Int. J. Indust. Entomol. Vol. 9, No. 1, 2004, pp. 47~51

International Journal of
Industrial Entomology

Evaluation of New Commercial F_1 Hybrids of Silkworm (*Bombyx mori* L.) with Participation of Sex-limited Lines

N. Petkov*, Z. Petkov, D. Grekov[1] and K. Arnaudova[1]
Regional Centre for Scientific-Applied Service, Vratza-3000, Bulgaria.
[1]Agricultural University of, Plovdiv-4000, Bulgaria.

(Received 15 March 2004; Accepted 5 July 2004)

To evolve silkworm hybrids with higher survival and productivity and easy and effective seed cocoon production, three new evolved bivoltine hybrids of silkworm (*Bombyx mori* L.), created with participation of sex-limited lines at eggs and larva stage were evaluated with control $Super_1 \times Hessa_2$ hybrid at Plovdiv Agrarian University during 2000 – 2002. ANOVA analysis was employed and the performance of hybrids was observed in respect of main quantitative traits. The results obtained show significant superiority of new hybrids $T_{15/4} \times TBV_{2/24}$, $XT_{215/38} \times TV_{3/2}$, $XT_{215/38} \times B_{2/6}$ and their reciprocal crosses over the control. They were characterized with 99% hatchability of eggs, 93 – 95% silkworm survival, 2.32 – 2.42 g cocoon weight, 53.5 – 55.7 cg shell weight, 22.9 – 23.1% shell ratio, 1,307 – 1,326 m filament length, 3.08 – 3.17 denier, 95 – 96% reelability, 44.4 – 45.1% silk ratio, 569 – 593 number of normal eggs per lying and 347 – 364 mg weight of normal eggs per lying. Newly evolved hybrids were manifested high productivity, 41.0 – 43.6 kg cocoon yield and 7.33 – 7.78 kg raw silk yield per one box (20,000 ± 200 viable eggs), which significant surpass the control $Super_1 \times Hessa_2$ hybrid with 7.6 – 14.4% and 10.8 – 17.6%, respectively. $T_{15/4} \times TBV_{2/24}$ and $XT_{215/38} \times TV_{3/2}$ were considered as highly productive hybrids to local conditions and found suitable to rear in spring season.

Key words: Silkworm, Hybrid evaluation, Economic traits, Sex-limited lines

*To whom correspondence should be addressed.
Regional Centre for Scientific-Applied Service, 24 Mito Orozov str., Vratza-3000, Bulgaria. Fax: +359-92-45154; E-mail: serivrbg@mbox.digsys.bg

Introduction

Under free marked conditions private farm is appear as main structure of sericulture organization in Bulgaria and used commercial hybrids is one of the fundamental elements of technology for intensification of cocoon production. The breeding experiments conducted during 1970s yielded bivoltine hybrids with cocoon shell ratio at commercial level and these hybrids could made much impact in the field. It was established (Akimenko *et al.*, 1994; Mano *et al.*, 1994; Petkov, 1995; Petkov *et al.*, 1998, 1999; Datta *et al.*, 2000; Singh, 2001; Greiss *et al.*, 2002) that the superiority of F_1 silkworm hybrids toward their initial races or lines has been reached 15 – 30%.

Systematic breeding approaches with appropriate selection procedures have contributed to amalgamate the major economic traits of choice from selected breeds and to synthesize genotypes of desirable constitution and expression in silkworm (Mano *et al.*, 1982; Tanaka and Ohi, 1994). Synthesis of new gene combinations by conventional breeding techniques is one of the important tools for exploiting the heterosis in the silkworm *Bombyx mori* L. (Harada, 1961; Petkov, 1995).

