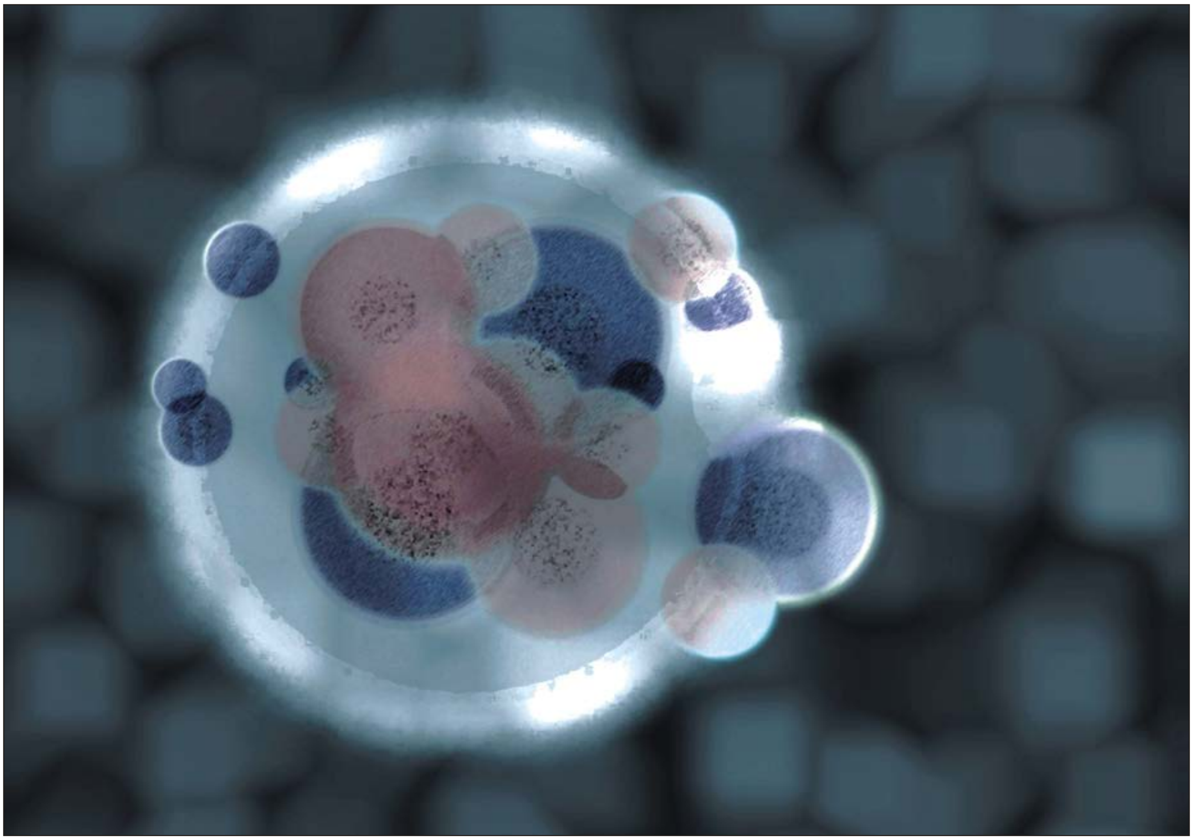

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Development of Bivoltine Pure Strains of Silkworm, *Bombyx mori* L. to Rear Eexclusively on Artificial Diet During Young Instar

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Abstract: As component bivoltine pure strains of existing productive silkworm hybrids did not accept the artificial diet, the study was taken up to create a pool of such strains which would feed on the artificial diet. Six strains viz., 5HT, GEN4, 8HT, B71, CSR3 and JPN8 were short-listed based on the results of an initial screening which recorded feeding response percentage of more than 20. These six strains were further subjected to continuous inbreeding and directional selection for 12 generations for improving the feeding response over the generations and stabilizing it at more than 85% so that they would form suitable breeding resource materials for preparation of commercial bivoltine hybrids for exclusively rearing on artificial diet during young instar. Care was taken so that breed characters in terms of economic traits were not adversely affected. At the end of ninth generation, the feeding response reached above 78% except in B71 and further stabilized at the higher level and thus forming prospective parents for bivoltine hybrid combinations. Data pertaining to G9~G12 were analyzed to check the stability in performance. The traits with particular reference to the diet phase such as feeding response, young instar larval duration and young instar larval weight have reflected non-significant differences in the last four generations clearly indicating the stability in these traits. Other traits such as cocoon weight, cocoon shell weight, shell percentage, survival and cocoon yield also did not vary among the generations. After the strains were stabilized for rearing on artificial diet, they were designated as 5HT (A), GEN4 (A), 8HT (A), B71 (A), CSR3 (A) and JPN8 (A) as these strains are different from the normal strains (5HT, GEN4, 8HT, B71, CSR3 and JPN8). The implications of the improved feeding response and stabilized economic traits in the context of this study are discussed.

