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Scanning Electron Microscopic Study on the Cross Sections of Cocoon Filament and Degummed Fiber of Different Breeds/Hybrids of Mulberry Silkworm, *Bombyx mori* Linn.

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Abstract: The cocoon filaments and degummed fibers of different breeds/hybrids of *Bombyx mori* Linn., viz., CSR_2 and CSR_4 (Pure bivoltine) $CSR_2 \times CSR_4$ (Bivoltine hybrid); PM × CSR_2 (Multivoltine × bivoltine hybrid) and Pure Mysore (Pure multivoltine) were examined using a scanning electron microscope. The study was under taken in order to clarify the characteristics of cocoon filament, degummed fibers and to confirm the presence of voids/striae. The morphological study reveals the presence of number of voids and variation in their shape and size at ultrastructure level. The number of voids in Pure Mysore is higher than those found in pure bivoltine and their hybrids. Further, the shape and size of these voids differ from that of the voids noticed in cross section of pure multivoltine and multi × bivoltine hybrids. The voids studied in all the breeds of *B. mori* were elongated in the direction of the fiber axis. Large sized and more number of voids were observed in male cocoon filament than that of female cocoon filament in all the breeds/hybrids of *B. mori* × bivoltine hybrids. Further, some voids were gradually flattened during the cocoon cooking and degumming process, which finally closed and transformed into striae resulting in an increased compactness of degummed fiber in all the breeds/hybrids studied.

Key words: Cocoon filament, degummed fiber, voids, striae, *Bombyx mori*, breeds/hybrids, SEM

INTRODUCTION

Certain species of Insecta and Arachnida spin a number of different types of silk for a variety of specialized purposes such as the fabrication of a cocoon and the construction of a nest. Among Insecta, *Bombyx mori* Linn., a species of the subfamily Bombycidae is the most familiar and extensively studied. Silkworm silk has been used as a luxury textile material since 3000 B.C. (Asakura and Kaplan, 1994), but it was not until recently that the scientific community felt the tremendous potential of silk as a structural material (Poza *et al*., 2002). Physical properties of silk thread spun by the mulberry and non-mulberry silkworm varies in different parameters. The granular spots/voids can be seen within cross sections of cocoon filaments in some wild silkworms. The structure and properties of the silk filaments of wild silkworms has been studied by a number of workers (Komatsu, 1984; Akai *et al*., 1991; Narumi and Kobayashi, 1995; Narumi and Kobayashi, 1997a, b). Narumi *et al*. (1992) have studied the cocoon filaments of various wild silkmoth species belonging to the family Saturniidae under a scanning electron microscope and found voids in the cocoon filaments of every species examined. Lin *et al*. (1993) described the cocoon filaments of five species of Taiwan Saturniidae, *Antheraea yamamai*, *Antheraea formosana*, *Attacus atlas*, *Caligula japania arisana* and *Caligula thibeta okurai* using scanning electron microscope (SEM). Voids were found in every species except *Attacus atlas*.

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The above observation made by various authors indicates that the presence of voids in cocoon filament is a characteristic of the fine structure of cocoon filament in wild silkworm belonging to the family Saturniidae. Akai *et al*. (1987) found many vacuoles in the liquid silk and cross sections of cocoon filaments of *Antheraea yamamai*. Further, the mechanism of the voids formations in Tussar silk fibroin was examined by the use of microscopes of small angle X-ray method by Hirabayashi *et al*. (1969). Narumi *et al*. (1992) reported the rare voids in cocoon filaments of *B. mori* and *B. mandarina*. Since the presence or absence of voids directly correlated with the quality of the silk fiber, it is felt to undertake the electron microscopic study on the cocoon filament and degummed fiber of different breeds/hybrids of mulberry silkworm. Therefore, the present study has been taken to investigate the voids or any other similar structure in different breeds/hybrids of *B. mori*, in both the sexes, using scanning electron microscope (SEM). The ultimate objective of the study is to identify a marker based on the number, shape and size of voids/striae present in the cocoon filament and degummed fiber, which ultimately will help in the selection process of good quality of silk cocoons for silkworm breeding and reeling parameters.

