# Relationship between the Amount of Silk and Nucleic Acids in the Silk Gland of *Bombyx Mori*

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Many kinds of varieties of silkworm, *Bombyx mori*, were elaborated in the history of sericultural industry and each one has the inherent characteristics in quantity or quality of the raw silk responding to the needs of the times. The silk yield per larva has increased owing to the breeding efforts continued for a long period. A variety of silkworm in the Edo era had cocoon-layer weight of 0.15 g but a variety at the present time has that of 0.75 g, and furthermore a parental variety for breeding having that of 1 g was developed.

Recently, a gene of fibroin which is a frame protein of silk fiber was identified[11] and was confirmed that the gene is only one per haploid without redundancy or multiplication[1]. By that time, it had been considered that varietal variation of silk productivity was controlled by a few main genes and minor polygenes concerning silk productivity.

Based on the evidence that a gene expression is performed along a flow scheme, DNA → RNA → Protein, we attempted to make clear quantitative relationship between cocoon-shell weight and bulk nucleic acids in the posterior silk gland of the silkworm.

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## Relation between RNA accumulation in silk gland and silk formation

The silkworms, *Bombyx mori*, used for an assay of hereditary control of silk production were 13 varieties of pure line: 7 of them belong to a line of low silk productivity, 6 of them that of high productivity and 2 hybrids as shown in Table 1. The total cellular RNA in the posterior silk gland accumulated when

**Table 1. Silkworm varieties used in the experiment**

| Line | Variety No. | Name of variety* | Silk productivity** (mg) |
|---|---|---|---|
| Low productivity | 1 | Matamukashi | 163 |
| | 2 | Akajuku | 182 |
| | 3 | Nichi 1 | 226 |
| | 4 | Ôkusa | 192 |
| | 5 | Nichi 106 | 203 |
| | 6 | Kohoku | 178 |
| | 7 | Giallo Ascoli | 186 |
| High productivity | 8 | Nichi 124 | 446 |
| | 9 | Shi 108 | 419 |
| | 10 | Shi 124 | 370 |
| | 11 | 610 | 570 |
| | 12 | Ô 16 | 401 |
| | 13 | 010 | 595 |
| | 14 | Nichi 140×Shi 145 | 577 |
| | 15 | Nichi 124×Shi 124 | 400 |

* Nichi 1 originated from Akajuku, Nichi 106 from Ôkusa, and Ô 16 and 010 from Giallo Ascoli.
** Values in terms of cocoon shell weight.

the stage reached the 5th instar, and it reached the maximum content on the 6th day[2]. The amount of RNA was measured quantitatively at that time of silk gland development in 13 varieties of silkworms. The weight of cocoon shell, the final product, was taken to express silk productivity. The cocoon shell weight per larva plotted against the total cellular RNA weight per larva showed apparently the linear relationship between them, as given in Fig. 1. The relation between RNA and silk was given by $Y=-37.4+73X$, with a correlation coefficient, r, of 0.959. Here, $X$ and $Y$ represent the total cellular RNA content in the silk gland (mg/larva) and the amount of cocoon shell (mg), respectively. The result shown in Fig. 1 demonstrates that the silk productivity depends primarily on the extent of RNA accumulation in the silk gland.

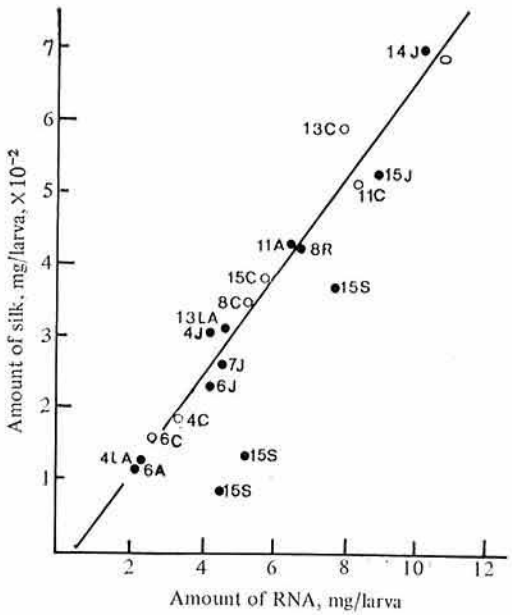


Fig. 1. Quantitative relationship between RNA accumulated in the posterior silk gland and the amount of silk produced by that gland