Key words: Artificial diet, bivoltine strains, cocoon traits, feeding response, silkworm

INTRODUCTION

In organized young instar silkworm rearing enterprise, easy and economic technologies hold influential impact. Rearing young silkworm on artificial diet offers clear advantages over the existing practice of raising exclusive mulberry garden and then rearing young silkworm on tender mulberry leaves since artificial diet ensures balanced nutrition irrespective of the seasons and disease free conditions. Despite the care taken for young instar rearing at the farmers' level, the success of the cocoon crop is not always assured. This is primarily because, the mulberry leaf grown particularly in tropical conditions is not assured of the required moisture and balanced nutrients for rearing young-instar silkworms throughout the year. The nutritional requirement of young instar silkworm is specific and to meet this, exclusive mulberry gardens are to be maintained. To avoid such preconditions, an artificial diet was developed for young instar silkworm rearing. The component bivoltine pure strains of existing productive silkworm hybrids commercially exploited in India did not accept the diet and the feeding response was low. The only option was to modify the selection response of silkworm for the feeding behaviour maintaining the breed characters intact. Thus, commercial breeds destined to be reared on mulberry leaf were converted gradually to feed on artificial diet through directional selection. This would ultimately facilitate to develop a common artificial diet for all the evolved silkworm breeds/hybrids.

When Japan faced a similar predicament, efforts were made to evolve exclusive silkworm strains for rearing on artificial diet and it centered mainly around the abnormal feeding behaviour of the strain, Sawa J. By exploiting the polyphagous nature of Sawa J, exclusive strains and commercial hybrids were developed for rearing on artificial

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diet (Tanaka and Midorikawa, 1984). Prior to the study, six bivoltine pure strains had been screened in, based on their initial feeding response to artificial diet (Nair *et al*., 2010). It was planned to make these pure strains stabilized as component races for the hybridization programme through which the better performing hybrid combinations would be evaluated and adjudicated.

MATERIALS AND METHODS

Six strains viz., 5HT, GEN4, 8HT, B71, CSR3 and JPN8 which were short-listed based on the results of previous screening with more than 20% feeding response were subjected to continuous inbreeding and directional selection for 12 generations for improving the trait over the generations and stabilizing it at more than 85%. Care was taken so that all other economic traits were within the stipulated level of breed characters. At the end of ninth generation, the feeding response reached above 78% except in B71 and further stabilized at the higher level and thus forming prospective parents for bivoltine hybrid combinations.

The rearing of young instar silkworm larvae was accomplished as described by Trivedy *et al*. (2003) in a rearing room designed for the purpose maintaining a temperature of 30°C and a relative humidity of 90%. On completion of 48 h from the time of brushing, the larvae which accepted the artificial diet, grown uniformly and remained healthy were counted. By this time, the number of hatched eggs also was counted. The Feeding Response (FR) percentage was calculated as described in our earlier studies (Nair *et al*., 2010).

On coming out of second ecdysis, the larvae were fed with tender mulberry leaves and transferred to ventilated plastic trays measuring 3×2 ft and reared on mulberry leaves following standard procedure. On maturation, the larvae were picked up and mounted on plastic collapsible mountages for cocoon spinning. The cocoons were harvested on the 6th day and assessed. Selected cocoons of the strains which recorded more than 20% feeding response in the screening process, were used to prepare layings for further breeding process.

Larvae of the six short listed bivoltine strains were brushed on artificial diet as already described. Twelve replications of the larvae of each strain were brushed as cellular batches. On completion of II instar, after careful examination of all the rearing beds, six beds with healthy and uniform larvae were retained for further rearing. Based on FR and the performance of the batches, cocoons conforming to the original characters were selected. Equal number of healthy males and females were selected for the preparation of DFLs for the next generation and the male and female pupae were separately incubated for eclosion. On moth emergence, healthy female and male moths were picked up and half sib-mating was followed. Twenty-five DFLs were prepared from each strain. The selected strains were continuously reared for 12 generations and until the FR of the strains stabilized above 75%. After the strains were stabilized for rearing on artificial diet, they were designated as 5HT (A), GEN4 (A), 8HT (A), B71 (A), CSR3 (A) and JPN8 (A) as these strains are different compared to the normal strains (5HT, GEN4, 8HT, B71, CSR3 and JPN8).

