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Mendelian inheritance of egg color in *Bombyx mori*: monohybrid segregation analysis of multivoltine and bivoltine crosses

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Abstract
The inheritance of egg color in the silkworm (*Bombyx mori* L.) is an important characteristic in breeding programs. It offers insights into Mendelian segregation patterns and hybrid performance. This study examined the monohybrid inheritance of egg color through a cross between multivoltine females (Pure Mysore) and bivoltine males (CSR2). F1 progenies were raised in controlled conditions. Economic factors such as fecundity, hatching percentage, larval duration, cocoon weight, shell weight, and filament length, etc were measured. F2 offspring displayed clear segregation of egg color, showing a 3.3:1 ratio of bivoltine-like (brown/violet) to multivoltine-like (yellow/light yellow) egg colors. This illustrates Mendelian inheritance of the trait. Fecundity and larval longevity were higher in F2. However, statistical analysis showed that F1 progeny performed better than F2 progeny regarding cocoon and post-cocoon parameters. These results affect egg quality in sericulture and how we approach hybridization strategies.

Keywords: Hybridization; egg color; monohybrid inheritance; *Bombyx mori*.

Introduction
The domesticated silkworm (*Bombyx mori*) is an important insect in the global silk industry. Its genetics have been studied extensively for over a hundred years. And its unique genetically controlled biological phenomenon “Voltinism” (number of generations per year) is classified into univoltine, bivoltine and multivoltine. (Saha *et al.*, 2013) [7] Egg colour is an important characteristic that is controlled by Mendelian inheritance and linked to voltinism. The univoltine and bivoltine breeds produce silk of superior quality, but they are less hardy. While the multivoltine breeds are more tolerant of environmental conditions, they produce silk of a relatively inferior quality ((Li *et al.*, 2005) [4].

Early century, Tazima (1982) [8] conducted interbreed crosses between univoltine and bivoltine races. This work marked the start of research into silkworm genetics. Thomson (2000) [9] and other researchers expanded Mendelian ideas to understand how different silkworm traits, including egg color, are passed down. Gamo (1983) [3] highlighted biochemical genetics as a strong tool for improving races. Meanwhile, research by Banno *et al.*, 2010 [1] showed how important quantitative genetics is in silkworm breeding. In order to attain the best possible balance between silk quality and environmental adaptability, breeding programs have relied heavily on the hybridization of multivoltine and bivoltine strains (Neshagaran Hemmatabadi *et al.*, 2016a and Thomson, 2000) [5, 9]. The most notable success stories in silkworm breeding came from Japan, China, and Soviet Russia. There, the systematic use of quantitative genetics changed hybrid development. These efforts led to measurable improvements in important commercial traits (Neshagaran Hemmatabadi *et al.*, 2016b). As a result, China and Japan became the world leaders in silk production, followed by the Soviet Union, Korea, and India. Notably, improvements in nutritional efficiency, especially in the quality of mulberry leaves, boosted the field performance of genetically altered silkworm races (Cappellozza *et al.*, 2023) [2].

This study looks at the monohybrid ratio for egg color segregation in the F2 generation from a cross between multivoltine (Pure Mysore) females and bivoltine (CSR2) males. Focusing on the economic traits in the F1 and F2 generations to measure hybrid performance and inheritance patterns.

Materials and Methods

Rearing and cross setup

Rearing and cross setup were conducted at the sericulture farm of Sri T. Shivakumar (Attahalli, Bannur, Karnataka) and subsequently in the department of sericulture, University of Mysore. 75 disease free layings (DFLs) of pure Mysore (Multivoltine) and CSR2 (Bivoltine) males were selected as a parental stock for crossing. Males were obtained from NSSO, Central Silk Board, Mysore, during the period of March and April.

Experimental design

Rearing of multivoltine × bivoltine hybrid crop, total larval duration, and weight of fifth instar larvae (on the fifth day prior to spinning have been carefully observed and recorded. A total of 100 cocoons were randomly selected from the rearer's house, from these 20 were assessed for their shape, colour, weight, shell weight, shell percentage, filament length and 80 were evenly arranged on a ventilated wooden tray, and after moth emergence, disease-free laying (DFLs) were prepared by inbreeding the moths.

After evaluating mother moths for pebrine illness, 20 disease-free laying's were split into two groups. Since F1 layings usually produce diapause eggs, one group of ten DFLs was treated with acid and incubated at room temperature using standard methods. The other group was set aside to study hibernation. Two disease-free layings were brushed and raised separately to examine eleven economic features, which are explained further below.

