Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 94, No. 4, August 1985, pp. 413–419.

Studies on the silk gland of *Bombyx mori*: A comparative analysis during fifth instar development

S K SARANGI

R & D Programme in Sericulture, Bangalore University, Bangalore 560009, India.

MS received 11 June 1984; revised 1 January 1985

Abstract. Middle and posterior parts of the silk gland of fifth instar bivoltine and multivoltine races of *Bombyx mori* and their hybrid were analysed for the concentration of fibroin, DNA, RNA and total protein. Fibroin content of the silk gland increased rapidly from the beginning of fifth instar upto the spinning stage. Concentration of DNA in the middle silk gland was maximum at 24 hr and decreased thereafter. In the posterior silk gland, the concentration of DNA increased upto 72 hr and then decreased. RNA concentration was maximum at 72 hr and 120 hr, in the middle and posterior silk gland respectively. The total protein content increased gradually upto the spinning stage in the middle silk gland whereas it increased upto 120 hr and decreased sharply thereafter in the posterior silk gland. The difference in the concentration of these constituents in the silk gland was correlated with the differential silk output in both the pure races and their hybrid.

Keywords. *Bombyx mori*; bivoltine; multivoltine; hybrid; silk gland; fibroin; DNA; RNA; total protein.

1. Introduction

Structural and functional aspects of the silk gland of silkworm *Bombyx mori* have thoroughly been investigated (Dhavalikar 1962; Lucas 1966; Machida 1970; Tashiro and Otsuki 1970; Sasaki and Noda 1973 a, b; Prudhomme *et al* 1973; Tashiro *et al* 1976). Variations in the concentration of fibroin, DNA and RNA in the silk gland of several strains of bivoltine race and their hybrids have also been reported (Shigematsu and Takeshita 1968; Tashiro *et al* 1968; Shigematsu and Moriyama 1970; Moriuchi *et al* 1972; Shigematsu *et al* 1974). However, no information is available on the quantitative variations in the silk gland of multivoltine race. Bivoltines are shown to produce more quantity of silk compared to multivoltines (Tanaka 1964). It is therefore interesting to undertake a comparative study on the quantitative analysis of the silk gland of both bivoltine and multivoltine races of *Bombyx mori* and their hybrid to highlight the influence of these variations on the differential silk output by the silkworm varieties.

The silk gland grows enormously during the fifth instar development of silkworm (Sakaguchi 1978). The middle and posterior parts of the silk gland are known to synthesize sericin and fibroin respectively (Machida 1927; Oba 1957; Shibukawa 1959). Further it has been shown that fibroin is synthesized very rapidly during the fifth instar development (Shimura *et al* 1955; Noguchi *et al* 1974) being associated with an increase in DNA, RNA and total protein content of the posterior silk gland (Tashiro *et al* 1968).

The present communication deals with a comparative account of the middle and posterior silk gland of the fifth instar bivoltine and multivoltine races of *Bombyx mori* and their hybrid. A few important commercial characters are analysed in different silkworm varieties to correlate with the variations in the silk gland.

2. Materials and methods

2.1 *Silkworms*

Bivoltine (NB_{18}), multivoltine (Pure Mysore) races of *Bombyx mori* and their hybrid (NB_{18} ♂ X PM ♀) were maintained under standard laboratory conditions at a temperature of 25–28°C and relative humidity of 75–90% with good quality mulberry leaves (M_5 variety). Fifth instar larvae of average body weight were used at different time intervals.

2.2 *Analyses of the silk gland*

The silk gland was dissected and washed with 0·9% NaCl. The intraglandular fibroin was extracted separately from the middle and posterior parts of the silk gland according to the procedure of Tashiro *et al* (1968). After complete extraction of fibroin, the extracts from both parts of the silk gland were pooled and weighed. The nucleic acid was extracted from the middle and posterior silk gland separately and estimated according to Schneider (1957). The concentration of DNA was determined by diphenylamine method using calf thymus DNA as standard and the concentration of RNA was determined by orcinol method using rat liver RNA as standard. The total protein from the middle and posterior silk gland was extracted by the reduction of disulphide bonds, according to the procedure of Gamo *et al* (1977) and estimated according to Lowry *et al* (1951). Usually 4–5 larvae were used for each determination and average value of four independent determinations was calculated.