Data pertaining to each strain for generations 9~12 after the stabilization of FR, were first subjected to descriptive statistical analysis. The mean, Standard Error (SE) and Coefficient of Variation (CV) were thus derived. The data on G9~G12 were then analyzed by employing one way ANOVA using analyse-It statistical package to ascertain statistical significance.

RESULTS

Dumbbell strains: Table 1 shows that the feeding response in the dumbbell strains viz., 5HT, GEN4 and B71 was stabilized during the Generations 9~12 because in all these strains, the FR was 80% and above. Among the three strains, however, 5HT was the best performer in respect of FR, the prime trait in the context of this study with a mean FR of 90.75% followed by GEN4 with 88.67%. B71 recorded the least FR among the three dumbbell strains (80.32%). The CV indicated that the FR had the least variation in 5HT while B71 had the highest variation. Mean young instar duration was the minimum in 5HT with 186 h whereas GEN4 had the maximum duration of 192 h. B71 recorded young instar duration of 190 h. The weight of ten young instar larvae on completion of second moult was maximum in GEN4 with 0.268 g whereas in the other two strains, it was marginally low. These three diet-phase traits were largely stabilized as revealed from the fact that the statistical variations in these traits among the generations were non-significant except for young instar larval weight in B71.

The post diet-phase traits such as 5th instar duration, pupation percentage, cocoon yield and the cocoon traits showed consistent results in all the three strains in accordance with the breed characters while the FR was stabilized. While GEN 4 recorded maximum mean 5th instar duration (161 h), B71 recorded the least (152 h). There was not much to differentiate in pupation percentage among the three strains with all of them recording a

Table 1: Rearing performance of dumbbell bivoltine pure strains during breed stabilization process

Generation	Feeding response (%)	Young instar duration (h)	Larval weight on II moult (10) (g)	V instar larval duration (h)	Pupation percentage	Yield/10000 larvae (kg)	Cocoon weight(g)	Shell weight (g)	Shell percentage
5HT									
9	90.790	186.000	0.2764	150.000	81.470	10.060	1.423	0.320	22.490
10	92.240	186.000	0.2530	152.000	82.180	10.840	1.498	0.346	23.080
11	89.880	186.000	0.2566	156.000	89.700	12.960	1.500	0.354	23.580
12	90.100	186.000	0.2481	155.000	85.830	13.130	1.602	0.357	22.270
Mean	90.750	186.000	0.2585	153.000	84.790	11.750	1.506	0.344	22.850
SE±	00.649	000.430	0.0070	002.101	01.504	0.484	0.023	0.005	00.165
CV	02.480	000.800	9.6500	004.780	06.14	14.280	5.220	5.220	02.510
Significance	NS	NS	NS	NS	NS	*	**	**	**
GEN4									
9	86.840	192.000	00.2399	165.00	88.270	12.710	1.464	0.329	22.470
10	91.210	192.000	00.2746	168.00	85.240	12.240	1.718	0.384	22.330
11	89.110	192.000	00.2781	169.00	76.550	11.500	1.760	0.392	22.250
12	87.500	190.000	00.2785	143.00	87.760	15.390	1.783	0.393	22.030
Mean	88.670	192.000	00.2680	161.00	84.460	12.960	1.681	0.374	22.270
SE±	00.838	000.440	00.0090	003.39	01.611	00.485	0.040	0.008	00.109
CV	03.273	000.800	11.6000	007.29	06.610	12.900	8.170	7.650	01.700
Significance	NS	NS	NS	**	**	**	**	**	NS
B71									
9	67.690	190.00	00.2040	156.00	82.600	10.780	1.472	0.305	20.740
10	81.170	190.00	00.2160	150.00	81.860	11.040	1.522	0.324	21.260
11	79.310	190.00	00.2202	156.00	81.180	11.040	1.512	0.325	21.500
12	89.320	190.00	00.2719	144.00	82.080	11.080	1.526	0.324	21.230
Mean	80.320	190.00	00.2280	152.00	81.930	10.990	1.508	0.320	21.180
SE±	01.316	000.70	00.0090	002.13	00.958	00.060	0.007	0.003	00.108
CV	05.680	001.30	13.2000	004.88	04.050	01.880	1.680	3.120	01.770
Significance	NS	NS	**	NS	NS	NS	**	*	NS

*Significant at $p<0.05$, **Significant at $p<0.01$, NS: Non-significant

mean pupation of 82~85%. While 5HT and B71 had non-significant variations in the 5th instar duration and pupation, that in GEN4 was significant. Cocoon yield in the last four generations ranged from 10.06 to 13.13 kg in 5HT which was significant at 5%. The higher CV of 14.28 also showed higher variation among the generations. Similar was the case in GEN4 with a mean cocoon yield of 12.96 and a high CV of 12.90%. The variation was significant at 1% level. At the same time, the mean cocoon yield in B71 was rather consistent at 10.99 kg, the variation being non-significant. The mean cocoon weight in 5HT, GEN4 and B71 was 1.506, 1.681 and 1.508 g, respectively and the shell weight was 0.344, 0.374 and 0.320 g, respectively. These traits conform to the original breed traits along with the shell percentage. However, variations seen in these traits among the generations showed significance except for shell percentage in GEN4 and B71.

