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SEM-Anatomical Studies of Hybrid Silkworm Cocoons.

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การศึกษารังไหมลูกผสมด้วยวิธีกายวิภาคศาสตร์-จุลทรรศน์อิเลคตรอนแบบสแกน

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**ผู้อำนวยการสถานีทดลองหม่อนไหมขอนแก่น

พวกเราได้ทำการศึกษารังไหมลูกผสม ด้วยวิธีการนำความรู้และเทคโนโลยีทางกายวิภาคศาสตร์มาประยุกต์ใช้ เพื่อแก้ปัญหาที่เกิดขึ้นกับเกษตรผู้ยากจนที่ผลิตรังไหมขาย ปัญหาคือเกษตรกรไม่สามารถกำหนดราคาขายรังไหมของตัวเองได้ เช่นเดียวกับผลิตผลจากการเกษตรชนิดอื่น ๆ ทั้งนี้เป็นเพราะเหตุผลหลายอย่าง กลุ่มนักอุตสาหกรรมผู้สาวไหมได้มีการนำปัญหาเรื่องการคัดขนาดของรังไหมขึ้นมา เพื่อเป็นวิธีการตีราคารังไหมที่จะกำหนดเพื่อซื้อจากเกษตรกร รังเล็กราคาต่ำ รังใหญ่ราคาสูง เรื่องนี้เกิดขึ้นในคราวการประชุมประจำปีของสถาบันวิจัยหม่อนไหมที่วิทยาลัยเกษตรตากในปี 2532 วิธีการตีราคารังไหมของสถาบันวิจัยหม่อนไหม ที่สถานีทดลองหม่อนไหมทั่วประเทศปฏิบัติอยู่นั้น คือวิธีการคิดเปอร์เซ็นต์เปลือกรังโดยน้ำหนักซึ่งเป็นอัตราส่วนของน้ำหนักเปลือกรังกับน้ำหนักตัวดักแด้นั้น หลาย ๆ คนก็เชื่อว่ายุติธรรมดีอยู่แล้ว หากนำวิธีคัดขนาดรังเข้ามาใช้ อาจจะทำให้เกษตรกรเสียเปรียบมาก

พวกเราใช้วิธีการศึกษาทางกายวิภาคศาสตร์-จุลทรรศน์อิเลคตรอนแบบสแกน สำรวจศึกษาขนาดและรูปร่างเส้นไหมแต่ละเส้นในชั้นนอก ชั้นกลาง และชั้นในของเปลือกรังไหมลูกผสมซึ่งสถานีทดลองหม่อนไหมขอนแก่นส่งเสริมให้เลี้ยงในเดือนตุลาคม 2533 เพื่อเปรียบเทียบกันระหว่างรังใหญ่กับเล็ก เราได้พบว่าขนาดของเส้นไหมจากรังขนาดใหญ่และขนาดเล็กในชั้นเดียวกันจะไม่แตกต่างกันอย่างมีนัยสำคัญ โดยเฉพาะในกำลังขยายขนาด 500 เท่า ซึ่งจะมองเห็นเป็นขนาด

เดียวกันด้วยสายตาเปล่า ๆ เพราะฉะนั้นเราจึงเสนอข้อสรุปว่า ตามที่เราทราบกันดีแล้วว่ารังไหมแต่ละรังนั้นจะประกอบด้วยเส้นไหมเส้นเดียว ที่บางทีอาจจะยาวถึง 1,500 เมตร รังไหมขนาดเล็กก็จะมีเส้นไหมที่สั้นกว่าเท่านั้นเอง น้ำหนักหรือมวลของเส้นไหมก็จะกำหนดโดยความยาวเท่านั้น เมื่อนำไปใช้ทอผ้าก็ต้องนำมาต่อกันอยู่แล้ว จึงไม่มีเหตุผลสนับสนุนในการที่จะรังเกียจรังขนาดเล็ก การคัดขนาดของรังไหมจะมีเหตุผลก็ต่อเมื่อได้มีการพิสูจน์ว่ารังใหญ่สาวได้ดีกว่ารังเล็กเท่านั้น ซึ่งยังไม่พบว่าเป็นเช่นนั้นเลย

Abstract

This study is designed and planned to apply anatomical knowledge and techniques to the problem imposing on the poor silk producer farmers in Thailand. The farmers have never been able to set the price for their farm products because of several reasons. To sell their silkworm cocoons is absolutely not an exception to this agrobusiness phenomenon. In the annual meeting of the Institute of Sericulture Research held at Tak Agricultural College in 1989, the idea of grading the size of the cocoon was brought up by a group of reeler businessmen-- the smaller the cheaper price. The problem was again intensified to cause more deterioration on the farmers' economy, and hence the Thai national economic retardation. The on-going role of the Institute of Sericulture Research as a referee or the price setter (by basing on the weight ratio of the cocoon and the pupa) is considered by many as a fair play. The cocoon size grading will worsen the situation to a greater extent. If we do not take any action; the situation would be under the superior hands in doing business. The farmers, therefore, are exploited as always. By using the Scanning Electron Microscopic Anatomical (SEM-Anat) technique, we examined silk fiber morphology and sizes of the outer layer, middle layer, and the inner layer from the small and large cocoons of the hybrid silkworms promoted by Khon Kaen Sericulture Experiment Station in October 1990.

