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Dulur Brahma
Department of Zoology,
Bodoland University,
Kokrajhar – 783370, India

Ananta Swargiary
Department of Zoology,
Bodoland University,
Kokrajhar – 783370, India

Karabi Dutta
Department of Zoology,
Gauhati University,
Guwahati – 781014, India

Correspondence:
Dulur Brahma
Department of Zoology,
Bodoland University,
Kokrajhar – 783370, India

A comparative study on morphology and rearing performance of *Samia ricini* and *Samia canningi* crossbreed with reference to different food plants

Dulur Brahma, Ananta Swargiary, Karabi Dutta

Abstract
Samia ricini is a non-mulberry, multivoltine, domestic moth, reared indoor. *S. canningi* is wild, bivoltine silkworm. Both the varieties are found in North-eastern states of India. Castor (*Ricinus communis*; Euphorbiaceae) and payam (*Evodia fraxinifolia*; Rutaceae) are the host plants of *S. ricini* and *S. canningi*, respectively. *S. ricini* is susceptible to unhygenic and poor environmental conditions. The present study was designed to produce a crossbreed between *S. ricini* and *S. canningi* and to study the differences in morphological and economic parameters between the parents and crossbreed. Experimental results have shown that the rearing and economic performance of crossbreed showed superior quality compared to parental species. However, crossbreed showed better result in payam leaves. The present study therefore suggest that the *S. ricini* x *S. canningi* crossbreed can be commercialized which may be highly productive to sericulture industry. However, further study regarding the productivity and food plants need to established.

Keywords: *Samia ricini*, *Samia canningi*, crossbreed

Introduction
Silk and sericulture has been a part of life and culture of the Indians. Today sericulture industry earns about 25 billion (2013-14) providing employment opportunity to approximately 7.85 million people in rural and semi-urban areas in India (Central Silk Board, 2015). The productivity of Sericulture mainly depends on high breeding stock of the silkworm. The hybridization is a technique to enhance the yield of silkworm and cocoon production. By crossing genetically distinct population and understanding the genetic mechanism of the silkworm, high yielding and disease tolerant races with distinct quantitative and qualitative traits can be achieved. The success in silkworm hybridization primarily depends on the selection of initial breeding materials followed by their effective utilization in different combinations to create genetic variability for selection [1]. In India, it is estimated that nearly 80% of the silk is produced by multivoltine × bivoltine hybrids where multivoltine races are used as female parent for commercial exploitation. The main reason attributed to this is that the contribution of bivoltine by virtue of its maternal inheritance may result in regular crop loss [2]. Suitable silkworm hybrids play a vital role in increasing the productivity and quality of silk which are important for sustainable sericulture industry [3].
Eri silkworm, *Samia ricini* is a domesticated multivoltine sericigenous insect largely reared by the farmers of North Eastern states of India, particularly Assam because of its easy rearing and availability of food plants [4]. *S. ricini* are susceptible to a disease flacherie. The productivity and quality of cocoon, however, depends upon quality food supply, favorable environmental conditions and utmost hygienic condition [5]. Scientists around the world are looking for silkworms which can withstand all the environmental conditions, resistant to diseases and easily cultivable and high productivity as well. *Samia canningi* is a wild variety of Eri silkworm with bivoltine nature that is susceptible to a bacterial disease called gracherie. In this connection, the possibility of enhancing the silkworm and cocoon production was experimentally attempted through crossing different variety of silkworms with selected traits. The present work was therefore designed to study the variation in morphology and productivity of *S. ricini* x *S. canningi* crossbreed and also to study variation in productivity as well as its adaptability to different diseases in relation to two food plants viz., castor (*Ricinus communi*; Family: Euphorbiaceae) and payam (*Evodia fraxinifolia* Hook; Family: Rutaceae).

