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SYNTHESIS OF PROMISING BIVOLTINE BREED UP_1 OF THE SILKWORM (*Bombyx mori* L.) FOR UTTAR PRADESH

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ABSTRACT
In silkworm *Bombyx mori* L. for high productivity and narrow range of adaptability in fluctuating environmental condition depends upon genetic stability of the breed. In tropical climate especially for Uttar Pradesh climatic conditions, it is necessary to synthesize silkworm breed with suitable genetic constitution. A breeding experiment was initiated to isolate robust bivoltine line of silkworm (*Bombyx mori* L.) by utilizing bivoltine breed CSR_2, NB_4D_2 and white multivoltine C. nichi by inbreeding the hybrids of the above pure breeds, recurrent back crossing followed by selection at each and every generation, a hardy bivoltine breed with white oval cocoon was isolated. This bivoltine line herein referred as UP_1 (Uttar Pradesh-1) has been bred through over 25 generations and revealed significant improvement in regard to viability and productivity compared to the control breed.

Keywords: silkworm, Bombyx, bivoltine breeding, viability, productivity.

INTRODUCTION
Hybridization coupled with selection as an important tool has been exploited by many breeders in the improvement of breed for their maximum economic grains in silkworm, *Bombyx mori* L. Breeders have extensively studied the silkworm breeds for recognition of their economic importance for the desirable traits through inbreeding. However continuous inbreeding results in the accumulation of many deleterious genes leading to inbreeding depression, thereby resulting in the deterioration of some commercial characters. However, the overall combination of beneficial traits could be achieved in a reasonable way by employing inbreeding techniques coupled with selection, as the silkworm breeder has at his disposal a diversified array of gene combination to manipulate and isolate new silkworm breeds having desirable qualities for commercial exploitation (Raju and Krishnamurty, 1993). The improvement of indigenous breed could be achieved through hybridizations utilizing exotic breeds (Koalov, 1970). Harada (1956) viewed that new silkworm breed has been evolved through hybridization followed by selection. India has many indigenous breeds but, it suffers for new silkworm breeds in competing with other Sericulturally advanced countries like china and Japan have commendable progress has been achieved in evolving robust and productive breed through hybridization (Yokoyama, 1956). Until the 1970s, Indian sericulture was mostly multivoltine-oriented and bivoltine rearing was restricted only for maintenance and multiplication of foreign breeds imported from other countries (Raju, 1990). During this period, Indian breeders evolved few bivoltine breeds like Kalimpong- A, Nan Nung 6D and new bivoltine series (NB), including NB_7, NB_{18} and NB_4D_2 breed (Narasimhanna *et al*., 1976; D. Gangopadhyay, 2003 and D. Raghavendra Rao, 2003). However, these breeds were mostly used for producing commercial crossbreed's cocoons with that of pure multivoltine pure Mysore female, but are not fully satisfied for the production of pure bivoltine silk in view of their poor adaptability to the fluctuating agro-climatic conditions of the tropics specially for Uttar Pradesh climatic conditions. Hence there is a necessity to formulate a need bases breeding programme to evolve robust bivoltine hybrid breeds to improve the productivity and viability traits.

MATERIALS AND METHODS
The present studies initiated during the year 2003 involve two bivoltine CSR_2 and NB_4D_2 and a white multivoltine breed C. nichi. The breeding plan of the present experiment represents the production of progeny from a cross between ♀ NB_4D_2 x CSR_2♂. The F1 was inbred and F2 hybrid female emerged were out crossed to C. nichi ♂ to obtain the F3 progeny, which was further inbred for analyzing cocoon shape till the 5^{th} generation. The F5 females emerged from the white oval cocoons were backcrossed to NB_4D_2 males, during the course of inbreeding from F6 onwards, careful selection was made to isolate oval white cocoon with good productivity and viability. The following selection parameters were applied at different stages of development to select the parent at every generation.

Egg Stage
During the course of breeding programme robust and fertilized female moth were placed on egg sheet for oviposition to replace around 50 layings at every generation. A definite period of 3 hours mating time of moth was followed at every generations as suggested by petkov *et al*., 1979 only the disease free layings (Dfls) exhibiting complete hibernation features were selected at every generation, in order to prevent the eggs from entering diapauses they were acid treated following the method of Yokoyama (1963) and incubated at 25 ± 1°C temperature and 60% to 70% relative humidity on the ninth day of incubation composite laying comprising approximately 1000 eggs were prepared in 3 replicates.