We have found that the fiber sizes of the cocoons of different sizes are not significantly different at 500 times magnification . The size would be definitely the same by the naked eyes. We, therefore, concluded that as we all know one cocoon is made up of only one single fiber of upto 1500 meters long, the smaller cocoon is made up of one shorter fiber that will weigh proportionally less than the longer fiber. The mass of the silk fiber is governed by the length in this case and all fiber are to join lengthwise together in the weaving. There is no reason to be prejudiced against the smaller cocoon. The cocoon size grading will be justified only when we have proved that the larger cocoons have advantage in reeling over the small ones-- this is not the case.

Background

It is a general phenomenon in the world of business that the price can not be controlled to please every one. The price depends on the balance of demand and supply and so little could be done to favour the producer or the dealer, let alone the consumers. Whatever the factors influencing the price fluctuation may be; it is necessary at least to agree upon or set a price acceptable by all concerned. It so happens that in the agro-business especially in Thailand the farmers have been exploited in the deal. When he is selling his agricultural products the buyers set the price and when he is buying fertilizers and pesticides he again has no bargaining power. The problem was taken seriously by the Institute of Sericulture Research of Thailand about ten years ago. The institute has been playing the role of a referee in calculating the price. That seemed to be working for some time until 1989 when the group of businessmen proposed the idea of grading the cocoons by size, the smaller the cheaper. If one looks at the situation superficially; that may seem fair. Looking at the proposal of size-grading from anatomical point of view, we feel that is by no means fair. The reasons we are offering are many :

1) The silk coccoons are not a solid mass that finally end up as an amorphous food product, but rather as continuous single fiber of each one and equally usable,

2) It is a raw material for a cloth industry which could be sold at high prices later on, and

3) The fibers to be reeled therefrom determine the weight that serves as a base to calculate the price employed by the Institute of Sericulture Research up to the present time.

We, therefore, set up this study to find out the following :

1) The fiber sizes, at the ultrastructural level, of the small cocoon and the larger cocoon should be the same,

2) The cocoon may be larger because the fiber is longer. This study is designed to prove our hypothesis that the silk fibers in the small and large cocoons at the ultrastructural level using the scanning electron microscope should not be significantly different enongh to cause undesirable quality of silk fiber at the level of naked eyes.

Material and Method

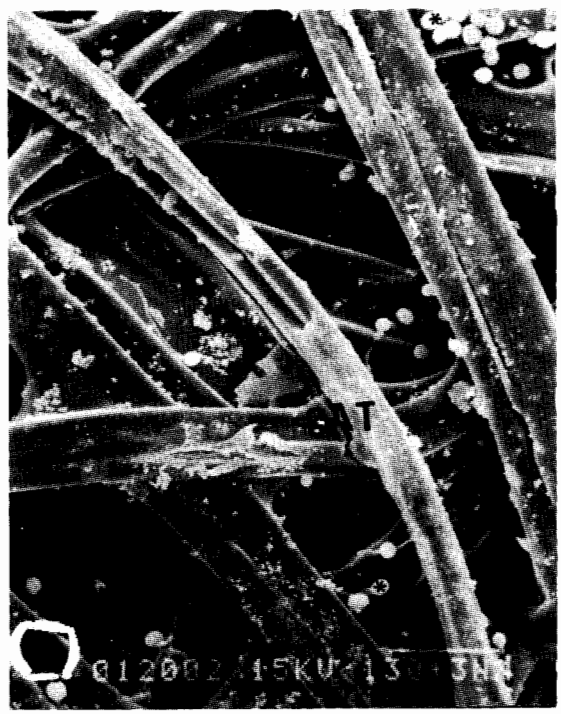
The silk cocoons produced by Khon Kaen Sericulture Experiment station in October 1990 were obtained. Each was cut in half to see the cut surface and identify the inner layer, the middle layer, and the outer layer of small and large cocoons. This could be done by naked eyes. The halves were furthered cut into small pieces of 1x1 mm. The pieces were fixed in 2% paraformaldehyde + 2.5% glutaraldehyde in 0.1 M. phosphate buffer at pH 7.2 overnight. Thereafter, the fixed specimens were washed twice in 0.1 M phosphate buffer at pH 7.2 and postfixed in 1% osmium tetroxide in phosphate buffer at the same pH for 2 hrs. After being washed again in the same buffer, the specimens were dehydrated in graded ethanols, transferred in to amylacetate and were critical-point dried by Hitachi HCP-2 apparatus. The obtained specimes were then mounted on the stubs, coated with gold in an ion coater EIKO IB-03. All specimens were examined under the Hitachi S-450 scanning electron microscope at 15 KV (electron accelerating voltage). The method was based on Hayat, 1970 (1) and Meek, 1970 (2).