Economic characteristics evaluated were

Fecundity: The total number of eggs laid by a female.

$$\text{Hatching percentage (\%)} = \frac{\text{Number of hatched larvae}}{\text{Total eggs}} \times 100$$

- **Weight of V instar larva (g):** Average weight of randomly collected larvae.
- **Larval duration (h):** The time it taken by a larva to hatch and spin its cocoon.
- **Cocoon weight (g):** The average weight of 50 cocoons, with 25 male and 25 female.
- **Shell weight (g):** The average shell weight from the same sample.

$$\text{Shell percentage (\%)} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \times 100$$

Shell weight=cocoon weight-pupal weight

Filament length (m): Silk reeled from ten cocoons.

$$\text{Denier} = \frac{\text{Weight of silk}}{\text{Total eggs}} \times 9000$$

(Where 9000 is the typical fiber length in meters, given in gram units). (Sado and Oishi, 1966) [6].

Statistical analysis

The data were analysed using Genstat 9th Version (2008), A chi-square test was used to confirm the observed 3:1 ratio in egg colour segregation.

Results

- **F1 Rearing Performance**

The rearing performance of the PM × CSR2 crossbreed is summarized in Table 1. The 75 DFLs had an average hatching percentage of 97.04±1.64% and a fecundity of 425±13.26. The weight of the V instar larva was 2.3±0.10 g, and it lived for 576±3.00 hours. During the cocoon assessment, found a single cocoon weighing 1.203±0.04 g, a shell weighing 0.209±0.03 g, and a shell percentage of 17±0.41%. Among the post-cocoon parameters, pupal weight was 0.992±0.009 g, the filament length was 438±6.33 m, and the denier of 1.99±0.06.

- **F2 Rearing performance**

The F2 generation represented in Table 2 produced 542±14.84 eggs, with a hatching rate of 96.00±2.64%. The V instar larval weight was 2.1±0.12 g, and the total larval length was 648±3.00 hours. The measured cocoon traits were: single cocoon weight 1.002±0.06 g, shell weight 0.170±0.01 g, shell percentage 16.96±0.41%, pupal weight 0.832±0.009 g, filament length 410±5.00 m, and denier 1.99±0.05. A comparison with F1 data shows that F1 outperforms all parameters except for fecundity and larval length. The differentiation of F1 and F2 rearing performance is represented in (Fig. 1-12).

- **Egg colour**

The data on the different egg colours produced from grainage operations is shown in Table 3 and Figures 11-14. Two sets of eggs were identified. All the bivoltine egg colours appeared in Group 1, while all the multivoltine egg colours were found in Group 2. The colours 910 and 170 are brownish and violet, respectively, in the bivoltine group. There were 201 light yellow and 119 yellow multivoltine eggs among the various colours. Additionally, the estimated total number of eggs in groups 1 and 2 is 1,080 and 320, respectively. The computed ratio for the two groups is 3.3:1.

Table 1: Rearing performance of F_1 cross breed (In the rearer's house)

Quantitative traits cross breed	Fecundity (No)	Hatching (%)	Weight of single V instar larva (g)	Larval duration in hr	Single cocoon Weight (g)	Single shell Weight (g)	Shell percentage	Single pupal weight (g)	Filament length (m)	Denier (d)
F_2	542±14.84	96.00±2.64	2.1±0.12	648±3.0	1.002±0.06	0.170±0.01	16.96±0.41	0.83±0.009	410±5.00	1.99±0.06