2. Materials and Methods

2.1. *Collection of sample and selection of food plants*

Healthy, disease free *Samia ricini* were collected at the cocoon stage from the local rearer of Kokrajhar district. *Samia canningi* were collected at cocoon stage from different places of Assam. Identification of the sample moth was done by the experts of Central Silk Board, Guwahati. Castor (*Ricinus communis*, Family: Euphorbiaceae) and payam (*Evodia fraxinifolia* Hook, Fam: Rutaceae) were taken as the food plants for the experiment.

2.2. *Preparation and isolation of pure line*

Parental seed cocoons of both the *S. ricini* and *S. canningi* were collected and laying of the races were done by adopting the method described by Tazima [6] and Rao and Mariswamy [7]. Briefly, Yellow Plain (YP) colored larvae with brick red cocoon (brc) of multivoltine *S. ricini* were selected and maintained up to eight generations. Similarly, bivoltine *S. canningi* with Greenish Blue Plain (GBP) larval color and dull brown cocoon (dbc) were selected for pure line maintenance.

2.3. *Hybridization and rearing*

After successful pure line maintenance of both the races, crossing was done by taking multivoltine female *S. ricini* and male bivoltine wild race *S. canningi* under controlled conditions of temperature and humidity following Doddaswamy *et al.* [2]. Rearing of silkworm was done following standard protocol of Grekov *et al.* [8] with little modification. Eggs were disinfected with 2% formalin and washed with tap water. *S. ricini* was fed on castor leaves while *S. canningi* was fed on payam leaves at outdoor condition. The *S. ricini* x *S. canningi* cross-breeds (C-breed) were reared indoor and fed on both castor and payam leaves throughout the experimental period (2008-2011).

2.4. *Morphological study*

***Eggs*:** Morphological variations of the eggs of *S. ricini*, *S. canningi* and C-breed were studied on the basis of its size, shape, shell color, weight and average fecundity following standard protocol.

***Larva*:** Morphological characters of the larvae were studied by observing its larval color, size (length and breadth) and body weight following standard protocol.

***Cocoon*:** The cocoon characters such as shape, size length and breadth (LxB), cocoon weight, shell weight, peduncle length and silk ratio (silk weight/cocoon weight) were taken as the parameters for comparing the cocoons of *S. ricini*, *S. canningi* and the crossbreed.

***Pupa*:** The pupal characters of all the three silkworms were studied on the basis of its size, shape and pupa weight following standard protocol.

***Moth*:** The morphological differences in the adult moths of *S. ricini*, *S. canningi* and crossbreed were studied on the basis of LxB of antennae, wing span, pattern and its coloration; and pattern of tufts and tergum in abdomen.

2.5. *Economic Characters*

The quantitative characters such as fecundity, hatching %, larval survivability %, pupation %, shell ratio %, moth emergence and effective rate of rearing (ERR) (%) were studied following the method as described by Tzenov [9].

2.6. *Statistical Analysis*

Data collected from the experimental observations were statistically analyzed using the statistical software SPSS programme and data were presented as means ± SE (standard error). Test of significance were made using student's t-test at $p < 0.05$ level. In all the cases experimental work was carried out for three replicates or three generations.

3. Results and Discussion

During maintenance of the pure line, the selected wild bivoltine GBP *S. canningi* showed no segregation whereas YP multivoltine *S. ricini* showed segregation of spotted yellow larvae for two generations i.e. in *F1* and *F2*-generation, but afterwards up to *F8*-generations it had not shown any further segregation. The crossing between the *S. ricini* and *S. canningi*, resulted in C-hybrid silkworm with GBP larval color with light reddish brown cocoon (rbc) were found in the *F1*-generation. No morphological changes were observed in the filial generations when the crossings were allowed between *F1* hybrid worms. All the offspring of *F1* x *F1* cross showed GBP larval color with rbc color and all the offspring were maintained indoor to study the morphological characters (Figure 1).