Result & Discussion

We found that the sizes of the fibers, which are made up of 2 strands of fibroin bound together with and covered by sericin as already estabished, were possible to compare at a high degree of confidence. All figures were magnified to 500 times. The fibers were measured and calculated for a sound comparision to be made therefrom.

As shown in figure 1 that the outer layers of the large (A) and small (B) cocoons illustrate no significant difference of the fiber sizes. Observe the drum-stick shaped

1A



1B

Fig. 1 *Shows the fibers sizes in the outer layer of the large cocoon (A) and small (B) cocoon structures (aste-*

risks) attaching to the fibers (IB) which is believed to be the bacteria that stained the cocoon brown as we observed before we processed the cocoons. A single fiber is seen clearly as 2 strands (SS) lengthwise with a shallow groove between them. Also notice how the fibers attach to each other to maintain the supportive structure (AT). In 1B we will see a crack of the attachment (CAT). This would be a site for the breaking-lose of the fibers in the process of reeling.

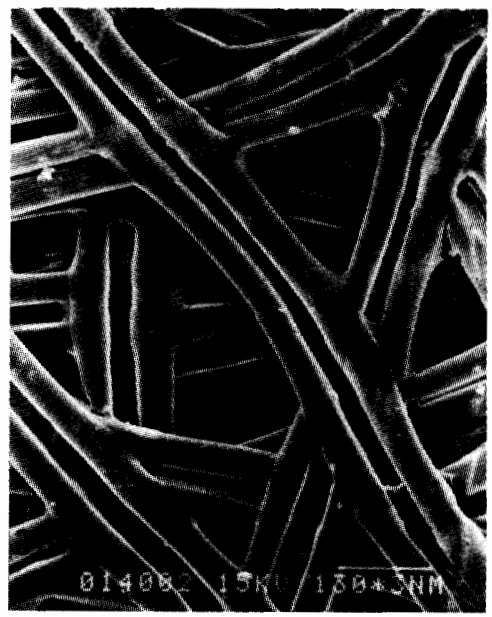
2A

2B

Fig. 2 *Reveals the fiber sizes in the middle layer of the large (A) and small (B) cocoons*

Figure 2 A is the SEM microphotograph of the middle layer of the large cocoon, and 2B of the small cocoon. Their fibers are clearly seen as smaller than that of the outer layer of the same group. Comparing the sizes of the fibers from the large and small cocoons, we could not find significant difference between the two. At this magnification of 500 times that reveals no significant difference; it, therefore, is unlikely to affect the quality of the cocoons to the degree that would be undesirable for the processing or industry to come.

3A



3B

Fig. 3 *demonstrates the fiber sizes in the inner layer of the large cocoon (A) and the small cocoon(B)*

If one looks at the fiber sizes of the large and small cocoon in the inner layer, one would tend to believe that the fibers of the small cocoon are somewhat thicker. But after a careful analysis that does not seem to be the case. The fiber sizes in the inner layer, nevertheless, are not significantly smaller than in the middle layer of the cocoon.

As postulated by Lucas in 1957 (3) the structure of the peptide chains making up the silk fibers is as follow:

Gly-Ala-Gly-Ala-Gly-(Ser-Gly-(Ala-Gly)n) 8-Gly-Ala-Ala-Gl y-Tyr. In which n is usually 2 and always has a mean value of 2 and the sequence Ala-Ala is either at the beginning or the end (1).

The structure of fibroin was subsequently postulated in 2 forms or models. The first model is in the form of a single chain in which crystalline regions alternate with amorphous regions. The second model is a multichain structure in which at least two separate chains are joined either by the hydrogen bonds or by special covalent bonds such as disulfide bonds or ester bonds involving serine residues (4). Whichever model is going to be confirmed as true, we could not present a conclusive evidence from this study. We can only demonstrate a regular sizes of the silk fibers within each layer, although those in the outer layer are larger than the rests. And the cocoon sizes do not seem to be because of the silk fiber sizes, and for that reason the cocoon sizes tell only the different length of the silk fiber. The small cocoon is made up of a single shorter fiber and weighs proportionally less than the larger cocoon which is made up of one single fiber. It seems to us that the larger cocoon has neither advantage over the small one nor vise versa. The size grading is certainly justified in cases of fruits or eggs where the whole mass is used in the subsequent processing. In determining the price of the silk worm cocoons, we actually weigh the silk fibers that should be fairest to all concerned. And it must be stated in closing that there is no material between the fibers making up the cocoon.

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