2.3 *Analyses of cocoon characters*

Four to five lots of 10 cocoons each, were taken after harvesting from bivoltine, multivoltine and the hybrid silkworm varieties for analysis of cocoon characters. Important characters like cocoon weight, shell weight percentage, floss weight percentage and filament length were determined. The shell weight percentage was calculated as the ratio between the cocoon shell and the whole cocoon, while the floss weight percentage was the ratio between the total floss and the whole cocoon. Mean values of the lots with standard deviation are presented in table 1.

3. Results

As shown in figure 1, total fibroin content increased significantly ($P = <0{\cdot}01$) from 24 hr to reach the maximum level prior to spinning in both the pure races. In hybrid male, a significant increase ($P = <0{\cdot}01$) in fibroin content was observed between 72 hr and 120 hr which remained more or less at the same level upto the spinning stage. On the other hand, the fibroin content increased significantly upto 120 hr followed by a slight increase thereafter in hybrid female. However, the concentration of fibroin at the final stages of fifth instar was high in bivoltine and low in multivoltine, while hybrid showed a sex difference in fibroin content being high in male and low in female.

Table 1. Important cocoon characters of bivoltine, multivoltine and the hybrid silkworm varieties

Silkworm variety	Av. Cocoon weight (g) Mean SD	Shell weight (%) Mean SD	Floss weight (%) Mean SD	Av. filament length (m) Mean SD
NB_{18} (bivoltine)	1·28 ± 0·06	14·35 ± 0·80	0·79 ± 0·08	780 ± 55
Pure Mysore (multivoltine)	0·88 ± 0·04	9·59 ± 1·02	2·25 ± 0·18	350 ± 25
Hybrid male	1·08 ± 0·06	12·02 ± 1·05	1·38 ± 0·09	620 ± 35
Hybrid female	0·94 ± 0·06	11·00 ± 0·90	1·40 ± 0·11	575 ± 50

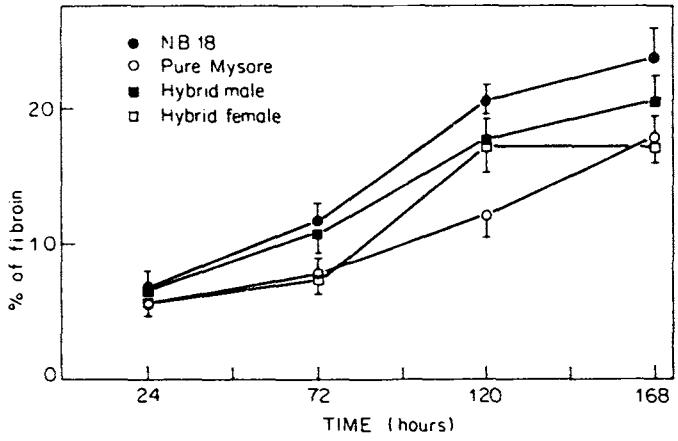

Figure 1. Increase in fibroin content of the silk gland (middle plus posterior) during fifth instar development.

The concentration of DNA in the middle silk gland was maximum at 24 hr which decreased gradually upto 120 hr and remained at a more or less constant level (figure 2A). The DNA concentration was high in bivoltine and both sexes of the hybrid while in multivoltine it was significantly low ($P = <0·005$). As shown in figure 2B, the concentration of DNA in the posterior silk gland increased to reach the maximum level at 72 hr and decreased thereafter. However, DNA concentration was different in different races being high in bivoltine and low in multivoltine. Further, at the final stages of fifth instar the DNA content remained constant between 0·4 and 0·5 mg/g wet wt in both the pure races and the hybrid.

RNA concentration increased upto 72 hr and then decreased to a more or less constant level by 120 hr in the middle silk gland of bivoltine and hybrid male, whereas it was maximum at the beginning of fifth instar (24 hr) and decreased thereafter in multivoltine and hybrid female (figure 3A). The level of RNA in the middle silk gland of bivoltine and both sexes of the hybrid was high compared to a significantly low ($P = <0·001$) level in multivoltine. The concentration of RNA in the posterior silk gland increased gradually to reach the maximum at 120 hr and decreased significantly