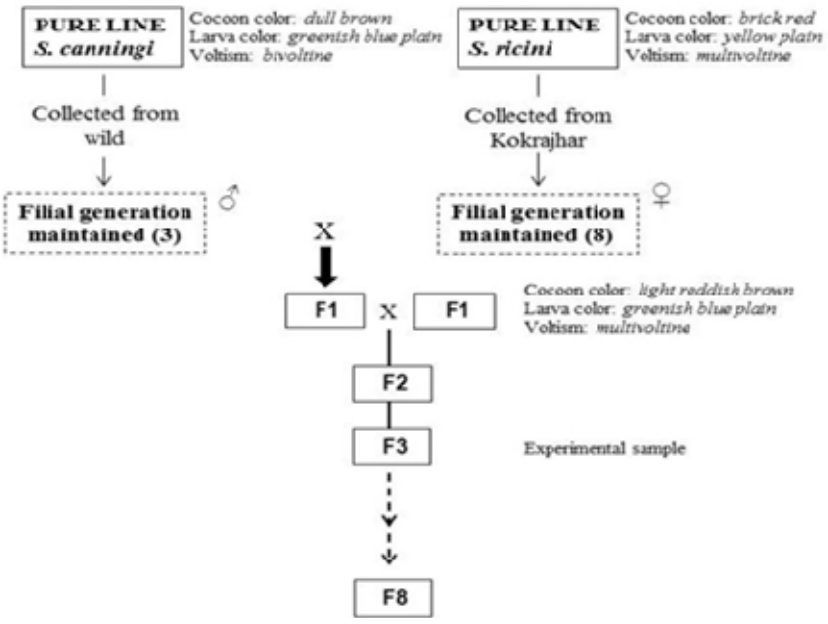

Fig 1: Maintenance of pure lines of *S. ricini* and *S. canningi* and hybrid.

Offspring of both the *S. ricini* and the C-breed silkworm were divided into two groups and each group were fed on castor and payam leaves separately to study the differences in pre-cocoon and cocoon parameters, if any. It has been reported that the variation of cocoon characters depends mostly on the type of food plants used for feeding [10]. It has been observed from the Table 1 that almost all the pre-cocoon and cocoon parameters showed significant differences between the local *S. ricini* and the C-breed. C-breed showed slightly better result in overall parameters under study. There was a significant fecundity difference between *S. ricini* and C-breed when the silkworms were fed on both castor and payam leaves (Table 1). Similarly, high degree of significance has also been observed in ERR%. However, there was no such significant difference between the pupation of *S. ricini* and C-breed (0.66) when they were fed on castor leaves. In terms of food preference, *S. ricini* was found to have more tendencies towards castor while C-breed showed more inclination towards payam leaves as their food plants. In terms of cocoon parameters, such as cocoon weight, pupal weight, shell weight and shell ratio the C-breed progeny were found to have better economic parameters compared to domesticated *S. ricini* in both the experimental plants. However, the cocoon productivity of *S. ricini* and C-breed was found to be higher in castor plant compared to payam (Table 1). The variability and productivity of cocoon have been found to be solely dependent of the food plants, good hygienic conditions and favorable environmental conditions [11].

The ERR% of *S. ricini* fed on castor and payam were found to be 83.87% and 81.74%, respectively. Similarly, variation in ERR% (65.0-79.5%) were observed in *S. ricini* when fed on different host plants, such as castor, kesseru, tapioca and barkesseru [12]. However, variations in ERR% (58-93.33%) have also been reported within the same species during different experimental seasons [13]. The leaves of payam have also been shown as potential food plant for *S. ricini* in terms of ERR% and SR%, respectively by different researchers [13, 14]. Several studies have reported that the foliage constituents have a positive correlation with the cocoon parameters such as weight, shell weight, etc. [15, 16]. The variation in ERR%, therefore, may be due to the presence of different nutritional content such as protein, calcium and phosphorus contents and fiber content etc. on the leaves of food plant during different seasons.

Table 1: Rearing performance of *S. ricini* and crossbreed on different food plants (pre-cocoon and cocoon parameters).