Oval strains: It is clear from Table 2 that the feeding response in the oval strains viz., 8HT, CSR3 and JPN8 was stabilized during the generations 9~12 as in the case of the dumbbell strains. All the strains showed consistent FR and the mean FR was the maximum in JPN8 with 91.53%. 8HT recorded the least FR among the three oval strains (83.42%). The CV recorded showed that the FR had the least variation in JPN8 while the other two strains viz., 8HT and CSR3 had slightly higher variation with a CV of 5.50 and 5.16%, respectively. While CSR3 took the longest duration during the young instar with 194 h, 8HT took only 186 h. The weight of ten young instar larvae on completion of second moult was maximum in JPN8 with 0.304 g whereas in the other two strains, it was marginally low. As in the case of the dumbbell strains, these three diet-phase traits stabilized to a considerable extent as revealed from the non-significant variations.

The post diet-phase traits showed that the 5th instar duration was the maximum in CSR3 with 176 h. The other two strains showed marginally low larval duration. Pupation percentage and the cocoon yield exhibited considerable variation among the generations and the variations were significant as well. This is also evident from the higher CV. There was no much difference among the three strains in respect of pupation percentage and the cocoon yield. The result showed that among the three strains, CSR3 had a higher potential with regard to the cocoon weight with a mean cocoon weight of 1.900 g whereas in 8HT, it was 1.594 g and in JPN8, it was 1.656 g. Shell weight also was the highest in CSR3 with 0.427 g. In all the three strains, shell percentage was considerably high with the highest value of 22.47% in CSR3.

Table 2: Rearing performance of oval bivoltine pure strains during breed stabilization process

Generation	Feeding response (%)	Young instar duration (h)	Larval weight on II moult (10) (g)	V instar larval duration (h)	Pupation percentage	Yield/10000 larvae (kg)	Cocoon weight (g)	Shell weight (g)	Shell percentage
8HT									
9	78.97	186.00	00.2249	144.00	80.190	11.430	1.567	0.345	22.040
10	84.05	186.00	00.2532	141.00	80.630	11.360	1.535	0.343	22.330
11	82.19	186.00	00.2728	168.00	79.670	11.030	1.640	0.378	23.050
12	88.46	186.00	00.2612	152.00	92.390	15.470	1.634	0.362	22.140
Mean	83.42	186.00	00.2510	151.00	83.220	12.320	1.594	0.357	22.390
SE±	01.323	000.55	00.0080	004.02	01.711	0.565	0.019	0.005	00.145
CV	05.50	001.00	11.5700	009.50	07.120	15.870	4.110	4.820	02.240
Significance	NS	NS	NS	**	**	**	NS	**	*
CSR3									
9	85.82	194.000	0.2700	180.00	78.890	12.180	1.723	0.387	22.460
10	82.03	194.000	0.2762	172.00	80.050	11.740	1.846	0.413	22.370
11	84.91	194.000	0.2880	184.00	79.200	13.190	2.009	0.449	22.350
12	89.72	194.000	0.3039	168.00	90.160	17.950	2.021	0.459	22.720
Mean	85.62	194.000	0.2850	176.00	82.080	13.760	1.900	0.427	22.470
SE±	01.275	000.370	0.0060	002.36	01.553	00.781	0.040	0.009	00.187
CV	05.16	000.700	7.3100	004.65	06.550	19.670	7.220	7.700	02.890
Significance	NS	NS	NS	*	**	**	**	**	NS
JPN8									
9	90.340	190.000	0.2690	171.00	73.250	10.370	1.496	0.315	21.090
10	94.720	190.000	0.3034	149.00	79.220	11.610	1.683	0.364	21.630
11	90.660	190.000	0.3237	144.00	74.450	11.750	1.703	0.368	21.630
12	90.390	190.000	0.3191	144.00	90.950	16.230	1.741	0.372	21.380
Mean	91.530	190.000	0.3040	152.00	79.470	12.490	1.656	0.355	21.430
SE±	01.024	000.250	0.0080	003.42	02.416	00.693	0.032	0.008	00.117
CV	03.876	000.400	9.5050	007.81	10.530	19.210	6.660	7.370	01.900
Significance	NS	NS	NS	**	**	**	**	**	NS