However during the production of F_1 silkworm eggs from hybrids, created with participation of non sex-limited initial forms, the percentage of eggs from pure parent races or lines reaches 5 – 15% irrespective of sex separation implementation. Ultimately about 25% of advantage of hybridization is used in field level only. Introduction and utilization of new hybrids, created with participation of sex-limited races or lines will led to significant improvement of productivity and cocoon and raw silk quality. Keeping this in view, an attempt has been made to evolve the productive performance of three new bivoltine hybrids of silkworm, *B. mori* L, created with participation of sex-limited at eggs and larva stage lines.

Materials and Methods

Experimental and theoretical work was carried out at Agrarian University of Plovdiv during the period of 2000 – 2002. Five newly evolved bivoltine breeds, *i.e.*, $T_{15/4}$ and $XT_{215/38}$ (Japanese type), $TV_{3/2}$, $TBV_{2/24}$ and $B_{2/6}$ (Chinese type) evolved by SES Vratza were formed experimental material. $T_{15/4}$ and $XT_{215/38}$ lines (Japanese type) are genetically sex-limited at eggs stage. Female eggs are lightly grey and male eggs are straw yellow. The larvae are marked. Cocoons are white and dumbbell. $TV_{3/2}$, $TBV_{2/24}$ and $B_{2/6}$ (Chinese type) are sex-limited for larval marking. The eggs are lightly grey. Female larvae are marked and male are white. Cocoons are white and oval. Three F_1 hybrids, $T_{15/4} \times TBV_{2/24}$, $XT_{215/38} \times TV_{3/2}$, $XT_{215/38} \times B_{2/6}$ and their reciprocal crosses were prepared and reared in 4 replicates of each having 150 larvae, counted after second molting. $Super_1 \times Hessa_2$ commercial hybrid, introduced into practice on a mass scale was used as control because of absent of F_1 hybrid from sex-limited initial forms. All hybrids along with control hybrid $Super_1 \times Hessa_2$ were reared at spring season as per standard techniques recommended by Petkov and Penkov (1980). The silkworms were reared at 26 – 27°C and 85% RH during the first two instars, at 25°C and 80% RH during the third larval instar, at 23 – 24°C and 70 – 75% RH during the last instars. During the first instars the silkworms were fed with sliced (3 – 5 mm) leaves from local varieties 4 – 5 times per day, with leaves three times per day at third instar and with whole branches two times per day at last instars. The rearing performance of the new and control hybrids were studied in respect to the main 16 quantitative traits: eggs hatchability, survival, larval duration, cocoon weight, shell weight, shell ratio, filament length, filament weight, denier, reelability, silk ratio, number and weight of normal eggs per lying, physiological waste in lying, cocoon and raw silk yield per 1 box of silkworms (20,000 ± 200 viable eggs).

ANOVA analysis was employed and the performance was observed in respect of main quantitative traits using LSMLWM&MIXMDL software (Harvey, 1990).

Results and Discussion

Average data for parent lines are presented inTable 1. It is evident that they were characterized with approximately high values of main biological characters and productivity, 98% hatchability, 91 – 94% survival, 2.06 – 2.22 g cocoon weight, 47 – 52% shell weight, 21.8 – 23.5% shell ratio and 34.2 – 38.0 kg cocoon yield per box of silkworm.

The data obtained from testing of new hybrids were pooled in Table 2, 3, 4, 5 and 6. New hybrids exceeded the parent lines for all tested traits. The analysis of variance (ANOVA) results showed significant differences among the hybrids and control for almost all tested traits. The laboratory performance of new hybrids is presented in Table 2. All tested hybrids were characterized with high eggs hatchability, 99% toward 98% in control (Table 2). $T_{15/4} \times TBV_{2/24}$, $XT_{215/38} \times TV_{3/2}$, $XT_{215/38} \times B_{2/6}$ hybrids and their reciprocal crosses had comparatively higher values of this character, which exceeded the control with 0.94 – 1.32%. With respect to silkworm survival, main biological character, that determine silkworm productivity to a great extent, the values obtained in new hybrids were comparatively high 93.4 – 95.5%, respective, compared to 93.6% in $Super_1 \times Hessa_2$ control hybrid. $T_{15/4} \times TBV_{2/24}$, $XT_{215/38} \times TV_{3/2}$ hybrids and their reciprocal crosses had comparative high survival, which excelled the control with 0. 55–1.97 points. Larval duration character was varied in narrow limits, 697 – 706 hrs, compared with 693 hrs for control. The differences obtained between new hybrids and $Super_1 \times Hessa_2$ control hybrid (4 – 14 hrs) were not proved and because of this we could nod established clear differentiation. The longer larval duration in newly evolved hybrids and control hybrid may be due to slow growth with reduced rate of metabolism (Morohoshi, 1969).