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Further, at every generation the replicate showing the highest percentage of hatching was selected for the next generation in order to improve the percentage of hatching.

Larval stage

The hatched larvae of evolved breed and hatched larvae of control breed were mass reared in three replicate up to the F6 generation. While fixing the base number of 800 larvae after 3rd moult, the comparatively more active larvae with a greenish colour and rough texture, medium weight exhibiting healthy and uniform growth were selected at every generation. Cellular rearing was conducted in five replicates from the F7 generation onwards until isolation of the lines with desirable genotype as achieved.

Pupal /Cocoon stage

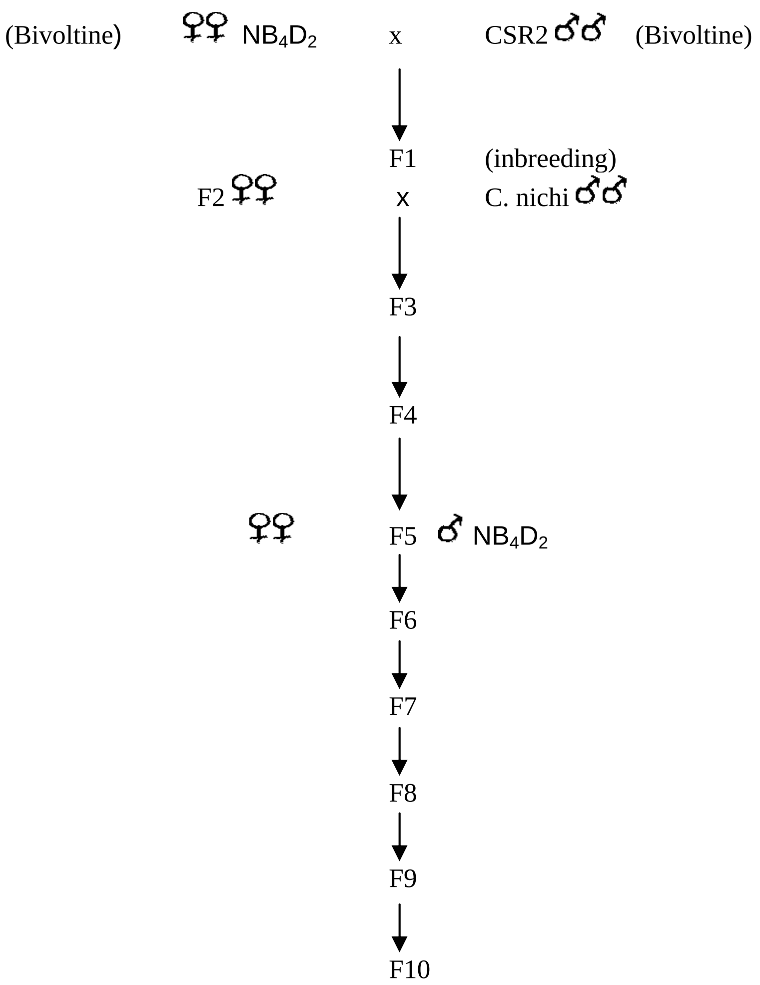
Around 2,000 cocoons were used for the measurement of cocoon traits. Individual cocoon weight and shell weight of the good cocoons were recorded and the shell % was calculated. On the basis of data obtained cocoons with weight, shell weight and shell percentage. Cocoons of uniform shape and size were chosen for breeding work at every generation. At the same time, the replicate showing highest pupation rate and cocoon yield by number/10,000 larvae brushed was retained for the next generation to improve the viability traits.

Moth stage

Robust male and female moths emerged out from selected good cocoons were selected at every generation and fertilizes female moth were allowed to lay eggs under ideal conditions. After the oviposition the females were kept under laboratory conditions until their death to record their longevity. The eggs laid by the moths exhibiting the highest longevity were retained for the next generation. The female moth was subjected to pebrine test to ensure the selection of disease free layings at every generation.

The rearing was conducted by improved rearing technology of silkworm for both chawki and adult rearing suggested by Yokoyama (1963) and Krishnaswami (1978). Observations were made on six commercial characters i.e. yield /10,000 larvae by number, by weight, single cocoon weight, single shell weight, and cocoon shell percentage. Data compared with new bivoltine line (UP_1) as well as its control breeds was analysed by statistical method. Evaluation of post cocoon parameters 4 kg of cocoons of evolved line and control breeds were reeled on multiend reeling machine at Babasaheb Bhimrao Ambedkar University, Lucknow.

