GENETICAL STUDIES OF THE DELETION, 'RETARDED GROWTH', IN THE THIRD CHROMOSOME OF *BOMBYX MORI*

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It has been demonstrated on the basis of genetic analysis that three dominant larval marking mutants, namely no lunule (*Nl*, Chikushi 1959), no lunule-1 (*Nl*¹, Tsujita 1961) and no lunule-2 (*Nl*², Tsujita 1961), and a dominant wing mutant of vestigial (*Vg*, Tazima 1944) are due to losses of chromosome segments. The genetic deficiencies have been recognized by lethalties of the homozygotes and by the phenomenon of pseudo-dominance revealed through crosses with recessive mutants. Precise location of a deficiency in a chromosome has been determined from the recombination value between other mutants in the vicinity. Only the left end of the deletion of a chromosome could have been traced back, while that for the right end has never been found except for speculative terminal deficiency.

In this paper we describe for the first time for the silkworm, *Bombyx mori*, a case of determination of the range of chromosomal deletion from a genetical analysis of a gamma-ray induced "Retarded growth" mutant having distinctly a prolonged larval life span.

MATERIALS

Origin and characteristics of "Retarded growth"

As reported earlier (Chikushi 1970), "Retarded growth" mutant was originated from a gamma-irradiated female pupa of *E^{vi}* strain which had been maintained at the National Institute of Genetics (N.I.G.), Japan. In spring of 1957, a twelve days old female pupa was exposed, at N.I.G., to ⁶⁰Co γ -rays at a dose rate of 1,000 R/min for 10 minutes; i.e. total dose administered was 10,000 R.

Among the progeny of the irradiated female crossed with a normal male of N 124 strain, Sakaguchi, during his service at N.I.G., discovered six extremely slow-growing larvae. This character of slow growth was transmitted to the offspring when outcrossed to "Daizo", a normal strain which is characterized by rapid growth. During early larval development up to the second molting, the growth of the mutant was delayed by 1 day as compared with the 7 days lapse of time of the segregating normals in the same brood. Further delayed growth in later stages of larval development was also clearly recognized. Consequently, whole larval life span of the mutant was prolonged by 3 days under usual rearing condition, i.e., 20 days of normal life as against that of 23 days of the mutant.

All slow growing larvae were dwarf. This character was inherited as an autosomal simple dominant with complete lethality of the homozygotes at an early embryonic stage. The mutant was named "Retarded growth", and the gene received the symbol *Rg*.

EXPERIMENTAL RESULTS

Linkage relations and locus of "Retarded growth"

Genetic analysis of the linkage relation of *Rg* was carried out. As it was already confirmed that *Rg* mutation does not locate on the sex-chromosome, mutant stocks for autosomal marker genes were crossed with *Rg*. Then the segregation in BF₁- or F₂-progenies was examined. If *Rg* were not linked to the other genes, it would have given the segregation ratio of 1 *A Rg* : 1 *a Rg* : 1 *A* +^{*Rg*} : 1 *a* +^{*Rg*} in BF₁, or 6 *A Rg* : 2 *a Rg* : 3 *A* +^{*Rg*} : 1 *a* +^{*Rg*} in F₂, respectively.

Independent relations of *Rg* from chromosome -2, -4, -5, -6, -7, -9, -10, -11, -12, -13, -14, -16, -17, -18, -19, -20 are clearly shown in Table 1. Though in some crosses the observed segregations were far from the expected ratio, it must be taken into consideration that in *Bombyx mori* recombinations occur only on the male

Table 1. Linkage test of *Rg* (Examined in F₂- or BF₁-generation)