Table 1. Characteristics of parent lines

Parent lines	Eggs hatch-ability (%)	Survival (%)	Larval duration (hrs)	Cocoon weight (g)	Shell weight (cg)	Shell ratio (%)	Filament length (m)	Filament weight (cg)	Filament thickness (denier)	Reelability of cocoons (%)	Silk ratio (%)	Cocoon yield per box (kg)
$T_{15/4}$	98.12	91.7	691	2.06	47.4	23.0	1256	39.9	2.86	94	43.43	34.2
$XT_{215/38}$	98.1	92.0	693	2.12	48.1	22.7	1283	40.0	2.81	94	43.58	35.3
$TBV_{2/24}$	98.4	94.0	686	2.21	52.0	23.5	1294	43.1	3.00	94	44.17	38.0
$TV_{3/2}$	98.5	93.1	685	2.22	51.0	23.0	1297	42.5	2.95	94	44.32	37.9
$B_{2/6}$	98.0	92.0	685	2.15	47.0	21.8	1231	37.9	2.77	93	42.49	36.1

Table 2. Silkworm biological characters of hybrids

Hybrids	Eggs hatchability (%)		Survival (%)		Larval duration (hrs)	
	x⁻ ± Sx	± D	x⁻ ± Sx	± D	x⁻ ± Sx	± D
$Super_1 \times Hessa_2$ (Control)	97.8 ± 2.17		93.6 ± 2.59		693 ± 11	
$T_{15/4} \times TBV_{2/24}$	98.8 ± 1.77	0.94*	94.1 ± 2.13	0.55	697 ± 9	4
$TBV_{2/24} \times T_{15/4}$	99.1 ± 1.93	1.32**	95.5 ± 1.99	1.97**	700 ±11	7
$XT_{215/38} \times TV_{3/2}$	99.0 ± 2.01	1.16*	94.5 ± 2.07	0.93	706 ± 8	14**
$TV_{3/2} \times XT_{215/38}$	98.9 ± 1.98	1.08*	95.3 ± 2.24	1.74**	699 ± 6	6
$XT_{215/38} \times B_{2/6}$	98.5 ± 2.13	0.72	93.4 ± 2.43	-0.17	701 ± 10	8
$B_{2/6} \times XT_{215/38}$	98.9 ± 2.04	1.10*	94.0 ± 2.51	0.47	697 ± 7	4
GD at P = 0.5		0.83		0.97		9
P = 0.1		1.22		1.43		14
P = 0.01		1.89		2.22		22

*Significant at 5% and **Significant at 1%.