Parameter	Species	Castor (A)		Payam (B)		Interaction
		Mean±SE	t-value	Mean±SE	t-value	t-value (A×B)
Hatching %	*S. ricini*	94.57±0.12	4.59**	93.76±0.10	-9.56**	6.07**
	C-breed	93.88±0.10		95.12±0.10		-10.81**
Larval Survivability %	*S. ricini*	85.12±0.14	-10.37**	83.39±0.15	-23.09**	10.30**
	C-breed	86.73±0.08		87.10±0.07		-3.98**
Pupation %	*S. ricini*	99.64±0.04	0.66	99.49±0.05	-2.25*	2.22*
	C-breed	99.60±0.04		99.64±0.04		-0.62
Emergence %	*S. ricini*	99.08±0.05	-1.89*	98.87±0.06	-6.33**	3.02**
	C-breed	99.20±0.04		99.33±0.04		-2.03*
Fecundity (nos.)	*S. ricini*	446.20±3.44	-16.99**	425.70±3.40	-21.31**	4.88**
	C-breed	532.07±3.71		533.06±3.72		-0.21
ERR%	*S. ricini*	83.87±0.14	-10.21**	81.74±0.14	-29.45**	13.48**
	C-breed	85.44±0.06		86.15±0.62		-8.62**
Cocoon weight (g)	*S. ricini*	2.81±0.19	-2.53*	2.71±0.18	-6.52**	6.09
	C-breed	2.85±0.19		2.80±0.20		3.91**
Pupal weight (g)	*S. ricini*	2.37±0.16	-2.76**	2.31±0.17	-3.66**	4.18
	C-breed	2.41±0.17		2.36±0.19		3.78**
Shell weight (g)	*S. ricini*	0.41±0.06	-0.20	0.37±0.05	-9.15**	8.80
	C-breed	0.41±0.05		0.41±0.05		0.57
Shell ratio %	*S. ricini*	14.65±1.57	1.08	13.77±1.83	-6.19*	6.29
	C-breed	14.52±1.50		14.74±1.93		-1.47

Data are mean ± SE, NS=Non-Significant; *significant level at p<0.05; **significant level at p<0.01 (highly significant): Note: C-breed: Crossbreed of *S. ricini* and *S. canningi.*

During the study, small variation in morphological characters such as color, size and weight in different stages of life cycle from egg to adult with specific adaptation and voltinism were observed. The *S. ricini* x *S. canningi* crossbreed were found to be multivoltine in nature giving more than two generations which went up to six generations a year. Many genetic experiments have established the fact that the voltinism is a maternal character [17, 18]. However, many of the characters of C-breed under study were found to be coming from both the parents and therefore showed mix combinations of characters in the C-breed silkworm.

Table 2: Comparative morphological characteristics of *S. ricini*, *S. canningi* and crossbreed.

Stages	Characters	*S. ricini*	*S. canningi*	C-breed
	Voltinism	Multivoltine	Bivoltine	Multivoltine
Eggs	Color	white	white	white
Larva	Body Color	yellow plain	greenish blue plain	greenish blue plain
	White waxy powder on body	mild	high	mild
Cocoon	Color	brick red	dull brown	light reddish brown
	Peduncle	absent	present (50-80 mm)	absent
Pupa	Color	copper brown	copper brown	copper brown
Moth	Abdominal Tergum	suffused with white scales	ground color with broad white belt on 1st abdominal segment	suffused with white scales
	Abdominal Tufts	flattened	white weak tufts in centers of segments, with larger white tufts on either sides	white weak tufts in centers of segments, with larger white tufts on either sides
	Antennae (Male)	broad	slender and elongated	slender and elongated
	Size (L × B)	13 x 4 mm	11× 3 mm	15 × 3 mm
	Antennae (Female)	slender and elongated	broad	broad
	Size (L × B)	13 × 3 mm	11 × 4 mm	15 × 4 mm

Eggs: The egg color of all the silkworm viz., *S. ricini*, *S. canningi* and the C-breed were all white (Figure 2). The egg size (LxB) of the C-breed was found to be bigger (1.9 ± 0.02 mm) compared to its parent *S. ricini* and *S. canningi* (Table 2). However, no significant difference was found in among the three grouped samples.