MATERIALS AND METHODS

The samples were collected form Silkworm Physiology Laboratory, Central Sericultural Research and Training Institute Mysore, India during the months of January-February 2006. Cocoon filament and degummed fiber of pure bivoltine breeds (CSR_2 and CSR_4), bivoltine hybrid ($CSR_2 \times CSR_4$), cross breed of multivoltine × bivoltine hybrids ($PM \times CSR_2$) and pure multivoltine (PM) were taken for the present study. The samples were cross sectioned by slicing them into small pieces of about 20 μm thickness with the help of a sharp blade. Three samples were taken for each breed from the middle of the cocoon for cocoon filament study. However, for the degummed fiber, the adhesive protein i.e., sericin was removed from cocoon filament by standard methods (Gulrajani, 1988) and the blocks were prepared using dental wax and then the blocks were sliced into cross sections. The sections of both cocoon filament and degummed fiber of both the sexes were mounted onto copper stubs using double side sticking tape. The mounted specimens were immediately coated with 20 nm thickness of gold on their cut surface, in a Sputter coater (EMS-550) to minimize charging under the electron beam. The gold coated samples were observed using scanning electron microscope (SEM) (JEOL 100 CX II ASID 4D, Tokyo Ltd., Japan) under an accelerating voltage of 20 kV with a beam current around 0.1 nA. Low magnification was used to show the cross sections and longitudinal sections of the cocoon shell however the higher magnification was selected to observe the number of voids/striae present in the cocoon filament and degummed fiber. The photographs were taken at different magnifications for observations.

RESULTS AND DISCUSSION

The cocoon filament and degummed fiber of different breeds/hybrids of *Bombyx mori* were examined under a scanning electron microscope in order to clarify the characteristics of the cocoon filaments and degummed fiber and to confirm the presence of voids or any other similar structures. Low magnification photomicrographs of the cross section of female cocoon of CSR_4 revealed that the cocoon filament had cut transversely and longitudinally (Fig. 1a). The cocoon fibers were tightly packed in the cocoons of pure bivoltine breeds (Fig. 1a and b) whereas the same filament observed loosely packed in the cocoons of pure multivoltine breed i.e., in pure Mysore (Fig. 1f).

The morphological studies on the cross sections of cocoon filaments in different pure breeds and hybrids reveals the presence of number of voids and their shape, size varies at ultrastructure level. The number of voids in pure multivoltine breed i.e., Pure Mysore, is much higher than those found in pure

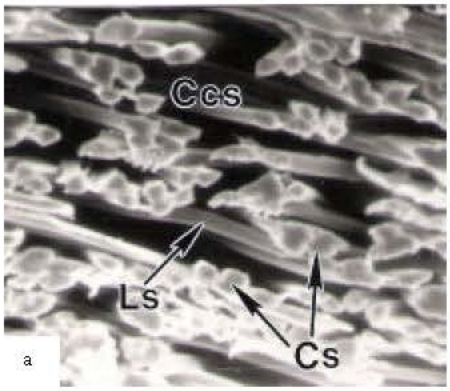


Fig. 1a: Cross section of the cocoon shell (Ccs) reveals the cross section (Cs) and longitudinal strands (Ls) of cocoon filament of female CSR4 breed. Note cocoon filaments are closely packed. (x 500)

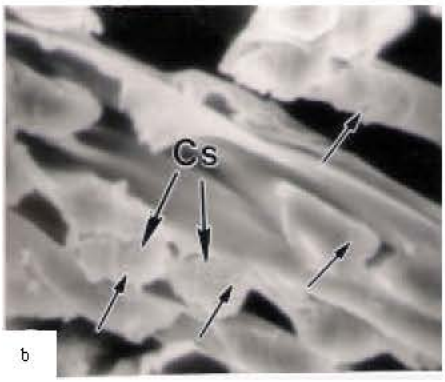


Fig. 1b: A magnified view of the cross section (Cs) of cocoon shell indicated the presence of voids (arrow) in male CSR_4 breed (x 1700)

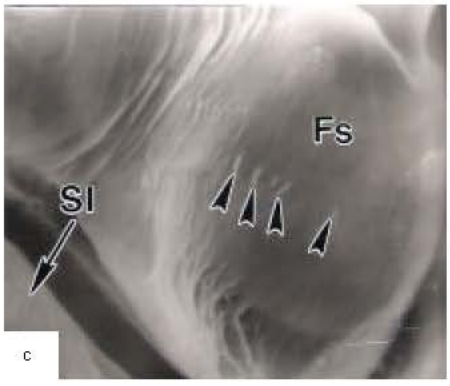


Fig. 1c: Cross section of fibroin strand (Fs) with a number of voids in the central part of filament (arrow heads) in female CSR_2 breed. (Sl = Sericin layer). (x 12800)

