)×f12 were not affected even by an irradiation of 100 Gy on day 2 of the fifth instar (Fig. 3A). We found a similar tendency in another BF_1 from the cross using m042. Compared with the above indicated BF_1 with p50, however, difference between f12×f12×m042×and (m042×f12)×f12 was rather small (Fig. 3B).

DISCUSSION

We reported here the differential response with regard to radio resistance of the wing among silkworm strains and described whether the radiation sensitivity was transmitted maternally or not. Genetic effects by IR were assessed in *Drosophila melanogaster* spermatogenesis (Aaron and Leigh, 1981; Cooper and Zimmering, 1981; Frei 1982; Zimmering, 1982; Ferro, 1983). In this species, X–ray exposure at a dose of up to 1,000 R (8.7 Gy) induced chromosomal deletion of up to 8% in the next generation as detected by a ring–X chromosome loss, although repair–deficient females were used as mating partners (Aaron and Leigh, 1981). In general, germ cells are thought to be one of susceptible targets for IR, because infertile insects used for pest control are generated routinely by γ–irradiation. However, we have noticed that almost all of silkworm individuals irradiated at a dose of 150 Gy underwent pupal–adult metamorphosis and produced almost fully fertile gametes. This is in contrast to the situation of human body, whose half lethal dose is 4 Gy. In the silkworm, the wing discs seemed to be the only regions that showed remarkable sensitivity to IR. The reason why the silkworm wing disc cells are more susceptible against IR than germ line cells remains unknown. It is, however, possible to provide a plausible explanation. In our studies, silkworm larvae were exposed to γ–ray on days 2 or 3 of the fifth instar, when a majority of germ cells are in the meiotic phase or shortly after the onset of meiotic division. Therefore, there was an enough time to recover the cell population entering meiosis, even if seriously damaged cells were eliminated through apoptic pathway. Our previous report demonstrated that cultured silkworm cells become arrested in the cell cycle progression at G_2/M phase after γ–irradiation but not at G_1 phase, and escaped from apoptic cell death (Takahashi *et al.*, 2006). However, spermatogonial cells and spermatocytes could be induced to enter G_1 arrest after γ–irradiation (Sado *et al.*, 1961), suggesting that silkworm germ cells might have a specific mechanism for monitoring or responding to DNA damages caused by IR. In contrast, the wing disc cells would not have a time to recover cell population after γ–irradiation on day 2 or 3 of the fifth larval instar. Moreover, it is possible that cytomorphologic differentiation of cell shape in wing disc confers the high susceptibility to IR.

In the present study, we found that the radiation susceptibility of the silkworm shows a tendency to inherit through the female parent as seen in the BF_1 generations, although this tendency was unclear in the F_1 generations. The genetic backgrounds of the stock strains used in this experiment were fairly pure, because of inbreeding for long term. Therefore, it is rational to suppose that hybrid vigor (heterosis) appeared in the F_1 phenotype, resulting from the heterozygosity in crosses between the different lines, and probably masked the distinctness of sensitivity to radiation. In fact, heterosis in the F_1 generations was apparent in their body size and resistance against diseases (data not shown). In the BF_1 progeny, the heterosis might be weakened and the maternal effect in radiation resistance could be detectable. In *D. melanogaster*, it is known that damages induced in the sperm nucleus are repaired using unidentified materials in oocytes (Muller, 1940; Aaron and Leigh, 1981). Moreover, the yields of induced genetic damage (mutation) are reported to be dependent on maternal genotype (Falk, 1962; Sobles, 1969). These notions appear to indicate that there are materials affecting or helping the damage repair in oocytes. Similar materials transmitted maternally might function in somatic cells of the silkworm for repairing damages.

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