Larva: The larvae of *S. ricini* x *S. canningi* C-breed showed GBP larval color similar to its parental character of *S. canningi*. The size of the larva was found to be almost similar in all the three groups of silkworm. However, female larvae showed slightly bigger in size compared to its male counterpart (Table 3). It has also been observed that the C-breed silkworms did not suffer from any kind of diseases during the entire experimental period.

Cocoon: The cocoon color of *S. ricini*, *S. canningi* and C-breed were found to be brick red, dull brown and light reddish brown, respectively (Figure 1g, h and i). The size of the C-breed cocoon was found to be almost in the middle range between *S. ricini* and *S. canningi* with cocoon size 60 ± 0.89 x 45 ± 1.60 mm and 70 ± 1.75 x 50 ± 0.89 mm for male and female, respectively (Table 3).

Pupa: The color of the pupa was copper brown in all three grouped samples. However, slight variation in the size (L × B) of the pupa was observed among the three groups. Pupal size of C-breed silkworm was found to be bigger (26 ± 2.07 mm and 15 ± 0.52 mm in male and 33 ± 1.83 mm and 18 ± 0.82 mm in female) compared to its parental race *S. ricini* and *S. canningi*.

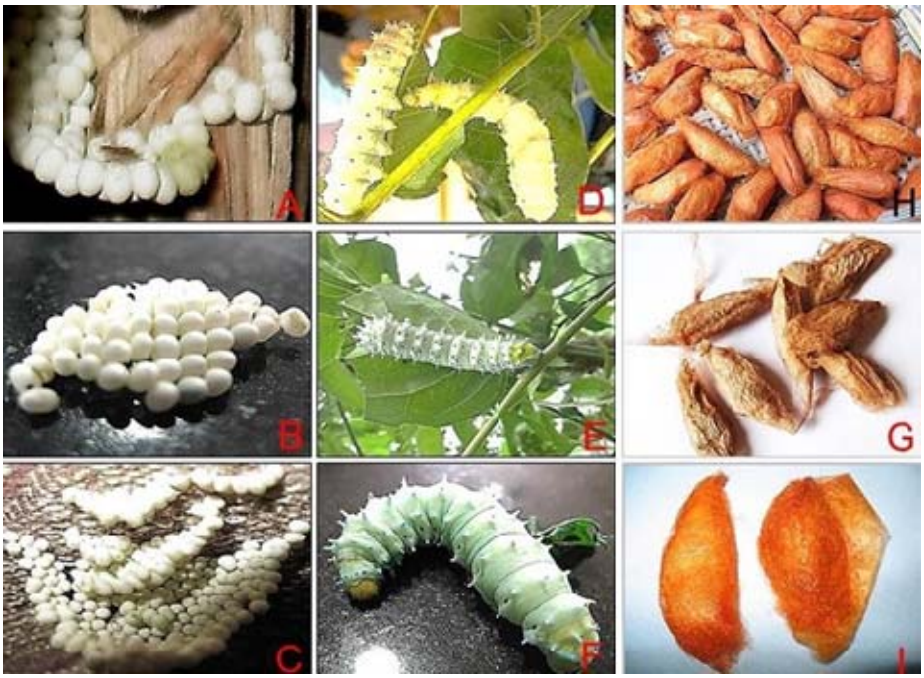

Fig 2: Photographs of eggs, larvae and cocoon of *S. ricini* (A, D and H), *S. canningi* (B, E and G) and cross breed (C, F and I).

Moth

Antennae: The antenna was prominent and serrate located at anterior portion of the head. The C-breed silkworms were found to possess similar type of antennae as *S. canningi* with slender and elongated structure (Figure 4). The length of the antennae was found to be longest (15 mm) in C-breeds silk moth (Table 3).

Table 3: Difference in the Morphometry (size in mm).

