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# Adoption of Cold Reeling as a tool of Selection in the Breeding of "Nistari": A Multivoltine Breed of Mulberry Silkworm (*Bombyx. mori*) in West Bengal, India

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Mulberry silkworm (*B. mori*) is an economic insect reared for silk. Economic traits of silkworm are highly influenced and deteriorated by fluctuating environmental conditions arising due to global warming. Artificial selection has always played a crucial role in silkworm domestication to improve economic traits. Filament length is important from the perspective of mulberry sericulture. Nistari lines ("Marked" and "Plain") which is a ruling multivoltine breed in West Bengal, India, have short filament length and economic traits are observed to be deteriorated. Selection of parents with high

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filament length is hard as the pupa corresponding with higher filament length is sacrificed during the process of reeling in hot temperature in conventional reeling. "Cold reeling is an alternative reeling, devoid of hot temperature, thereby enabling the rescue of pupae for further metamorphosis. Cold reeling can be adopted to select parents with higher filament lengths in mulberry silkworm for successive generations. The present study attempted to increase the filament length of "Marked" and "Plain" lines of Nistari, by selecting parents with high filament length for six consecutive generations by implementing cold reeling. A gradual increase in filament length was observed, in both the Nistari lines over six generations. This approach could enhance the economic value of Nistari silkworms and may be applicable to other breeds, potentially boosting silk production in West Bengal."

*Keywords: Filament length; bombyx mori; selection; cold reeling; nistari lines.*

## 1. INTRODUCTION

The domestic silkworm is an economically important insect [1]. Mulberry silkworms are reared for silk. More than 95% of natural silk, all over the world is obtained from this insect [2]. Fluctuating climatic conditions affect the success of cocoon production [3] and decline in economic traits. Economic traits are also affected by inbreeding depression and rearing conditions. Artificial selection plays an important role in moulding economic traits of choice for desired characters. During the process of domestication, silkworms have evolved rapidly under human-preferred selection [4]. In silkworm domestication, different modes of artificial selection come to play. Conventional selection plays a pivotal role where economic traits are selected visually based on shape, size, colour, weight, larval marking etc. Marker assisted selection also plays an important role in silkworm breeding. Some of the widely employed markers are SSR markers and ISSR markers [5,6,7,8]. Filament length is an important economic character. It is the fibrous length reeled out from a single cocoon [9]. Filament length varies in different breeds of silkworms. Environmental factors affect filament length [10]. Application of selection pressure, in economic traits like cocoon weight of parental lines was shown to improve the performance of the cocoon weight of hybrids [11]. The scenario of mulberry sericulture in West Bengal, India is dominated by Crossbreeds of Nistari lines "Marked" and "Plain". Nistari is broadly classified into Nistari "Marked" and Nistari "Plain" lines based on presence of larval marking. Nistari Marked lines possess black larval making, which is devoid in the Plain lines.

Nistari lines are characterized by its high survival in drastic climatic conditions. One of the downsides of this preferred breed among many is low filament length. In this study, an attempt was made to improve the filament length of Nistari lines, by subjecting selection pressure in different generations. Cold reeling was adopted where single cocoons are reeled manually without subjection of high temperature to rescue the pupa for further metamorphosis, unlike conventional reeling. Gradual improvement in filament length was observed. Without selection pressure, gradual increase in filament length was absent, but the filament length was higher from the initial generation when selection was initiated.

## 2. MATERIALS AND METHODOLOGY

Cocoons (5-6 day aged after spinning) of Nistari "Marked" and "Plain" lines with its racial characteristics were selected based on visual observation. The cocoons were labelled individually and placed in small chambers separately. The cocoons were soaked for 10-15 minutes in 0.25% NaOH solution. The cocoons were reeled individually in eprouvette in 0.25% NaOH solution. The filament length of each cocoon was calculated. The individual live pupa after reeling was dipped in 0.1% glacial acetic acid for 1min and later washed thoroughly in tap water and dried with paper towel. The sex of the pupae was recorded and kept for further metamorphosis. 70-75 cocoons of both Marked and Plain lines were reeled as described above. 20-25 each of male and female pupae with the highest filament length were selected as parent for the next generation. The average filament length of the corresponding males and females' pupae were taken as filament length of the particular generation. Selection for six generations was conducted in a similar protocol as described.

The Selection was retracted after six generations. Cold reeling was conducted as described above, from generation 7 to generation 13 without selection. The average filament length of unselected cocoons was recorded from equal numbers of male and female cocoons in each generation.

## 3. RESULTS

The cocoons of Nistari lines Plain and Marked were cold reeled. The average filament length of equal number of males and females was taken (Tables 1 & 2). In Nistari "Marked" lines it could be observed that there is a gradual improvement of filament length from generation 1 (G1) to generation 6 (G6). There was an overall increase in filament length from 378m to 454m (Table 1). In Nistari Plain line the average filament length increases from 348 m to 361 m though a decline could be seen in generation 4 (Table 2) during the period.