Figure-1. **Breeding plan**



F11

F12

New Breed UP_1

RESULTS

Statistical analysis calculated for the six characters of new evolve breed and controls over 12 generations are given in Tables 1 to 6. The F1 hybrids of the cross female NB_4D_2 x male CSR_2 (Figure-1) showed mean yield of 9532, which showed a little difference in F2 generation (9516 cocoons). The F2 females out crossed with C. nichi males showed a mean yield of 9156 cocoons in F3 generations. The F5 females backcrossed with NB_4D_2 males and recorded a mean of 9110 cocoons in F6 generation. Further inbreeding resulted in a gradual increase in the cocoon yield by number from F7 to F12 generation, the difference between last two generations was observed to be insignificant (P> 0.05), showing 9333 and 9349 cocoons in F11 and F12 generation, contributing to the most stabilized in the isolated line UP_1 (Table-1)

Cocoon yield/ 10,000 larvae by weight

Data presented in Table-2 showed that the isolated line has insignificant (P > 0.05) differences between the F11 (15.30 kg) and F12 (15.10 kg) generations, which indicates an almost stabilized nature of the said characters in the isolated line, UP_1.

Single cocoon weight

The mean values of the single cocoon weight as shown in Table-3 reveal that the evolved line UP_1 has insignificant (P > 0.05) difference between the F11 (1.78 g) and F12 (1.77g) generations, which indicates an almost stabilized nature of the said characters in the isolated line, UP_1.

Single shell weight

The mean values of the single shell weight as shown in Table-4 reveal that the evolved line UP_1 has insignificant (P > 0.05) difference between the F11 (0.29 g) and F12 (0.30 g) generations, which indicates an almost stabilized nature of the said characters in the isolated line, UP_1.

Cocoon shell percentage

The mean values of the cocoon shell percentage as shown in Table-5 reveal that the evolved line UP_1 has insignificant (P > 0.05) difference between the F11 (22.42 %) and F12 (22.50 %) generations, indicating a gradual stability of the said characters in the new line, UP_1.

Pupation rate

The mean values of the pupation rate as shown in Table-6 reveal that the evolved line UP_1 has insignificant (P > 0.05) difference between the F11 (90.78 %) and F12 (90.50 %) generations, which indicates an almost stabilized nature of the said characters.

In addition, the silk fibre technological characters of the control breeds NB_4D_2 and CSR_2 and that of the synthesize new bivoltine line UP_1 showed higher values for average filament length, denier, renditta and evenness than the control breeds (Table-7).

DISCUSSIONS

In the fluctuating environmental conditions of Uttar Pradesh, poor quality of mulberry leaves coupled with inferior management in the silkworm rearing practice emphasized the need to synthesized new bivoltine breeds with suitable genotype that showed higher viability and moderate productivity. The main aim of the silkworm breeders is primarily to evolve a breed, which can give rise to stabilized crops and secondly to improve both the quality and the quantity of the silk (Tazima, 1984). In the studies of silkworm carried out various characters have shown that they could be changed to suit the breeders' choice. Selection of one character has a correlation with genetic changes for other characters (Gamo, 1976; Raju, 1990). Utilization of both bivoltine and multivoltines inbreeding programmes could provide combinations of genomes leading to synthesis of robust bivoltine breeds with higher viability and moderate productivity. Inbreeding the hybrids to isolate and stabilize silkworm breeds for the desired traits has been well documented by many breeders (Hirobe, 1968; Kovalov; 1970; Gamo, 1976). During the course of breeding method will help in the improvement of the cocoon qualities (Tazima, 1964).