Variety	Eggs	Larva		Cocoon		Pupa		Wing Span	
		Male	Female	Male	Female	Male	Female	Male	Female
	L x B	L x B	L x B	L x B	L x B	L x B	L x B		
S. ricini (A)	1.7±0.02 x 1.1±0.06	85±1.41 x 18±0.82	86±1.03 x 18±0.52	74±1.72 x 50±1.94	82±1.17 x 56±1.84	24±0.82 x 14±0.82	30±1.47 x 17±0.82	115±2.23	120±2.25
S. canningi (B)	1.7±0.02 x 1.1±0.08	69±1.47 x 17±0.82	71±1.63 X 18±0.75	32±2.07 x 31±1.41	36±1.55 x 34±1.03	23±1.17 x 14±1.17	26±0.82 x 16±0.82	117±1.60	155±1.67
C-breed (C)	1.9±0.02 x 1.2±0.08	95±1.41 x 18±0.75	96±0.82 x 19±0.82	60±0.89 x 45±1.60	70±1.75 x 50±0.89	26±2.07 x 15±0.52	33±1.84 x 18±0.82	158±1.97	162±2.10

Data are mean ± SE, L - length and B - breadth

Abdomen: Distinct differences were observed in the pattern of the arrangement of tufts and tergum in abdominal region. The abdominal tergum of both *S. ricini* and C-breed were similar i.e., suffused with white scales whereas the wild breed *S. canningi* had ground color scales with broad white belt scales extending to first abdominal segment (Table 3). There were differences in the pattern of arrangement of the abdominal tufts among the three groups (Figure 3). Both *S. canningi* and C-breed had white tufts in the center of the abdominal segments and large patch of white tuft on either side of the segments. In contrast, *S. ricini* had flattened and fused abdominal tufts in both male and females.

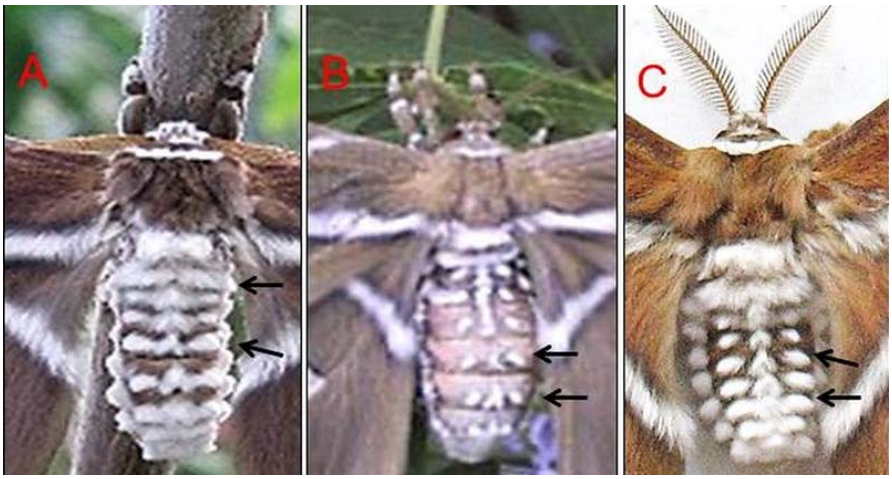

Fig 3: Photographs of the abdomen (black arrows) of *S. ricini* (A), *S. canningi* (B) and C-breed (C).

Wings: Striking variation in the colors of wings among the three grouped samples was observed (Figure 4). The wingspan was recorded highest in both length and breadth in C-breed 158 ± 1.97 and 162 ± 2.10 mm and lowest in *S. ricini* (115 ± 2.23 and 120 ± 2.25 mm) (Table 3). The dorsal view observation of the forewing and hindwing of *S. ricini* x *S. canningi* C-breed showed mixed combination of both the parental characters (Figure 4). Out of the 27 dorsal wing characters under study only one character, i.e., the size of the ocellus (7 x 4 mm) is found to differ from either of the parents (Table 4).

Table 4: Morphological analysis of wings of *S. ricini*, *S. canningi* and C-breed.