*Significant at $p<0.05$, **Significant at $p<0.01$, NS: Non-significant

DISCUSSION

Silkworm needs a balanced nutrition during young stage for a robust growth. Mulberry leaves consumed by the young instar silkworm should be succulent with high moisture (>75%) and rich in proteins (>30%) and carbohydrates (>19%). But the Indian mulberry leaves fed to young instar silkworm is inconsistent in its nutrient level in relation to the dietary requirement (Trivedy *et al*., 2001; Kamble *et al*., 2008). To tide over this constraint, a viable artificial diet for the young instar (up to the second moult) was developed for rearing young instar silkworm. The rearing technology for such a practice was developed simultaneously. The reduction in the labour involvement in the initial stages of rearing has been an added advantage. To develop silkworm strains and hybrids for commercial exploitation which would feed on artificial diet during the young instars remained a major challenge.

All the silkworm strains are not equally good in accepting the artificial diet although the diet contains all the required nutrients in the right proportion. Initially, their Feeding Response (FR) varied from among the six strains and thus a single diet formulated was not suitable for all the silkworm strains and the productive silkworm hybrids as reported by Magadum *et al*. (1994) in a similar effort. Thus the strategy of transforming the existing promising pure strains to that with high level of acceptance to artificial diet was resorted to, in the present study. Park and Kang (1981) had in fact opined that it was necessary to improve new silkworm varieties suitable for the artificial diet which was different from the mulberry leaves in physical and chemical nature.

In the context of the present work, it was prudent to make the promising pure strains already in use to adapt to the physiological condition of feeding on artificial diet so that they could be used as breeding resource materials for hybridization resulting in exclusive hybrids for rearing on artificial diet. This was also a model tried by Trivedy *et al*. (2003, 2004).

The breeding programme concentrated on isolating the better performing pure lines which are components of existing programmes of breeding laboratories of CSRTI, Mysore. An initial screening followed by short listing based on their inclination towards artificial diet was carried out. The initial response of the different silkworm strains to artificial diet feeding showed that prominent inter-strain difference exists in silkworm. This can be attributed to the stress placed on several physiological systems while attempting to accumulate different levels of major nutrients available in the artificial diet. It was clear from the results that 5HT, had the highest FR among the dumb-bell races (90.75%). In the case of oval strains, JPN8 recorded the highest FR (91.53%). Although there is not much to differentiate among the strains with regard to FR, dumbbell strains showed marginally better FR compared to oval strains. A line of similarity could be drawn between these results and a few in the past. Shinbo and Yanagawa (1994) had reported in their effort to breed polyphagous silkworm strains to rear on artificial diet that almost all Japanese strains had accepted the artificial diet and could grow well unlike most of the Chinese strains. Asaoka and Mano (1992) also had reported that the feeding ability of Chinese strains on the artificial diet was apparently low and conspicuous.

In a similar effort, many productive breeds having low response to artificial diet were made to adapt to the artificial diet over generations through directional selection. Five multivoltine and six bivoltine strains were thus evolved by Trivedy *et al*. (2001, 2003). The hybrids developed from these improved strains accepted the diet and performed well with respect to their economic traits and almost at par with that of mulberry reared counterparts (Trivedy *et al*., 2001, 2003). The present study thus created a larger pool of potential bivoltine strains for rearing exclusively on artificial diet during the young instar which will form breeding resource material for prospective hybrids.

One of the main considerations in the present study was that while improving the feeding response over a period though directional selection and inbreeding, the other important economic traits are not adversely affected or rather such traits are maintained as per the breed characters. This is fairly clear in the traits of cocoon yield, pupation percentage, cocoon weight and shell weight.

Similarly, the duration for young instar was slightly more in the diet reared batches than that usually reared on mulberry leaf. But this meager extension is offset once the larvae are switched over to mulberry. All the other traits were within the breed traits. Furthermore, there was no deliberate attempt to increase the cocoon traits such as cocoon weight, cocoon shell weight and shell percentage through selection as this could have an adverse impact on the hybrid vigour in the resultant hybrids when these pure strains are used for hybridization.

It is concluded that six bivoltine silkworm pure strains were subjected to continuous inbreeding and directional selection up to 12th generation for improving and stabilizing their feeding response to create a pool of breeding resource material for prospective exclusive hybrids meant for rearing exclusively on artificial diet during young instar. While improving the feeding response, care was taken to retain all the other economic traits conforming to the breed characters.

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