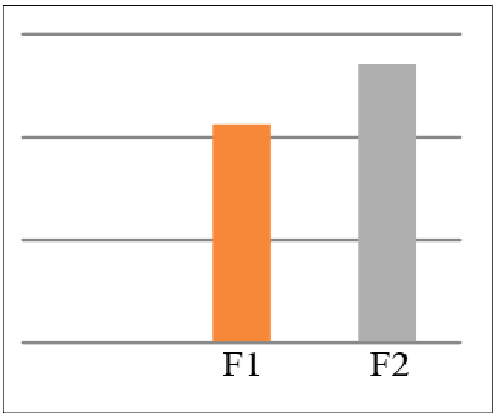

Fig 1: Fecundity

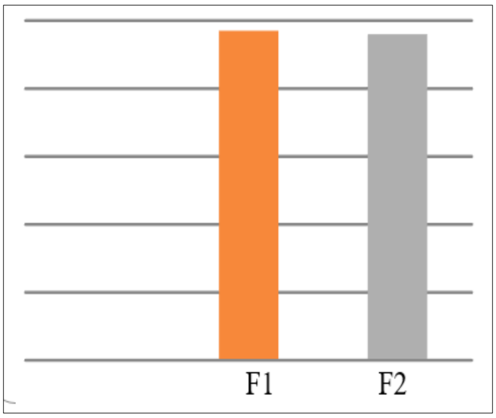

Fig 2: Hatching percentage

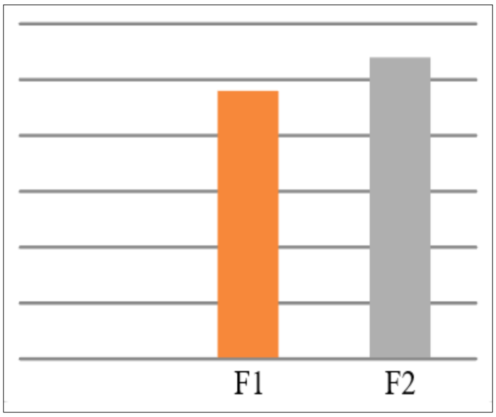

Fig 3: Larval duration

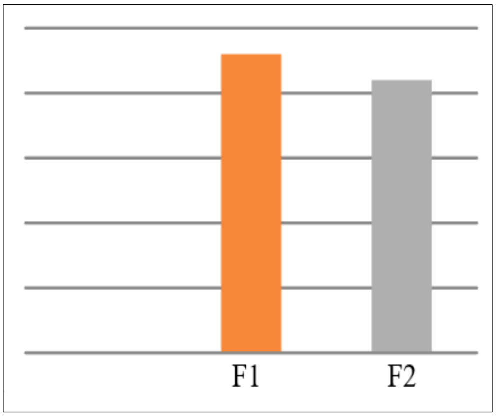

Fig 4: Larval weight

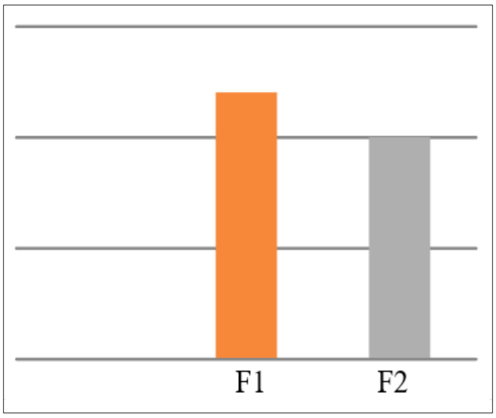

Fig 5: Cocoon weight

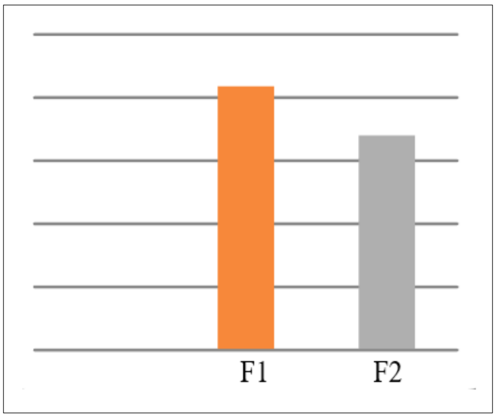

Fig 6: shell weight

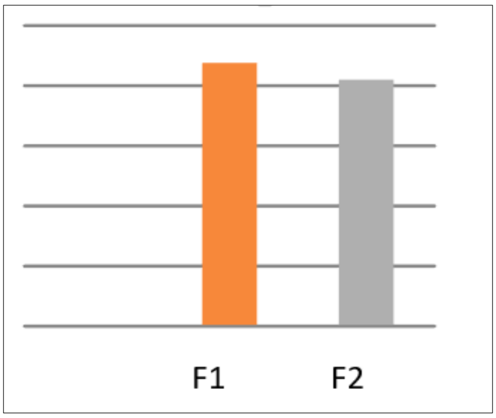

Fig 7: Filament length

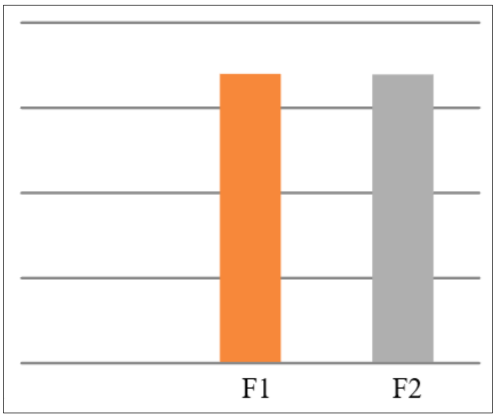

Fig 8: Shell percentage

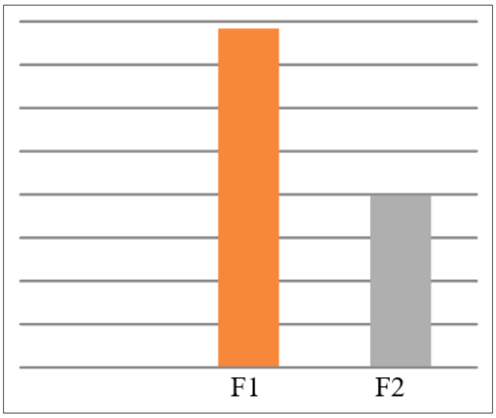

Fig 9: Denier

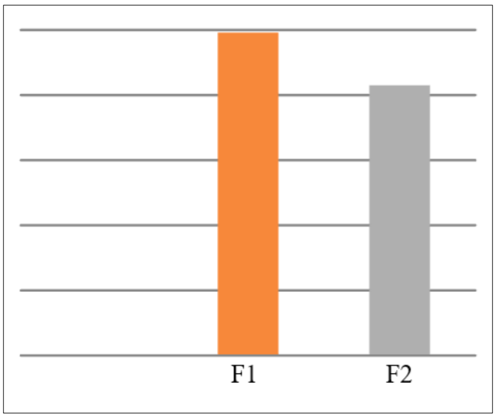

Fig 10: Pupal weight

Fig 1-10: Histrograms comparing key traits between F1 and F2 generations

Table 3: Segregation of egg color in the F2 generation from a cross between Pure Mysore (♀) and CSR2 (♂) strains of *Bombyx mori*.

Sl.no	Colour	Number of eggs
EGG Colour of Bivoltine		
1.	Brownish	910
2.	Violet	170
	Total	1080
Egg colour of Multivoltine		
3.	Light yellow	201
4.	Yellow	119
	Total	320
	Ratio (BV:MV)	1080:320(3.3:1)

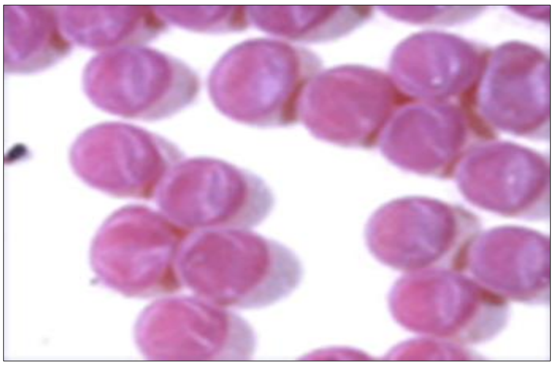

Fig 11: Brownish

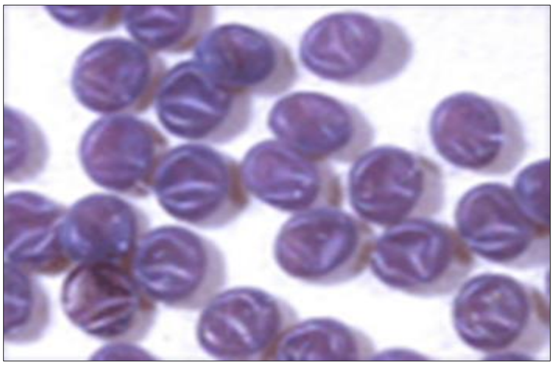

Fig 12: Violet

Fig 13: Light yellow

Fig 14: Yellow

Fig 11-14: Different Egg colours observed during the experiment (Segregation of egg colour at F_2)

Discussion

The current study's findings show that the color traits of cocoons and eggs in *Bombyx mori* follow a Mendelian inheritance pattern. Green cocoons, usually associated with bivoltine lines, appeared in a 3:1 ratio compared to white cocoons in the F2 generation. This indicates that the green cocoon trait was dominant. Likewise, the egg color segregation demonstrated that bivoltine traits, such as brown or violet eggs, were dominant over multivoltine traits, which include light yellow or yellow eggs. This confirms monohybrid inheritance controlled by a single dominant gene.

The better performance of F1 hybrids in key economic factors like filament length and shell percentage matches earlier studies on hybrid vigor in silkworm breeding. However, the decline seen in the F2 generation reflects a common loss of heterosis due to genetic segregation. This highlights the need for careful hybridization strategies to keep desirable bivoltine traits while taking advantage of the flexibility of multivoltine lineages. Future research using molecular markers could help us understand the genetic roots of these traits. This would enable more accurate selection in breeding programs, especially for traits like egg colour, cocoon quality, and disease resistance.

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