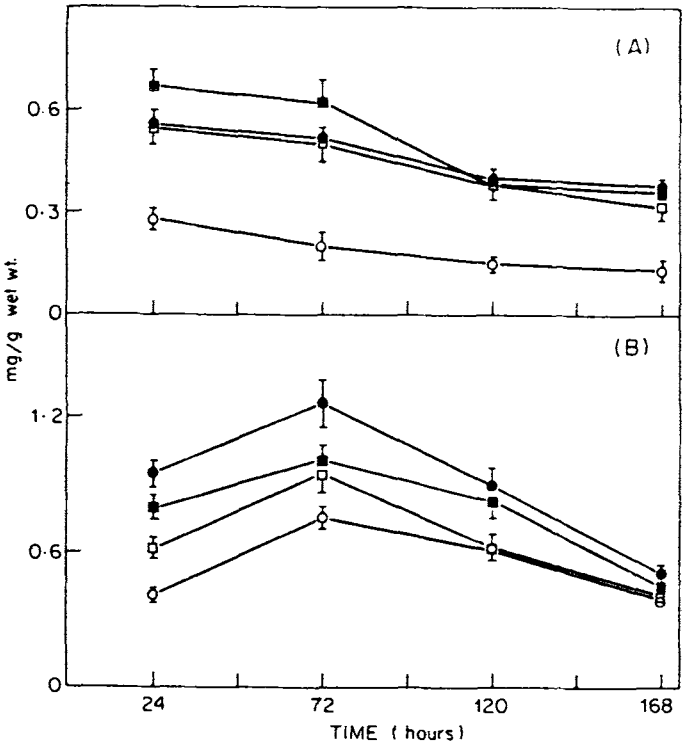

Figure 2. Change in DNA content of the middle (A) and posterior (B) silk gland during the fifth instar.

($P = <0{\cdot}001$) thereafter in bivoltine and hybrid male (figure 3B). But in multivoltine and hybrid female, the RNA content was maximum at 72 hr and then decreased gradually. The concentration of RNA in the posterior silk gland was significantly high ($P = <0{\cdot}01$) in bivoltine and hybrid male as compared to multivoltine and hybrid female.

There was a significant increase ($P = <0{\cdot}001$) in the amount of total protein in the middle silk gland from the beginning to the end of fifth instar (figure 4A). The final concentration was about five times that at the beginning of the fifth instar. Further, a significant difference in the concentration of total protein was observed in different races, being high in bivoltine (160 mg/g wet wt) and low in multivoltine (112 mg/g wet wt) prior to spinning. As shown in figure 4B, the concentration of total protein in the posterior silk gland increased upto 120 hr, reached the maximum level and then decreased significantly ($P = <0{\cdot}005$), unlike in the middle silk gland. Further, the concentration of total protein in the posterior silk gland at 120 hr was significantly different in the silkworm varieties studied being high in bivoltine and low in hybrid female.

Table 1 shows the difference in a few important commercial characters of the cocoon from different silkworm varieties. The average cocoon weight, shell weight percentage and the average filament length were significantly high in case of bivoltine as compared to multivoltine. But the hybrid showed an improvement of all these characters over the

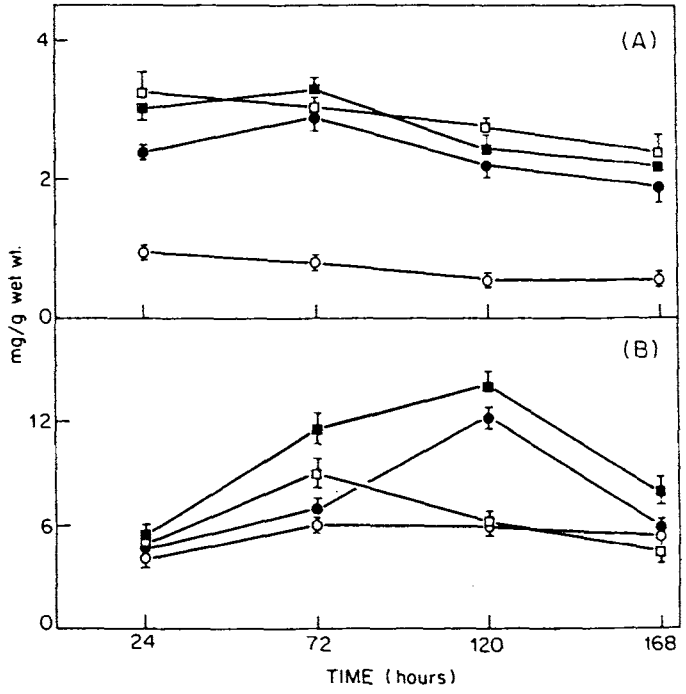

Figure 3. Change in RNA content of the middle (A) and posterior (B) silk gland during the fifth instar.

multivoltine parent. Further, a difference, though not significant, was observed between both sexes of the hybrid.