1) The numerals refer to the varieties shown in Table 1.
2) Treament:
LA ; Late autumn rearing
A ; Actinomycin D application
R ; $\gamma$-ray irradiation
S ; Forced starvation
J ; JHA application
C ; Control (no treatment)

## Quantitative relation between RNA an DNA in the posterior silk gland

The quantitative relationship between RNA and DNA in the posterior silk gland measured on the 6th day of the 5th instar is shown in Fig. 2, in which all the varieties used are divided into two groups showing the average amount of DNA of 121 $\mu$g/larva and 212 $\mu$g/larva. These two groups correspond to the group of low silk productivity and that of high silk productivity, respectively. This result suggests that the DNA content of the

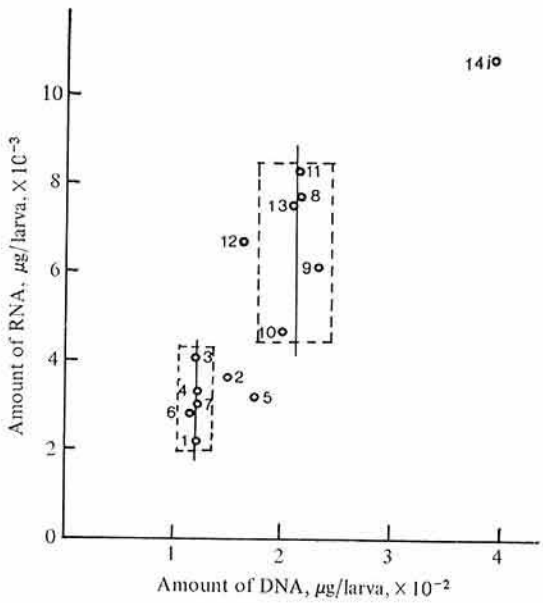


Fig. 2. The amount of RNA in the posterior silk gland corresponding to the quantitative level of DNA in lines of low and high productivity

1) DNA and RNA are shown by $\mu$g of the total cellular DNA or RNA in the posterior silk gland/larva.
2) Numerals: Varieties shown in Table 1.
3) j: Treated with JHA.

silk gland of high productivity is as much as twice that of low productivity, and that an extent of the RNA synthesis in the silk gland is controlled by the level of the amount of DNA.

It was estimated that one molecule of DNA synthesized 4 to 8 pg of RNA. It is suggested that the reason why the RNA content ranged from 4 to 8 pg in several varieties was due to the difference in the extent of accumulation of the genes, supposedly controlling silk productivity, in different varieties.

## Silk formation under the controlled accumulation of RNA in the silk gland

Under the following conditions, the activities of silk formation and RNA accumulation in the silk gland were measured quantitatively.

*1) Effect of tetraploid*

It was known from the data that varieties with a large amount of DNA showed high RNA content in the posterior silk gland. In this connection, the RNA content in tetraploid silkworms produced artificially was observed[3] (Table 2). Diploid larvae hatched from eggs treated to induce tetraploids and were used as control. In the tetraploid silkworms, the number of cells in the silk gland was about half that of diploid ones. The RNA content in a silk gland cell was equal to that of control larvae, although the DNA content in each cell was twofold that of diploid ones. Consequently the RNA content in the silk gland (as expressed by RNA/cell × the number of cells) of the tetraploid silkworms was 64% of that of control larvae and the cocoon shell weight of the former was 78% that of the latter[3].

*2) Effect of unfavorable rearing condition*

The condition of autumn rearing is less favorable for the silkworm than that of spring rearing so that neither animal growth nor cocoon production can fully function. Two varieties of silkworm, Ôkusa and 010, were used for assay of nucleic acids. It was observed that replication of DNA in the silk gland was unaffected, whereas the accumulation of RNA was significantly diminished, under the unfavorable rearing condition. The change of silk productivity was related to the extent of RNA accumulation[10]. This effect was clearer in 010. The examples are shown as 4LA and 13LA on the line of the silk vs RNA relationship in Fig. 1.

*3) Effect of actinomycin D*

Actinomycin D was administered orally to larvae at a dose level of 1 μg/g body weight on the 4th day of the 5th instar. This dose was equivalent to the value which could inhibit by half the fibroin synthesis in the posterior silk gland. Actinomycin did not disturb DNA replication in the silk gland but suppressed the accumulation of RNA on the next day of the treatment. Silk formation suffered to an extent corresponding to the suppression of RNA accumulation[10]. The relation of silk to RNA in the treated larva can be plotted as 11A and 6A on the line in Fig. 1.