Fore Wing	***S. ricini***	***S. canningi***	**C-breed**
Basal Area	Brown	Brown	Brown
Ground Colour	Brown	Brown	Brown
Costal Line	Brown	Brown	Brown
Antemedial band	White Black	White Black	White Black
Median area	Dark brown	Brown and yellow	Dark brown
Crescent	Black white and yellow bands	Black white and yellow bands (prominent)	Black white and yellow bands (prominent)
Postmedial band	White grey white	White purple white	White purple white
Subterminal area	Dark brown	Yellow	Dark Brown
Subterminal line	Mild brown	Black	Black
Terminal area	Plain light brown	Grey	Grey
Apex	Gray patches with white zigzag line with yellowish brown outer tip	Purple patches with white zigzag line with greenish yellow outer tip	Purple patches with white zigzag line with yellowish brown outer tip
Ocellus			
Anterior outer line	Black	Black	Black
Anterior inner line	White	White	White
Anterior half	Grey	Grey	Grey
Posterior outer line	Black	Black	Black
Posterior inner line	Absent	Absent	Absent
Posterior half	Black	Black	Black
Shape of Hyaline spot	Oval	Triangular	Triangular
Area of ocellus	(6 × 4) mm	(6 × 5) mm	(7 × 5) mm
Hind wing			
Basal area	Brown	Brown and yellow	Brown
Antemedial band	White Black	White Black	White Black
Median area	Dark brown	Brown and ochre yellow	Dark brown
Crescent	Black white and yellow bands	Black white and yellow bands (prominent)	Black white and yellow bands (prominent)
Postmedial band	White grey white	White purple white	White purple white
Subterminal area	Dark brown	Yellow	Dark Brown
Fringe	Light brown with light stripes and broad	Grey with dark narrow stripes	Grey with dark narrow stripes
Ocellus	Absent	Absent	Absent

Fourteen (14) numbers of wing characters were found to be common in all the three sample groups. While, the C-breed silk moths were found to have carried 5 characters from *S. ricini* such as median area of subterminal area forewing and basal area, median area, subterminal area of hindwing. Seven dorsal wing characters of *S. canningi* were found to be carried by C-breed (Table 4).