4. Discussion

The amount of fibroin in the silk gland increases significantly from the beginning to the end of fifth instar. This can be correlated with the high rate of fibroin synthesis in the silk gland during fifth instar development (Shigematsu and Takeshita 1968; Tashiro *et al* 1968; Noguchi *et al* 1974). A higher concentration of fibroin in the silk gland of bivoltine might account for more quantity of silk produced as compared to multivoltine. The concentration of fibroin in the silk gland of hybrid male is more concomitant with larger quantity of silk produced as compared to the female. However, no significant sex difference in the concentration of fibroin is observed in pure races.

High level of DNA at the beginning of fifth instar shows that the synthetic activity starts early in the middle silk gland. In contrast, there is a time-lag for the increase in DNA content of the posterior silk gland suggesting that the fibroin synthesis starts a little late during the fifth instar. Incorporation studies have also shown that the synthesis of fibroin is significant only from 96 hr of the fifth instar development (Fukuda and Florkin 1959). This result, however, contradicts the earlier reports of Tashiro *et al* (1968) who reported no time-lag for the increase in DNA content of the posterior silk gland. The difference in the DNA content of both middle and posterior silk gland accounts for the

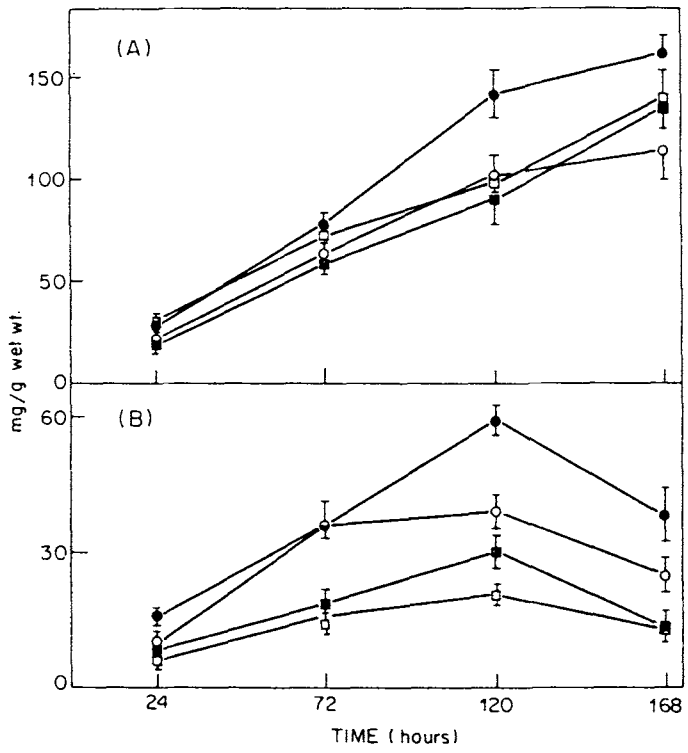

Figure 4. Change in total protein content of the middle (A) and posterior (B) silk gland during the fifth instar.

difference in the degree of their synthetic activity. Further, the DNA content of the silk gland of both the races is different showing that it is probably specific to the race.

The concentration of RNA in the middle silk gland of multivoltine is low concomitant with a low DNA concentration. This reflects the lower rate of synthetic activity in the middle silk gland of multivoltine compared to bivoltine and both sexes of the hybrid. Maximum level of RNA at 72 hr and 120 hr in the posterior silk gland of multivoltine and bivoltine respectively, suggests that the synthetic activity in the posterior silk gland starts later than in the middle silk gland.

The initial increase in the total protein content of the middle silk gland might be due to the synthesis and accumulation of sericin as suggested by an early increase in its DNA and RNA contents. The rapid increase in the amount of protein after 72 hr, supports the view that there is an inflow of fibroin to the middle silk gland for storage (Fukuda and Florkin 1959; Gamo *et al* 1977; Sakaguchi 1978). The significant decrease in total protein content of the posterior silk gland after 120 hr is in accordance with earlier reports (Tashiro *et al* 1968). This suggests that the outflow of fibroin from posterior silk gland is more rapid during the later part of the fifth instar.

Thus it is evident that the concentration of fibroin, DNA, RNA and total protein in the middle and posterior silk gland is different in both the pure races and that the hybrid shows an increase in the concentration of these constituents over the multivoltine parent. The final silk output, however, is directly correlated with the concentration of these constituents in the silk gland of the silkworm varieties studied.

Acknowledgement

Grateful acknowledgements are due to the World Bank for financial assistance and to the Co-ordinator, R & D Programme in Sericulture, Bangalore University for facilities. The author thanks Mr P M Chandrasekhar for technical help.