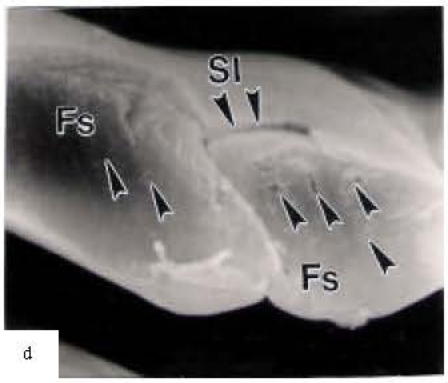


Fig. 1d: Two fibroin strands (Fs) coated with a sericin layer (Sl). Arrow heads indicated the voids in the cross section of fibroin strands of male CSR_2 x CSR_4. (x 6400)

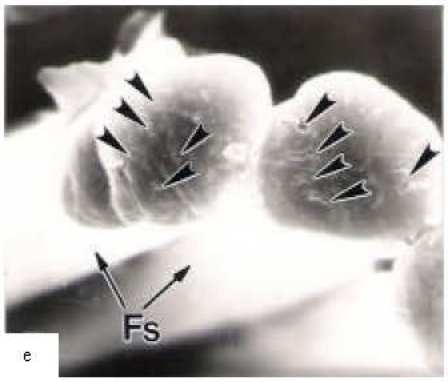


Fig. 1e: Long channel shaped voids (arrow heads) in the cross section cocoon filament of PM x CSR_2. (x 5000)

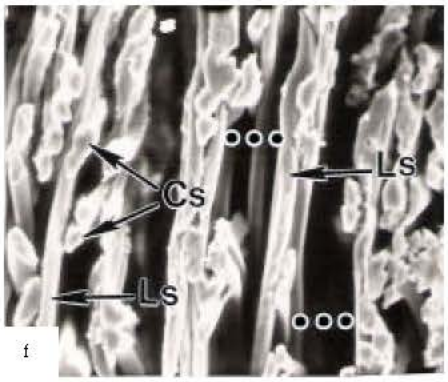


Fig. 1f: Cross section of the cocoon shell showing the cross section of cocoon filament (Cs) and longitudinal strands (Ls) of Pure Mysore. The cocoon filaments are loosely packed (ooo) in multivoltine. (x 500)

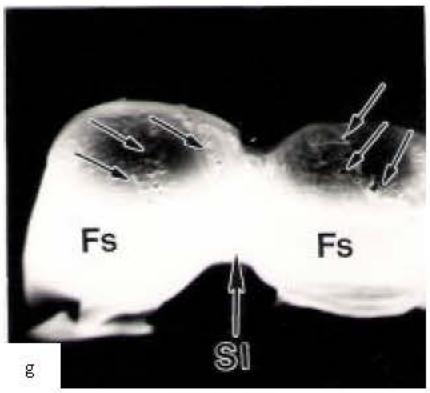


Fig. 1g: Two fibroin strands (Fs) coated with sericin layer (SI) revealing a large number of voids (arrows) in the female cocoon filament of Pure Mysore (x 5000)

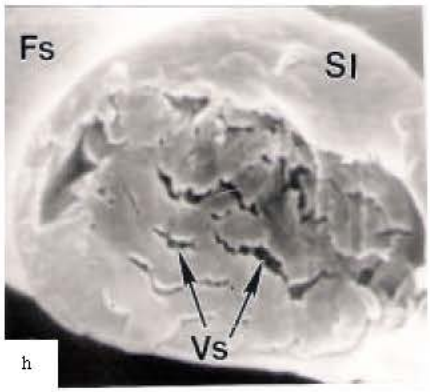


Fig. 1h: A large number of channel shaped voids (Vs) in the central part of the cross section of fibrion strand in female Pure Mysore (x 12800)

breeds and hybrids of bivoltine. The number of the voids in the cross sections of pure breeds of bivoltine i.e., CSR_2 and CSR_4 were observed relatively very less in both the sexes. Further, the shape and size of these voids also differ from that of the voids noticed in cross section of cross breed of multivoltine and pure multivoltine. The shape of the voids in pure bivoltine and their hybrids were observed to be round or elliptical and sometime two or three small voids joined to form a small channel shaped void in CSR_2 and $CSR_2 \times CSR_4$ breeds (Fig. 1c-e). However, a large number of bigger voids were noticed in the central part of cocoon filament of Pure Mysore and its hybrid (Fig. 1g and h). Narumi *et al*. (1992, 1993) and Lin *et al*. (1993) stated that the voids were distributed in the central part of the filament cross section and were typically circular or elliptical in the cocoon filament of wild silkworms. The voids studied in all the breeds of *B. mori* were elongated in the direction of the fiber axis. It is also observed that the diameter of the voids distributed in the central part of the filament was larger than that of the voids near the surface in the cocoon filament. Large sized and more number of voids in male cocoon filaments was observed than that of female cocoon filament in all the breeds/hybrids studied. Narumi *et al*. (1994) reported that the number of voids in male filament was larger than that of female ones and there were many large voids in the central part of male cocoon filaments of wild silkworms.