**Table 2. Nucleic acid content in a silk gland cell and silk yield of the tetraploid and diploid silkworm**

| Ploidy | Middle silk gland | | | Posterior silk gland | | | Cocoon-shell (mg) |
|---|---|---|---|---|---|---|---|
| | No. of cells* | RNA (μg/cell) | DNA (μg/cell) | No. of cells* | RNA (μg/cell) | DNA (μg/cell) | |
| Tetraploid | 78 | 6.5 | 0.24 | 145 | 5.6 | 0.27 | 250 |
| | 66 | 7.1 | 0.26 | 124 | 5.0 | 0.27 | — |
| Diploid | 117 | 6.7 | 0.14 | 241 | 5.6 | 0.16 | 320 |

* The numerals represent the number of cells in one side of the paired cell layers constituting the tubular gland.

*4) Effect of genetic constitutions*

To clarify whether the RNA content influencing the amount of silk is controlled by genetic constitutions in larval body or controlled directly by genes, hybridization between KATSUKI's Mosaic and Nichi 124 was made to produce silkworms with the mosaic silk gland, i.e., in a pair of silk glands, one has homogeneous genotype and another has heterogeneous genotype[3]. KATSUKI's Mosaic belongs to a line of low silk productivity. Genotype of the posterior silk gland in such a mosaic silkworm consisted of KK and KN, where K and N represent genotype of KATSUKI's Mosaic and Nichi 124, respectively.

The data obtained are shown in Table 3. There was definite difference in the RNA content in the posterior silk gland of the mosaic silkworm according to the genotype of the silk glands. The RNA content in the silk gland having the genotype KN was larger than that of the silk gland having the genotype KK. The RNA content of the silk gland with the genotype KK in the mosaic silkworm was larger than that of the silk gland (KK) of KATSUKI's Mosaic silkworm. The silk gland with the KN genotype of the mosaic silkworm had smaller content of DNA than that of the non-mosaic hybrid between Nichi 124 and KATSUKI's Mosaic, which had also genotype KN. From these results, it is considered that the amounts of DNA and RNA are controlled by inherence and are influenced by genetic physiological conditions in the larval body.

*5) Effect of starvation*

Three methods were adopted to make larvae starved. Larvae under forced starvation produced smaller cocoons than the control larvae. In these larvae, the RNA accumulation in the silk gland decreased but the replication of DNA was affected little. Complete cut of feeding after the 4th day caused a degradation of DNA. When the worms were fed less every day, a delay of increase of RNA and DNA occurred[12]. The relation of silk to RNA in the treated larva deviated from the line shown in Fig. 1 (as 15S).

**Table 3. Nucleic acid content of mosaic silk glands obtained from the mating of Nichi 124 to KATSUKI's Mosaic**

| | Nucleic acids | Individual No. | Middle silk gland | | Posterior silk gland | | Ratio of right to left | |
|---|---|---|---|---|---|---|---|---|
| | | | Right | Left | Right | Left | Middle silk gland | Posterior silk gland |
| | | | (μg) | (μg) | (μg) | (μg) | | |
| a. | KATSUKI's Mosaic (Control) | | | | | | | |
| | RNA | Average | 813 | 816 | 1,005 | 951 | 1.00 | 1.06 |
| | DNA | Average | 30 | 33 | 37 | 36 | 0.91 | 1.03 |
| b. | Nichi 124 (Control) | | | | | | | |
| | RNA | Average | 988 | 1,085 | 2,251 | 2,098 | 0.91 | 1.07 |
| | DNA | Average | 47 | 51 | 77 | 78 | 0.92 | 0.99 |
| c. | KATSUKI's Mosaic×Nichi 124 (Control silk gland) | | | | | | | |
| | RNA | Average | 1,604 | 1,610 | 2,266 | 2,220 | 1.00 | 1.02 |
| | DNA | Average | 38 | 43 | 76 | 72 | 0.88 | 1.06 |
| d. | KATSUKI's Mosaic×Nichi 124 (Mosaic silk gland) | | | | | | | |
| | RNA | 1 | 2,002(KN) | 1,829(KK) | 2,450(KN) | 1,691(KK) | 1.09 | 1.45 |
| | | 2 | 1,519(KN) | 1,380(KK) | 2,174(KN) | 1,467(KK) | 1.10 | 1.48 |
| | | 3 | 1,726(KN) | 1,311(KK) | 2,217(KN) | 1,415(KK) | 1.32 | 1.75 |
| | | 4 | 1,139(KK) | 1,588(KN) | 1,346(KK) | 2,209(KN) | 0.72 | 0.61 |
| | DNA | 1 | 55(KN) | 60(KK) | 58(KN) | 47(KK) | 0.92 | 1.23 |
| | | 2 | 68(KN) | 51(KK) | 53(KN) | 37(KK) | 1.33 | 1.43 |
| | | 3 | 49(KN) | 43(KK) | 53(KN) | 35(KK) | 1.14 | 1.51 |
| | | 4 | 42(KK) | 42(KN) | 37(KK) | 46(KN) | 1.00 | 0.80 |