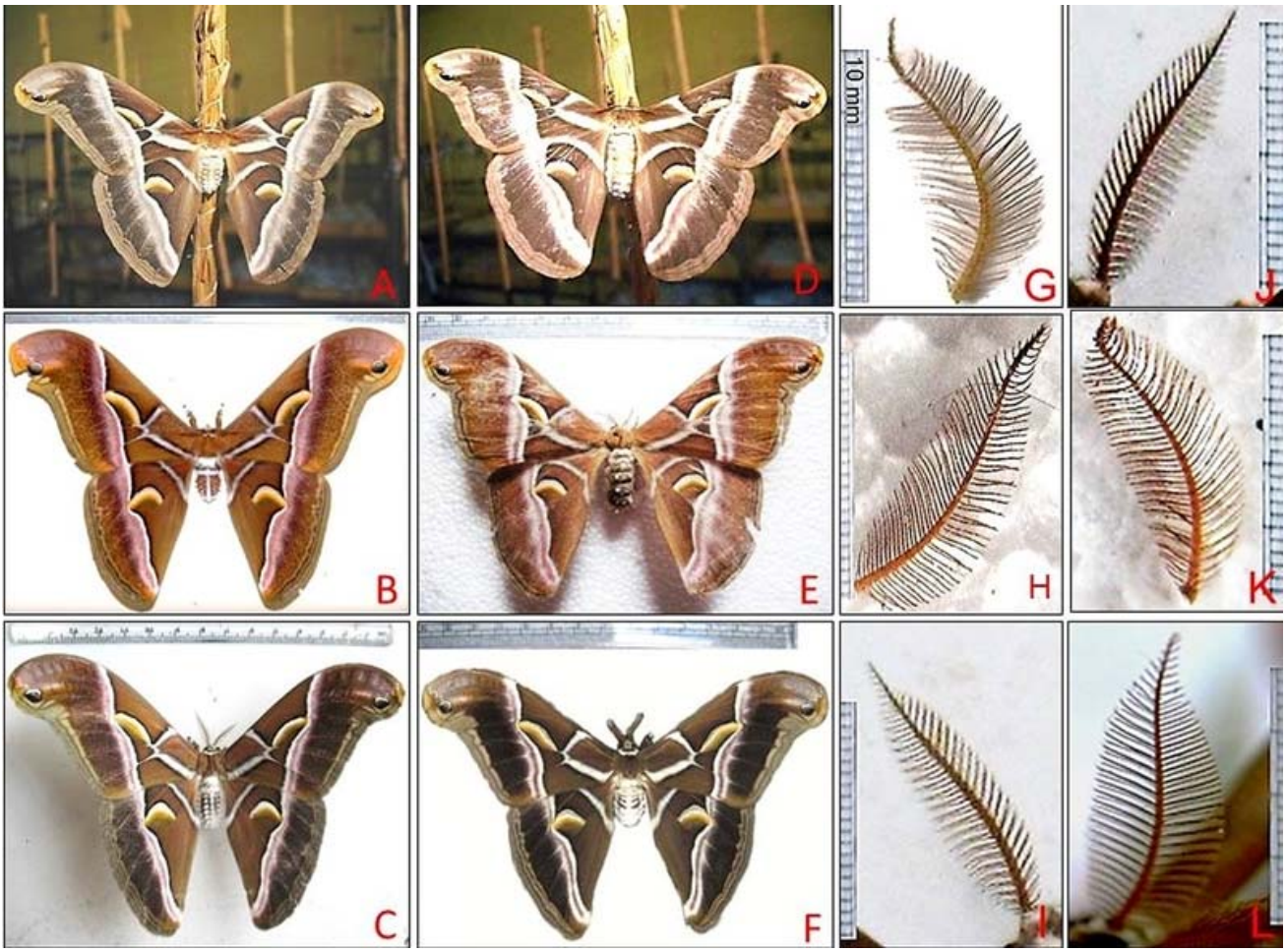

Fig 4: Dorsal view of the wings and antennae of *S. ricini*, *S. canningi* and C-breed of male (adult moth - A, B and C; antennae - G, H and I) and female (adult moth - D, E and F; antennae - J, K and L), respectively.

The resultant greenish blue plain C-breed larvae with brick red cocoon in the F1 generation of the cross between *S. ricini* × *S. canningi* may be due to the fact that greenish blue plain color of the larvae is dominant over yellow color. The allele for GBP may be dominant over the yellow plain. It has been studied that the gene for the skin colors and markings of silk worms are located on different chromosomes [18]. The morphological characters such as blue skin color is dominant over yellow, zebra marking is dominant over double-dotted and double dotted character is dominant over plain larval color in almost all the silkworms. Our investigations have showed better performance of C-breed silkworm in all the food plants in terms of cocoon parameters. Similarly, increase of larval weight, cocoon and pupal weight and SR% exhibited by Eri silkworm fed on castor leaf have been correlated with higher rate of food ingestion, food assimilation and respiratory activity [19].

4. Conclusion

Hybridization is an important technique in the realm of life sciences including sericulture industry. The hybridization experiment in the present study between local multivoltine variety *S. ricini* and wild bivoltine *S. canningi* showed better economic parameters almost all aspect of experimental parameters. Both the *S. ricini* and C-breed showed good cocoon productivity when reared on castor and payam leaves. Better survivability and resistance to diseases during the entire experimental period can also be seen as a good indication for silk industry. The results of the present study, therefore, throw considerable light on the potentiality of the *S. ricini* x *S. canningi* crossbreed in increasing the cocoon productivity and also suggest the possibility of introducing new host plant payam. However, detailed molecular level study need to be carried out to establish the relationship between the silkworm productivity and the food plants.

5. Acknowledgment

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Conflict of Interest:

Authors declare no conflict of interest.

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