Genes and linkage groups tested	Mating	Segregation of characters						Remarks
		<i>Rg</i>			+ <i>Rg</i>			
<i>Y</i> (2)	(<i>Y</i> /+, +/ <i>Rg</i>) × (+/+, +/+)	<i>Y</i> 152	+	149	<i>Y</i> 140	+	158	independent
<i>Ze</i> (3)	(<i>Ze</i> ++ <i>Rg</i>) × (+ ++ +)	<i>Ze</i> 0	+	122	<i>Ze</i> 102	+	0	linked
	(<i>Ze</i> ++ <i>Rg</i>) <i>inter se</i>	<i>Ze</i> 140	+	1	<i>Ze</i> 91	+	0	
<i>L</i> (4)	(<i>L</i> /+, +/ <i>Rg</i>) × (+/+, +/+)	<i>L</i> 101	+	122	<i>L</i> 115	+	120	independent
<i>oc</i> (5)	(<i>oc</i> /+, +/ <i>Rg</i>) × (<i>oc oc</i> , +/+)	+	206	<i>oc</i> 205	+	170	<i>oc</i> 165	"
<i>E^{Kp}</i> (6)	(<i>E^{Kp}</i> /+, +/ <i>Rg</i>) <i>inter se</i>	<i>E^{Kp}</i> 256	+	78	<i>E^{Kp}</i> 91	+	37	"
<i>q</i> (7)	(<i>q</i> /+, +/ <i>Rg</i>) × (<i>q q</i> , +/+)	+	41	<i>q</i> 17	+	30	<i>q</i> 20	"
<i>I-a</i> (9)	(<i>I-a</i> /+, +/ <i>Rg</i>) × (+/+, +/+)	<i>I-a</i> 79	+	88	<i>I-a</i> 88	+	84	"
<i>w-3</i> (10)	(<i>w-3</i> /+, +/ <i>Rg</i>) <i>inter se</i>	+	54	<i>w-3</i> 15	+	18	<i>w-3</i> 12	"
<i>K</i> (11)	(<i>K</i> /+, +/ <i>Rg</i>) × (+/+, +/+)	<i>K</i> 101	+	146	<i>K</i> 81	+	65	"
<i>Ng</i> (12)	(<i>Ng</i> /+, +/ <i>Rg</i>) × (+/+, +/+)	<i>Ng</i> 36	+	40	<i>Ng</i> 29	+	35	"
<i>ch</i> (13)	(+/ <i>ch</i> , <i>Rg</i> /+) <i>inter se</i>	+	107	<i>ch</i> 49	+	52	<i>ch</i> 23	"
<i>U</i> (14)	(+/ <i>U</i> , <i>Rg</i> /+) × (+/+, +/+)	<i>U</i> 75	+	80	<i>U</i> 59	+	52	"
<i>cts</i> (16)	(+/ <i>cts</i> , <i>Rg</i> /+) × (<i>cts cts</i> , +/+)	+	94	<i>cts</i> 75	+	78	<i>cts</i> 77	"
<i>bts</i> (17)	(<i>bts</i> /+, +/ <i>Rg</i>) × (<i>bts bts</i> , +/+)	+	16	<i>bts</i> 17	+	47	<i>bts</i> 25	"
<i>bl</i> (18)	(<i>bl</i> /+, +/ <i>Rg</i>) × (<i>bl bl</i> , +/+)	+	13	<i>bl</i> 24	+	14	<i>bl</i> 19	"
<i>mln</i> (19)	(<i>mln</i> /+, +/ <i>Rg</i>) × (<i>mln mln</i> , +/+)	+	80	<i>mln</i> 53	+	87	<i>mln</i> 59	"
<i>nb</i> (20)	(<i>nb</i> /+, +/ <i>Rg</i>) × (<i>nb nb</i> , +/+)	+	88	<i>nb</i> 46	+	95	<i>nb</i> 83	"
<i>Nd</i> (?)	(<i>Nd</i> /+, +/ <i>Rg</i>) × (+/+, +/+)	<i>Nd</i> 89	+	70	<i>Nd</i> 51	+	93	"
<i>so</i> (?)	(<i>so</i> /+, +/ <i>Rg</i>) × (<i>so so</i> , +/+)	+	27	<i>so</i> 38	+	50	<i>so</i> 29	"
<i>ge</i> (?)	(+/ <i>ge</i> , <i>Rg</i> /+) × (<i>ge ge</i> , +/+)	+	55	<i>ge</i> 21	+	38	<i>ge</i> 10	"

side. *Rg* is also independent from the genes for Naked pupa (*Nd*), sooty (*so*) and geometrid (*ge*). Although twenty linkage groups have already been established in the silkworm, those three genes belong to some still unknown groups among the remaining eight chromosomes.