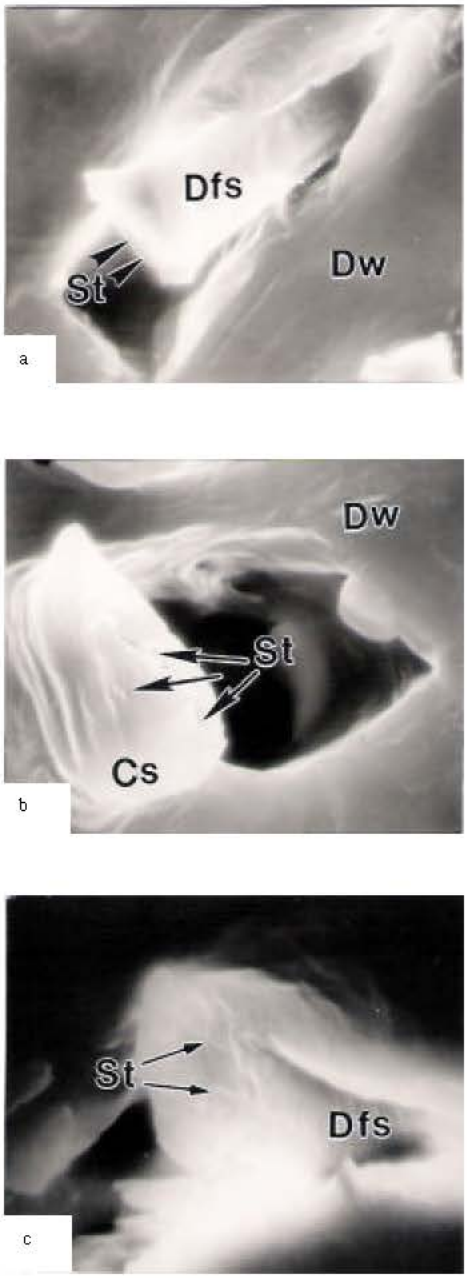


Fig. 2a-c: Cross sections of degummed fiber (Dfs) in the dental wax media (Dw) reveals the striae (St) in CSR_2 female (2a), CSR_2 male (2b) and male CSR_4 (2c). (x 3300 for Fig. 2a; x 6660 for Fig 2b and x 4270 for Fig. 2c)

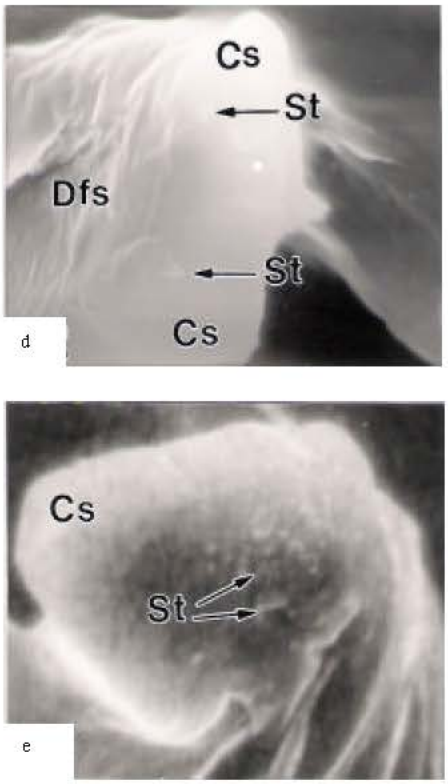
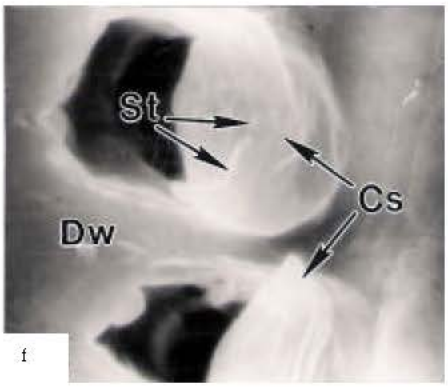