**Table 4. Effect of starvation on accumulation of RNA species in the posterior silk gland**

| Starvation treatment | Age in the 5th instar | mRNA | rRNA | sRNA |
|---|---|---|---|---|
| | day | % | % | % |
| No treated | 5 | 3.6 | 86.2 | 10.2 |
| | 6 | 3.5 | 84.8 | 11.6 |
| | 7 | 4.1 | 80.9 | 15.0 |
| | 8 | 5.3 | 81.1 | 13.6 |
| | 9 | 5.2 | 65.7 | 29.7 |
| Treated for 24 hr from the 3rd to the 4th day | 5 | 1.8 | 93.0 | 5.2 |
| | 6 | 1.9 | 88.5 | 9.6 |
| | 7 | 2.8 | 91.8 | 5.9 |
| | 8 | 3.0 | 81.5 | 15.5 |
| | 9 | 2.4 | 79.8 | 18.8 |

RNA species accumulation in the posterior silk gland was observed in larvae starved for 24 hr from the 3rd day of the 5th instar[9] (Table 4). Although, recovery of total RNA accumulation in the silk gland delayed 2 days from the 5th day of the 5th instar, individual RNA species varied in their quantity, even when larvae were re-fed. After starvation, the percentage of the crude fibroin mRNA was half that of the control larvae and did not recover throughout the larval stage. The percentage of the sRNA was quickly reduced by the starvation. It seemed to be due to alteration of mRNA and sRNA amounts in the total RNA that the relation of silk to RNA content deviated from the regression equation in the starved silkworm.

### *6) Effect of γ-irradiation*

In this experiment an attention was focused on the variation of silk content of the larvae as influenced by decreased amount of nucleic acids in the silk gland, as a result of decrease in primer activity of DNA by γ-irradiation. The γ-irradiation was performed on the 2nd day of the 5th instar larvae with $^{60}Co$ γ-rays. There was no effect on larval growth at the dose range employed. The effect of γ-irradiation was to increase the amount of nucleic acids contrary to the expectation. The highest increase in DNA and RNA amounts was observed by the irradiation with 3 kR in two varieties. The increment of RNA content depended on the increment of DNA content in the silk gland. The increase in cocoon-shell weight by the irradiation was also observed in both pure varieties used, but not in the hybrid between Nichi 124 and Shi 124 at any dose employed[7].

### *7) Effect of juvenoid*

When larvae were given juvenile hormone (juvenoid, JHA) in the early period of the 5th instar, especially on the 3rd day, the larvae produced larger cocoons than those produced by the control larvae or the larvae treated in the late period of the 5th instar[4,6]. Juvenoid used in this experiment was methoprene. The stimulative efficiency of juvenoid supply was compared between varieties with low silk productivity and ones with high silk productivity regarding the silk formation and nucleic acid accumulation in the silk gland. The result is presented in Table 5. Juvenoid supply was most effective in increasing silk productivity of Giallo Ascoli. In general, it was more effective with varieties of low silk productivity than those with high productivity.