As is shown in the table, complete linkage relation between growth rate and larval marking of Zebra (*Ze*) localized at 20.8 position on the third chromosome was shown by the female test cross of the type *Ze* +/+ *Rg* × + +. Upon inbreeding the *F*₁ *Ze* +/+ *Rg* moth, it was found that very few individuals of +*Rg* segregated with no + + individuals. Hence, *Rg* has its locus on the third chromosome, quite closely to the *Ze* locus.

In order to determine chromosome mapping involving *lem* (0.0), *Ze* (20.8) and *Rg* on the third chromosome, a linkage experiment was carried out. Female test-crosses of the control + *Ze Rg/lem* + + × *lem* + + gave progenies only of the parental genetic constitutions, namely 348 + *Ze Rg* and 412 *lem* + + in totals of 3 batches. Precise localization of *Rg* was made by *lem* + + × + *Ze Rg/lem* + +. The data are shown in Table 2.

Table 2. Crossing over experiment between *lem-Ze-Rg*

Non-crossovers		<i>lem-Ze</i> crossovers		<i>Ze-Rg</i> crossovers		Double crossovers		Total
+ <i>Ze Rg</i>	<i>lem</i> + +	+ + +	<i>lem Ze Rg</i>	+ <i>Ze</i> +	<i>lem</i> + <i>Rg</i>	+ + <i>Rg</i>	<i>lem Ze</i> +	
2,345	3,017	927	710	178	117	21	10	7,325

(The number of segregants are shown by the summation from 22 batches)

From this testcross the recombination value between *lem* and *Ze* was 22.8%, the value of *Ze* and *Rg* was 4.5% and *lem* and *Rg* was 26.4%. Hence the arrangement of these three gene loci is in the order of *lem-Ze-Rg*. Since the distance between *lem* and *Ze* is already known as 20.8 units on the third chromosome, the locus of *Rg* is indicated by the correction factor as 4.1 to the right of *Ze*. It can be concluded from these evidences that the locus of *Rg* is at 24.9 on the third chromosome.

"Retarded growth" as a deletion in the third chromosome

The question still remains unresolved from the previous experiments, whether *Rg* is a gene mutation or a kind of chromosomal aberration. Cytological observations to detect an aberrant chromosome in the mutant of *Rg* were not yet successful, similarly to other cases of chromosomal deletions in *Bombyx mori*. The possibility, however, that *Rg* is not a gene mutation but a deletion in its genetic nature is suggested from its origin being induced by irradiation as well as from the complete lethality of homozygotes. Females of *Ze Rg*/+ + were crossed with such recessive mutants on the third chromosome as apodal (*ap*, 22.3), small egg (*sm*, 41.8) and flimsy cocoon (*flc*, 49.0).

The cross of *Ze* + *Rg*/+ + + × + *ap* + gave no *ap* individuals, *ap* character was not manifested either in +^{*Ze*} +^{*Rg*} progeny or in *Ze Rg*. In the cross of *Ze Rg* +/

+ + + × + + *flc*, neither was *flc* character manifested in both types of the growth rate and was just the same as in the cross for *ap*.

The situation was completely different in the cross with *sm*. The mutant *sm* which was discovered in 1927 by Tanaka (1955) is inherited pseudo-maternally and eggs laid by *sm/sm* female are small and completely lethal regardless of the genotype of the expected embryo (Hayashi 1937). Two separated lines of *sm* were used in this experiment. One was the original "small egg forming" line, which is tentatively referred to as *sm*(S). The other was the "no egg forming" line, which was first selected by Otsuki in this laboratory, 1958, and later by us from the original *sm* strain. This type of *sm* is referred to as *sm*(N).