Fig. 2d-e: Magnified views of cross sections showing the striae (St) which were formed after cooking and degumming of cocoon filament in male CSR_4 (2d) and in the female of $CSR_2 \times CSR_4$ (2e) (x 8530 for Fig. 2d and × 8530 for Fig. 2e)

The cross sectional areas of filament after cooking showed about 6% reduction compared to the raw cocoon filaments. Cocoon cooking removes sericin from the peripheral areas of filaments, resulting in a decrease in the cross sectional area of the filament. Following cocoon cooking, the number of voids was found to have reduced to about 30% in the cocoon filament and about 20% after degummed cocoon filament in all the breeds/hybrids. Some voids were gradually flattened during the cocoon cooking and degumming process and finally closed and were transformed into striae in all the breeds/hybrids studied, except in the females of Pure Mysore (Fig. 2a-h). Assuming that striae is a closed void, we found that the total number of voids and striae observed after cooking and degumming was smaller than the number of voids in the cocoon filament. This suggests that the closer of voids has progressed further and they have fused into fibroin to such an extent that they were not detected in the SEM. After degumming, the number of voids in the filament was further reduced, while the number of extremely flat voids and streak-like striae increased in all the breeds/hybrids except in Pure Mysore. The number of the striae, their shape and size were found almost similar in CSR_2, CSR_4 and $CSR_2 \times CSR_4$ and $PM \times CSR_2$ (Fig. 2a-f). However, more number of striae were found in $PM \times CSR_2$ male degummed fiber (Fig. 2g). Narumi and Kobayashi (1997b) have studied the morphological charactertics of voids in wild silks used in woven fabrics and mentioned that the larger diameter voids found in cocoon filaments of wild silkworms are flattened at a significant degree in woven fabrics structure and transformed into striae.



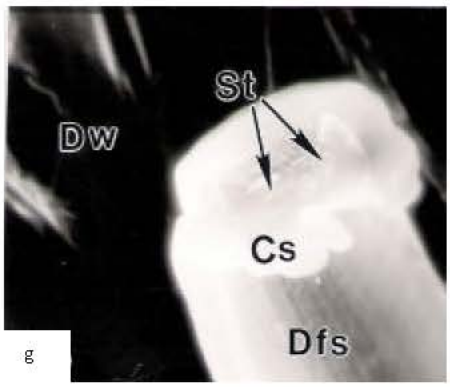


Fig. 2f and g: Cross sections of the degummed fibers of male CSR_2 x CSR_4 (2f) and female PM x CSR_2 (2g) reveals the striae (St) in the central part of degummed fiber. (x 4270 for Fig. 2f and x 6660 for Fig. 2g)

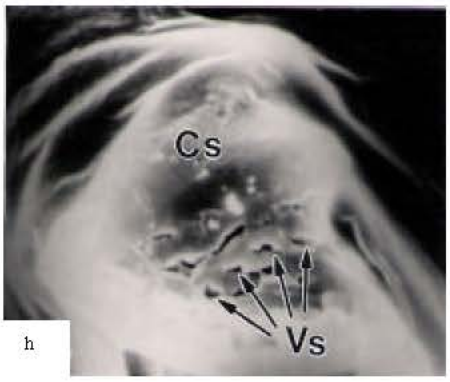


Fig. 2h:Magnified view of a cross section of degummed fiber of female Pure Mysore showing a large number of voids (Vs) in the peripheral area of cross section. (x8530)

The present findings clearly indicate that the large sized and more number of voids present in male cocoon filaments than in female in all the breeds/hybrids of *B. mori* studied. Further, there are considerable differences in shape, size, number and distribution pattern of the voids in the cocoon filaments of different breeds/hybrids of mulberry silkworm. However, the number of voids/striae was higher in wild silkworms than that of the breeds/hybrids of *B. mori*. Further, the voids gradually flattened during the cocoon cooking and degumming processes and finally closed and transformed into striae resulting in an increased compactness of degummed fiber. In conclusion, the results of the present study confirmed that the presence of more number of voids/striae in multivoltine and less number in bivoltine cocoon filament and degummed fiber. More number of voids/striae found in inferior silk hence bivoltine silk is superior over the multivoltine silk. Therefore, the results of the present study will find use in the selection of the breeds/hybrids of superior quality silk for breeding programmes in addition to other reeling parameters.

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