According to Gupta *et al.*, 1992 multi x bi hybrids were gave the highest cocoon yield/10,000 larvae in spring and autumn. Sekharappa *et al.*, 1999 evolved bivoltine silkworm breeds with better survival and high shell content for tropics by conventional breeding, the performance of evolved breed revealed that the larval yield/10,000 larvae is 12 to 13 Kg. and ERR/ 10000 larvae in both the isolated lines is 8680 and 8700 respectively but finding of this experiment revealed that the larval yield/10,000 larvae is 15 to 16 kg. Which is higher than other popular breeds and ERR/10000 larvae is 9349, which shows better survival in tropics. Raju *et al.*, 1993 evolved two bivoltines MG511 and MG512 of silkworm for higher viability and silk productivity by conventional breeding. These breeds' shows increase in single cocoon weight (1.80 and1.809 gm.), shell weight (0.32 and 0.33 gm.) and shell % (18.21 and 18.40). In present study also showed similar result, the evolved breed UP_1 showed the single shell weight (1.77 gm), single shell weight (0.30gm) and shell % (22.10), which is higher than the parent. The correlation for some traits in silkworms in negative and some for it is positive ((Stsuchiya and Kurashima, H., 1960; Suzuki and Ichimaru, 1961; Gamo and Ichiba, 1971; Lekuthai and Butrachand, 1974; Sturnnikov and Sturnnikov, 1986; Raju and Krishnamurthy, 1993). The survival and productivity traits, which are of high economic value, are negatively correlated with each other as seen in the present study. The new evolved breed is superior to control breed in many quantative and qualitative character.

The present breeding programme (Figure-1) main aim was to isolate a more resistant breed that can assure stable cocoon crops with better viability than the existing bivoltines. An attempt has been made to increase the viability of the evolved breed without sacrificing much of the productivity characters. The present findings with regard to the new bivoltine breed UP1 with good racial characters spinning white oval cocoons were found to be superior in term of viability traits (cocoon yield by number and pupation rate) over their controls (Tables, 1-7). There is a slight decrease in the cocoon weight, shell weight and cocoon shell percentage (Table-3 to 5) in the new synthesized breed UP_1as compared to the controls, but it exhibits better viability characters to assured cocoon crops. This improvement in the cocoon yield by number and pupation rate contributes the cocoons yield by weight. The new bivoltine breed UP_1 has recorded better fiber technological characters *i.e.* average filament length, denier, renditta, neatness, evenness and cleanness (Table-7).

Hence, in view of the above findings, the newly synthesized breed UP_1 of silkworm *Bombyx mori* L. could be effectively used for commercial exploitation in Uttar Pradesh.

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Table-1. Mean values of yield/10,000 larvae by number of the evolved bivoltine line and the control from F1 to F12 generations.

Generations	Control breeds			Evolved breed
	NB_4D_2	**CSR_2**	**C. nichi**	**UP_1**
F1	8270	8290	9430	9532
F2	7842	7856	8910	9516
F3	7640	7668	9220	9156
F4	8130	8140	9050	9140
F5	7780	7795	8072	9280
F6	7685	7698	7935	9110
F7	8460	8490	9240	9290
F8	8510	8540	9450	9215
F9	7740	7835	9335	9182
F10	7690	7712	8420	9210
F11	8395	8470	8980	9333$
F12	8021	8126	8640	9349$
F value S.E. C.D. at 5%	12.548 137.254 405.562	12.782 138.655 409.512	14.256 151.784 446.316	2.564* 55.724 158.893

*Significant (P< 0.01)
$ Insignificant (P>0.05)

Table-2. Mean values of yield/10,000 larvae by weight (kg) of the evolved bivoltine line and the control from F1 to F12 generations.

Generations	Control breeds			Evolved breed
	NB_4D_2	**CSR_2**	**C. nichi**	**UP_1**
F1	13.75	14.12	8.85	14.56
F2	11.92	13.89	7.79	14.10
F3	10.65	13.56	7.56	15.23
F4	13.30	13.23	7.52	15.35
F5	10.57	12.98	6.84	14.86
F6	10.12	12.65	6.76	14.52
F7	13.52	14.50	6.51	14.98
F8	14.00	14.05	8.25	15.50
F9	10.78	13.88	7.43	14.90
F10	12.51	12.45	6.24	15.43
F11	11.53	11.97	7.82	15.30$
F12	12.89	11.80	7.46	15.10$
F value S.E. C.D. at 5%	13.786 0.563 1.635	15.122 0.129 1.382	18.209 0.226 0.652	2.651* 0.123 0.344

*Significant (P< 0.01)
$ Insignificant (P>0.05)

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Table-3. Mean values of single cocoon weight (g) of the evolved bivoltine line and the control from F1 to F12 generations