References

Dhavalikar R S 1962 Amino acid composition of Indian silk fibroins and sericins. II. Sericins; *Indian J. Sci. Ind. Res.* **C21** 303–304

Fukuda T and Florkin M 1959 Contributions to silkworm biochemistry, (VII) ordered progression of fibrinogen in the reservoir of the silk gland during the fifth instar. *Arch. Int. Physiol. Biochem.* **67** 214–222

Gamo T, Inokuchi T and Laufer H 1977 Polypeptides of fibroin and sericin secreted from the different sections of the silk gland in *Bombyx mori*; *Insect. Biochem.* 7 285–295

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with folin phenol reagent; *J. Biol. Chem.* **193** 265–275

Lucas F 1966 Cystine content of silk fibroin (*Bombyx mori*) *Nature (London).* **210** 952–953

Machida J 1927 On the secretion of the silk substance in the silkworm (*Bombyx mori* L.); *J. Coll. Agric. Imp. Univ. Tokyo* **9** 119–138

Machida Y 1970 Studies on the silk glands of silkworms *Bombyx mori* L; *Fukuoka Women's junior coll. Studies.* **3** 1–21 (not referred to in original)

Moriuchi A, Koga K, Yamada T and Akune S 1972 DNA synthesis and activities of pyrimidine synthesizing enzymes in the silk gland of *Bombyx mori*; *J. Insect Physiol.* **18** 1463–1476

Noguchi A, Takeshita H and Shigematsu H 1974 Interrelationship between the silk gland and other tissues in protein metabolism in the latest larval stage of the silkworm, *Bombyx mori*; *J. Insect Physiol.* **20** 783–794

Oba H 1957 Studies on the secretion and the current of liquid silk during spinning period in the silkworm *Bombyx mori* L; *Bull. Nagano Sericult. Exp. Station* **10** 9–25

Prudhomme J C, Sridhara S and Daillie J 1973 The bio-synthesis of fibroin 1. The intracellular form of silk fibroin of *Bombyx mori* L; *Arch. Biochem. Biophys.* **159** 97–104

Sakaguchi B 1978 In: *The Silkworm: An important laboratory tool.* (ed.) Y Tazima (Tokyo: Kodansha Ltd) pp. 31–51

Sasaki T and Noda H 1973a Studies on silk fibroin of *Bombyx mori* directly extracted from the silk gland; *Biochim. Biophys. Acta* **310** 76–90

Sasaki T and Noda H 1973b Studies on silk fibroin of *Bombyx mori* directly extracted from the silk gland; *Biochim. Biophys. Acta* **310** 91–103

Schneider W C 1957 Determination of nucleic acids in tissues by pentose analysis; *Methods Enzymol.* **3** 680–684

Shibukawa A 1959 Studies on the silk substance within the silk gland of the silkworm, *Bombyx mori* L; *Bull. Sericult. Exp. Sta. Tokyo.* **15** 383–401

Shigematsu H and Moriyama H 1970 Effect of ecdysterone on fibroin synthesis in the posterior division of the silk gland of the silkworm, *Bombyx mori*; *J. Insect Physiol.* **16** 2015–2022

Shigematsu H and Takeshita H 1968 Formation of silk proteins by the silkworm, *Bombyx mori*, after gamma ray irradiation in the embryonic stage; *J. Insect Physiol.* **14** 1013–1024

Shigematsu H, Moriyama H and Arai N 1974 Growth and silk formation of silkworm larvae influenced by photoecdysones; *J. Insect Physiol.* **20** 867–875

Shimura K, Koide F, Itabashi H and Fukai H 1955 Protein synthesis in excised silk gland of silkworm; *J. Biochem.* **42** 285–294

Tanaka Y 1964 In: *Sericology. V. Silkworm races* (Bombay: Central Silk Board) pp. 99–104

Tashiro Y and Otsuki E 1970 Studies on the posterior silk gland of the silkworm *Bombyx mori*; *J. Cell. Biol.* **46** 1–16

Tashiro Y, Morimoto T, Matsumura S and Nagata S 1968 Studies on the posterior silk gland of the silkworm, *Bombyx mori*; *J. Cell. Biol.* **38** 574–587

Tashiro Y, Matsumura S and Hata K 1976 Ultrastructural observations on the organogenesis of the posterior silk gland of the silkworm, *Bombyx mori*; *J. Insect Physiol.* **22** 273–283