The cross *Ze Rg* +/+ + + × + + *sm*(N) gave progenies of *Ze Rg* +/+ + *sm*(N) and + + +/+ + *sm*(N). Female moths of the former *Ze Rg* laid no egg, while those of the latter +^{Ze} +^{Rg} laid normal eggs. It was revealed on dissecting that the no egg laying *Ze Rg* moths had well developed ovarioles but mature egg cells were not found in the organ. That indicates that *sm*(N) is manifested "dominantly" in the females heterozygous for *Rg*, though *sm*(N) itself is recessive. The data are shown in Table 3. It is a very common phenomenon of pseudo-dominance that the recessive character of *sm*(N) is manifested in heterozygotes combined with *Rg*.

Somewhat different and unexpected results were obtained in the progenies of the cross *Ze Rg* +/+ + + × + + *sm*(S). Some of the female moths of the type *Ze Rg* +/+ + *sm*(S) laid small lethal eggs as was expected from the cross of *sm*(N). The other segregated females having the same genetic constitution in the same batch laid normal eggs. The result, shown in Table 4, means that expected pseudo-dominance of *sm*(S) in the heterozygotes combined with *Rg* is incompletely expressed.

Female moths having the genotype *Ze Rg* +/+ + *sm*(S) were crossed with + + *sm*(N) males. As expected, all of the small eggs derived from the cross were lethal. On the contrary, normal eggs laid after the above mating were viable. They were

Table 3. Segregation in the cross of the type *Ze Rg* +/+ + + × + + *sm*(N)

Larval character	<i>Ze Rg</i>	+ +
Egg type	+ <i>sm</i> (N)	+ <i>sm</i> (N)
Total of 2 batches	0 63	63 0

Table 4. Segregation in the cross of the type *Ze Rg* +/+ + + × + + *sm*(S)

Larval character	<i>Ze Rg</i>	+ +
Egg type	+ <i>sm</i> (S)	+ <i>sm</i> (S)
Total of 2 batches	32 37	83 0

Table 5. Segregation in the cross of the type *Ze Rg* $+/+ + sm(S) \times + + sm(N)$

Larval character	<i>Ze Rg</i>			+ +		
Egg type	+	<i>sm(S)</i>	<i>sm(N)</i>	+	<i>sm(S)</i>	<i>sm(N)</i>
Total of 3 batches	0	0	127	0	154	0

reared in separate batches and compared with the egg type of the emerged female moths to check the homozygosity of the "grand father" of *sm(S)*. The data are shown in Table 5. All of the female moths showed the tendency to lay eggs of *sm(S)* or *sm(N)* type. None of the female moths laid normal eggs.

It may be safely concluded from these crossing experiments that *Rg* mutant is not a gene mutation but a deletion in the third chromosome, ranging from 24.9 for the left end to 41.8 for the right end of the chromosome.

DISCUSSION

The evidence presented above in three-point experiments suggests that *Rg* mutation locates at 24.9 in the third chromosome. The closest neighbors, shown on the standard map, are apodal (22.3) to the left and small egg (41.8) to the right (Hirobe 1947; Chikushi and Doira 1970). The pseudo-dominant expression of *sm(N)* in the heterozygote *Rg* $+/+ sm(N)$ confirms that *Rg* mutation is a chromosomal deletion in its genetic nature. It was shown from the cross carried out to clarify the relation between *Rg* and *ap* that *ap* locus is outside the deletion. This agrees with the observation that the *Rg* locus is at 24.9. Therefore 24.9 is a very probable position of the left breakage point of *Rg* deletion. The second neighbor to the right of *Rg* is *flc* (49.0) which also is outside the deletion (Doira 1970). From these results it is evident that *Rg* is neither a gene mutation nor a loss of the terminal chromosomal segment. But indeed it is a loss of an intercalary chromosomal segment, *i.e.*, it is a deletion.