There was a comparable increase of cellular RNA accumulation in the silk gland. The relation of cocoon-shell weight vs the RNA content can be plotted on the mathematical line, as shown in Fig. 1. In several cases of the silkworm, DNA in the silk gland of the treated larvae reached almost twice that of the control. Juvenoid, in this case, temporarily diminished the nucleic acid synthe-

**Table 5. Stimulative effect of JHA on the amount of nucleic acids in the posterior silk gland and cocoon shell**

| Variety | Treatment | Weight of cocoon (g) | | Nucleic acids in the silk gland (μg/larva) | |
|---|---|---|---|---|---|
| | | Cocoon | Cocoon shell | RNA | DNA |
| Nichi 140×Shi 145 | Control | 2.40 | 0.577 | 8,640 | — |
| | V-3 | 2.74 | 0.701 | 10,000 | — |
| 610 | Control | 2.71 | 0.657 | 8,500 | — |
| | V-3 | 2.88 | 0.715 | 11,000 | — |
| Kohoku | Control | 1.25 | 0.159 | 2,700 | — |
| | V-1, 2, 3 | 1.77 | 0.226 | 4,300 | — |
| Giallo Ascoli | Control | 1.35 | 0.157 | 2,900 | — |
| | V-1, 2, 3 | 2.00 | 0.260 | 4,350 | — |
| Ôkusa | Control | 1.61 | 0.192 | 2,900 | 94 |
| | V-1, 2, 3 | 2.65 | 0.309 | 4,100 | 105 |
| Nichi 124×Shi 124 | Control | 2.44 | 0.400 | 5,600 | 206 |
| | V-1, 2, 3* | 3.30 | 0.430 | 6,000 | 195 |
| | V-3 | 2.62 | 0.514 | 9,000 | 395 |

Larvae were given topically JHA of 1 μg/g body weight.
V-3: Larvae were given JHA on the 3rd day of the 5th instar.
V-1, 2, 3: Larvae were given JHA every day from the 1st to the 3rd day of the 5th instar.
* JHA was applied to a larva at an excess dose of 8.7 μg/g body weight every day from the 1st to the 3rd day of the 5th instar.

sis, but thereafter appeared an additional increase of the synthesis[5,8].

## Summary

The capacity of silk production was essentially controlled by the replication frequency of DNA of the silk gland throughout its development, although productivity of silk, properly speaking fibroin of silkworm, *Bombyx mori*, was in proportion to the amount of RNA accumulation in the posterior silk gland. From the data on the amount of silk gland DNA in the fully grown larva, its replication frequency was calculated as 29 times and 30 times for a line of low productivity and a line of high productivity of silkworm varieties, respectively. In some cases, juvenoid would be able to make a chance to increase a replication frequency of chromosomal DNA. A DNA molecule can transcribe RNA ranged from 4 to 8 pg.

Certain alteration in the capacity of RNA synthesis took place among silkworm varieties, due to genetic difference of larval body and to different rearing environment. It is essential to take account the transcriptional control.

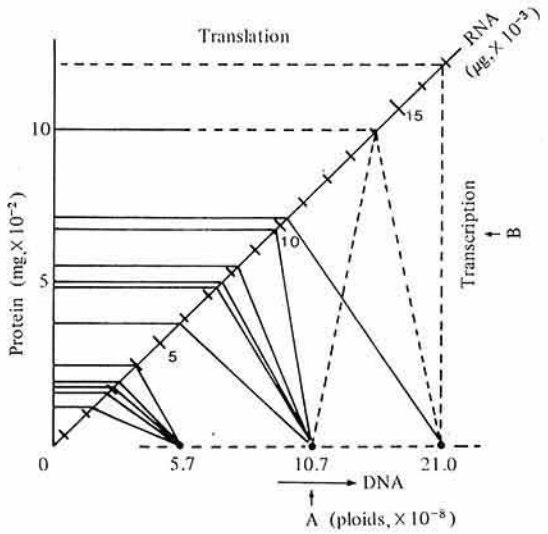


Fig. 3. Model illustration of quantitative amplification of silk information framed in DNA strands
A : Factors promoting frequency of DNA replication, as shown in a history of breeding, or by the effect of juvenoid application.
B : Factors controlling the capacity of translation, as shown by genetic and environmental conditions of silkworm body, or action of antibiotics or juvenoid.

Abnormal states such as starvation caused a severe alteration in translation phase and the efficiency of RNA for silk synthesis was reduced remarkably. Thus lowering translation activity may be brought about by quantitative imbalance of RNA species.

From our experiments quantitative relationship between nucleic acids and silk production of the silkworm is synthetically presented as shown in Fig. 3.

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