Generations	**Control breeds**			**Evolved breed**
	NB_4D_2	**CSR_2**	**C. nichi**	**UP_1**
F1	1.72	1.77	0.98	1.71
F2	1.79	1.82	0.85	1.79
F3	1.71	1.80	0.87	1.72
F4	1.75	1.71	0.86	1.81
F5	1.78	1.72	0.80	1.90
F6	1.61	1.84	0.77	1.82
F7	1.78	1.87	0.84	1.78
F8	1.71	1.91	0.96	1.80
F9	1.62	1.71	0.96	1.78
F10	1.53	1.69	0.83	1.85
F11	1.68	1.74	0.89	$1.78^{\$}$
F12	1.64	1.79	0.86	$1.77^{\$}$
F value S.E. C.D. at 5%	12.564 0.061 0.183	20.409 0.049 0.154	10.543 0.026 0.223	6.523^{**} 0.034 0.092

**Significant (P< 0.01)
$ Insignificant (P>0.05)

Table-4. Mean values of single shell weight (g) of the evolved bivoltine line and the control from F1 to F12 generations

Generations	Control breeds			Evolved breed
	NB_4D_2	CSR_2	C.nichi	UP_1
F1	0.34	0.35	0.14	0.30
F2	0.32	0.33	0.13	0.31
F3	0.34	0.31	0.12	0.30
F4	0.30	0.32	0.10	0.30
F5	0.30	0.31	0.11	0.29
F6	0.32	0.32	0.12	0.28
F7	0.31	0.34	0.11	0.30
F8	0.32	0.35	0.12	0.31
F9	0.33	0.33	0.12	0.30
F10	0.31	0.32	0.13	0.29
F11	0.32	0.33	0.12	$0.29^{\$}$
F12	0.31	0.32	0.12	$0.30^{\$}$
F value S.E. C.D. at 5%	12.569 0.014 0.042	15.231 0.004 0.0013	10.326 0.006 0.017	4.562** 0.004 0.012

**Significant (P< 0.01)
$ Insignificant (P>0.05)

Table-5. Mean values of cocoon shell percentage of the evolved bivoltine line and the control from F1 to F12 generations.

Generations	Control breeds			Evolved breed
	NB_4D_2	**CSR_2**	**C. nichi**	**UP_1**
F1	19.80	20.56	13.90	19.56
F2	17.50	20.66	14.20	20.80
F3	20.35	19.98	12.40	20.32
F4	17.12	20.86	12.75	18.81
F5	16.60	21.24	12.59	18.06
F6	19.50	20.51	12.78	18.89
F7	19.48	19.98	13.07	19.90
F8	17.38	20.81	12.26	21.10
F9	20.60	20.90	12.90	21.16
F10	20.12	21.00	12.12	22.15
F11	17.65	21.86	12.50	$22.02^{\$}$
F12	18.70	22.15	12.67	$22.10^{\$}$
F value S.E. C.D. at 5%	6.489 0.235 0.684	15.526 0.213 0.625	11.821 0.323 0.948	8.845** 0.512 1.523

**Significant (P< 0.01)
$ Insignificant (P>0.05)

Table-6. Mean values of pupation rate (%) of the evolved bivoltine line and the control from F1 to F12 generations.

Generations	Control breeds			Evolved breed
	NB_4D_2	**CSR_2**	**C. nichi**	**UP_1**
F1	76.50	79.12	91.56	90.40
F2	77.59	77.58	92.43	88.50
F3	75.50	76.35	90.12	88.40
F4	76.12	72.29	91.87	89.50
F5	77.34	71.95	93.29	87.86
F6	79.81	75.86	94.05	89.42
F7	74.92	73.59	92.34	86.50
F8	72.56	74.86	94.51	89.56
F9	76.65	75.66	93.89	90.52
F10	78.84	73.68	95.65	89.54
F11	74.42	77.48	96.42	$90.78^{\$}$
F12	78.12	78.57	94.68	$90.50^{\$}$
F value S.E. C.D. at 5%	8.143 2.472 5.984	21.214 1.528 4.623	18.632 1.352 4.093	7.524** 0.624 1.846

**Significant (P< 0.01)
$ Insignificant (P>0.05)

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Table-7. Mean values of silk fibre technological characters of SUP-1 and the bivoltine control breeds.

Breed	**Fibre technological characters**					
	Avg. filament length (m)	**Denier** (D)	**Renditta** (kg)	**Neatness** (%)	**Evennes s** (%)	**Cleanness** (%)
NB_4D_2	890.56	2.39	8.10	84.50	82.50	84.80
CSR_2	947.65	2.57	7.82	90.80	89.10	86.65
UP_1	980.20	2.72	7.32	92.35	90.35	89.50