The right breakage point of *Rg* deletion was evident from the cross with *sm(S)*. Heterozygous female moths of the genotype *Rg* $+/+ sm(S)$ laid either normal or small and lethal eggs. Expected genotype of the moths which laid normal eggs was ascertained by a further cross with *sm(N)*. This unexpectedly low penetrance of "pseudo-dominance" of *sm(S)* in the heterozygotes combined with *Rg* means that *sm(S)* locus, or at least most of it, is outside the deletion, while the locus of *sm(N)* is inside it. Dominant nature of *sm(S)* over *sm(N)* was also demonstrated. As it seems, we may be allowed to consider *sm(N)* to be a "repeat" of two identical doublets, though they may be somewhat differently modified. The contradiction between these two types of *sm* crossed with *Rg* is explainable when we consider that only the doubled left half of the repeat is inside the deficiency of *Rg*, while the ancestral right half is on its outside. Then, the actual position of the right breakage point of *Rg* is between the doublet of *sm(N)*, or more precisely just at the left of the *sm(S)* locus.

In conclusion, the limits of *Rg* on the normal map are about 24.9 for the left end to 41.8 for the right end. The length of the deficient section is represented as : 41.8–24.9=16.9. The interpretation to which we are inclined is that the “dominantly” manifested small egg character in a heterozygous female of the genotype *Rg* +/+ *sm*(*S*) is due to a type of position effect, but not to a pseudo-dominance and only the case with *sm*(*N*) is due to pseudo-dominance.

It has been suggested that “repeats” offer a generalized mechanism for further steps in rearrangements through a preliminary synapsis of homologous sections. Further studies of the deletion of *Rg* are in progress and may provide information based on some striking evidences.

SUMMARY

The dominant mutant “Retarded growth” (symbol *Rg*) was found as a gamma-ray induced phenomenon. It is characterized by a departure from the normal type representing distinctly delayed larval growth. The homozygote is completely lethal in embryonic stage.

Linkage test showed the *Rg* locus to be in the third chromosome at 24.9. Further crosses with the recessive gene *sm*(*N*) gave without exception pseudo-dominantly manifested *sm*(*N*) character in *Rg* females. While the crosses with allelic *sm*(*S*) gave *sm*(*S*) character in only a part of *Rg* females, which is explained as due to a position effect. It is concluded from these results that *Rg* is not a gene mutation but a deletion of intercalary chromosomal segment. The left break is at 24.9 and the right break is just left by the *sm*(*S*) locus at 41.8. The deficit is about 17 units of the length of the third chromosome.

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LITERATURE CITED

- Chikushi, H., 1959 Contributions to genetics of *Bombyx*, with special reference to mechanisms of manifestation of characteristics, 1. Sci. Bull. Fac. Agr. Kyushu Univ. **17**: 171-187 (in Japanese).
- Chikushi, H., 1970 Genetic analysis of the *Rg* deficiency. Proc. Sericult. Sci. Kyushu **1**: 6 (in Japanese).
- Chikushi, H. and H. Doira, 1970 The third linkage map of silkworm, *Bombyx mori*. J. Sericult. Sci. Japan **39**: 319-320 (in Japanese).
- Doira, H., 1970 Inheritance of flimsy cocoon. Proc. Sericult. Sci. Kyushu **1**: 5 (in Japanese).
- Hayashi, T., 1937 Morphological studies on abnormally shaped eggs in *Bombyx mori*, 1. Sci. Bull. Fac. Agr. Kyushu Univ. **7**: 359-372 (in Japanese).

- Hirobe, T., 1947 Mutants and linkage data. *Silkworm Information Service* **1**: 4 (in Japanese).
- Tanaka, Y., 1955 "Small egg", a mutant in the silkworm. *Ann. Rep. Nat. Inst. Genet. Japan* **5**: 28-29.
- Tazima, Y., 1944 Studies on chromosomal aberrations involving the second and the W chromosomes. *Bull. Sericult. Expt. Sta.* **12**: 109-181 (in Japanese).
- Tsujita, M., 1961 Investigations in *Nl* type lethal mutants of the silkworm, with special reference to *Nl*, *Nl*¹ and *Nl*² embryo. *Japan. J. Genetics* **36**: 235-243.