**Table 1. Average filament length of selected males and females of Nistari "Marked" lines from generation 1 to generation 6, by conducting cold reeling which shows a gradual increase in filament length over the generations (G represents generation)**

| Generation | Average Filament Length (m) |
|---|---|
| G1 | 378 |
| G2 | 384 |
| G3 | 409 |
| G4 | 412 |
| G5 | 430 |
| G6 | 454 |

**Table 2. Average filament length of selected males and females of Nistari "Plain" lines from generation 1 to generation 6, by conducting cold reeling which shows a gradual increase in filament length over the generations (G represents generation)**

| Generation | Average Filament Length (m) |
|---|---|
| G1 | 348 |
| G2 | 379 |
| G3 | 395 |
| G4 | 379 |
| G5 | 452 |
| G6 | 461 |

**Table 3. Average filament length of males and females of Nistari "Marked" line from generation 7 to generation 13, by conducting cold reeling without subjecting to selection (G represents generation)**

| Generation | Average Filament Length (m) |
|---|---|
| G7 | 452 |
| G8 | 427 |
| G9 | 436 |
| G10 | 426 |
| G11 | 467 |
| G12 | 448 |
| G13 | 431 |

**Table 4. Average filament length of males and females of Nistari "Plain" line from generation 7 to generation 13, by conducting cold reeling without subjecting to selection (G represents generation)**

| Generation | Average Filament Length (m) |
|---|---|
| G7 | 420 |
| G8 | 417 |
| G9 | 447 |
| G10 | 422 |
| G11 | 467 |
| G12 | 442 |
| G13 | 437 |

Filament length was also estimated without selection, by cold reeling in both the lines of Nistari (Table 3 & table 4). It can be observed that in absence of selection there is no gradual increase in the length of the filament length, but changes over generations. But it is also prominent from the study that even in absence of selection, an improvement of filament length can be observed from the initial generation of G1in both the lines of Nistari.

## 4. DISCUSSION

Artificial selection has always played a key role in shaping traits of choice. In domesticated animal and plant breeding, a breeder can change genetic characteristics of a population in two ways. The first way is by selection of individuals as parents and the second way is controlling of mating of parents which includes inbreeding and outbreeding [12], which illustrates the importance of selection in breeding. Studies have shown the adoption of directional selection to produce insect strains with higher tolerance to extreme environmental conditions, such as low humidity, for extended periods [13]. Economic insects are domesticated for economic traits and selection plays a crucial role in improving the economic traits. Evidence on an increase in cocoon weight by selection was observed in silkworm [14]. Gradual improvement of economic traits is pivotal for silkworm rearing to increase the profit of the sericulture industry [15].

Filament length is an important economic trait in silkworms. High filament length is an important characteristic of an elite silkworm breed. Among the mulberry silkworm breeds bivoltine breeds have higher filament length than multivoltine breeds [16]. Filament length like other traits can be improved by selection. In a silkworm breed, a parent with a higher filament length from a population can be selected over generations to yield progeny with higher filament length.

Conventional selection for high filament length is hard, unlike other silkworm economic traits. Estimation of filament length requires reeling of the cocoons. The process of reeling in mulberry silkworm (*B. mori*) involves cooking up of the cocoons in hot water [17]. During the process of reeling the metamorphosing pupae are killed. This makes it hard to select better parents with longer filament lengths. “Cold reeling” is an alternative reeling where the subjection of heat during the process of reeling is avoided. Here the cocoons are reeled individually, in alkaline solution, thereby rescuing the pupae and allowing it for further metamorphosis to silkworm moths and oviposition. Cold reeling is labour intensive and time-consuming compared to the conventional reeling. The process of cold reeling facilitates conventional artificial selection for filament length. It enables the selection of the pupa corresponding to high filament length as a parent for next generation.

It was observed from the present study that filament length could be improved by selection. A gradual increase in filament length could be observed in both Nistari “Marked” and “Plain” lines with continuous subjection of selection pressure from generation 1 to generation 6 (Tables 1&2). Without the subjection of selection pressure gradual improvement was not observed (G7 to G13), but the filament length in both the Nistari lines, Marked and Plain were higher than the filament length of the initial generation (G1) (Tables 3 & 4). Hence, the experiment on selection implies that cold reeling could be employed as a selection tool to improve filament length in mulberry silkworm “Nistari”.

Nistari, plays a leading role in mulberry sericulture of West Bengal, India. The Farmers’ field are dominated by Nistari and its cross breeds. Multivoltine x Bivoltine hybrids (Nistari x SK6.7) and Multivoltine x Multivoltine (Nistari x M12W), crosses are widely preferred depending upon the seasons. Improving the filament length of Nistari can be one of the factors to improve production of raw silk in West Bengal.

The present study was conducted on “Nistari” a multivoltine breed, but similar attempts can be conducted to other parental silkworm breeds. The commercial sericulture sector depends on hybrids, viz., double hybrids, bivoltine hybrids, multivoltine x bivoltine hybrids, multivoltine x multivoltine hybrids depending upon the climatic conditions. Enhancing the filament length of the parental breeds is expected to improve the filament length of the hybrids too.

Economic insects like mulberry silkworms due to continuous inbreeding and domestication are usually associated with deterioration of economic traits and fluctuating environmental conditions. Economic traits suffer from inbreeding depression [18]. Filament length also suffers the same fate of inbreeding depression and decline due to harsh and unpredictable environmental factors like temperature and humidity. Directional selection can be used to restore those characters lost during the mass rearing process in insects [13]. The process of cold reeling can also be employed to rejuvenate filament length suffering the above factors. In the present study, an attempt has been made to improve filament length by selection, adopting cold reeling in the multivoltine breed "Nistari". The observations from the study, present an increase in filament length which implies the application of cold reeling as a "selection tool" in Nistari and the possibility of adopting it in other breeds of mulberry silkworm rearing.

## 5. CONCLUSION

Selection plays a significant role in silkworm breeding. In the present study filament length of "Nistari" lines was improved by adopting cold reeling. Nistari being the backbone of mulberry sericulture in West Bengal, improving the filament length is expected to increase the raw silk production. Similar approach can be also be adopted for other silkworm breeds.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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