Table 3. Technological characters of fresh cocoons

Hybrids	Cocoon weight (g)		Shell weight (cg)		Shell ratio (%)	
	x⁻ ± Sx	± D	x⁻ ± Sx	± D	x⁻ ± Sx	± D
$Super_1 \times Hessa_2$ (Control)	2.20 ± 0.05		48.8 ± 1.1		22.1 ± 0.53	
$T_{15/4} \times TBV_{2/24}$	2.32 ± 0.06	0.12***	53.5 ± 1.5	4.7**	23.1 ± 0.63	0.95*
$TBV_{2/24} \times T_{15/4}$	2.38 ± 0.06	0.18***	55.1 ± 1.0	6.3***	23.1 ± 0.57	0.93*
$XT_{215/38} \times TV_{3/2}$	2.37 ± 0.05	0.17***	54.2 ± 1.2	5.4***	22.9 ± 0.49	0.71*
$TV_{3/2} \times XT_{215/38}$	2.42 ± 0.06	0.22***	55.7 ± 1.4	6.9***	23.0 ± 0.61	0.82*
$XT_{215/38} \times B_{2/6}$	2.26 ± 0.04	0.06**	52.4 ± 1.3	3.6**	23.2 ± 0.55	1.02**
$B_{2/6} \times XT_{215/38}$	2.29 ± 0.06	0.09***	51.8 ± 1.4	3.0*	22.6 ± 0.57	0.41
GD at P = 0.5		35		24		0.70
P = 0.1		52		35		0.99
P = 0.01		81		54		1.54

*Significant at 5%, **Significant at 1% and ***Significant at 0.1%.

Table 4. Filament technological characters

Hybrids	Filament length (m)		Filament weight (cg)		Filament thickness (denier)		Reelability of cocoons (%)		Silk ratio (%)	
	x⁻ ± Sx	± D	x⁻ ± Sx	± D	x⁻ ± Sx	± D	x⁻ ± Sx	± D	x⁻ ± Sx	± D
$Super_1 \times Hessa_2$ (Control)	1280 ± 31		43.1 ± 1.1		3.04 ± 0.08		95 ± 1.97			
$T_{15/4} \times TBV_{2/24}$	1307 ± 32	27*	44.7 ±1.1	1.6	3.08 ± 0.05	0.04	95 ± 1.78	0.33	44.8 ± 1.03	1.28**
$TBV_{2/24} \times T_{15/4}$	1323 ± 37	43***	46.5 ± 1.0	3.4	3.16 ± 0.04	0.12*	96 ± 2.03	1.35**	45.1 ± 0.99	1.57***
$XT_{215/38} \times TV_{3/2}$	1319 ± 28	39**	45.9 ± 1.2	2.8	3.13 ± 0.07	0.09*	95 ± 2.22	1.07*	44.5 ± 1.17	1.02**
$TV_{3/2} \times XT_{215/38}$	1326 ± 39	46***	46.7 ± 1.3	3.6*	3.17 ± 0.08	0.13*	96 ± 1.99	1.46**	44.4 ± 1.22	0.88**
$XT_{215/38} \times B_{2/6}$	1288 ± 24	8	44.2 ± 1.1	1.1	3.09 ± 0.09	0.06	95 ± 2.14	0.18	44.7 ± 1.19	1.23**
$B_{2/6} \times XT_{215/38}$	1287 ± 29	7	44.0 ± 1.2	0.9	3.08 ± 0.05	0.05	94 ± 2.53	0.02	43.5 ± 1.20	0.05
GD at P = 0.5		19		35		0.09		0.71		0.59
P = 0.1		28		52		0.14		1.05		0.75
P = 0.01		43		81		0.22		1.62		1.35

*Significant at 5%, **Significant at 1% and ***Significant at 0.1%.

The primary goal of silkworm breeding includes simultaneous genetic improvement in the economic value of the population and to exploit it commercially. From the data in Table 3 it is evident that all newly evolved hybrids manifest comparative high values of cocoon weight characters, varied between 2.26 g and 2.42 g, compared with 2.20 g for control. $T_{15/4} \times TBV_{2/24}$, $XT_{215/38} \times TV_{3/2}$ hybrids and their reciprocal crosses were characterized with markedly high cocoon weight and statistically proved exceed the control ($p < 0.01$) with 0.12 – 0.22 g (5.32 – 9.87%). In respect to shell weight character, analogically to cocoon weight character, all new hybrids were manifested higher values, but the differences obtained toward control were bigger. The highest value of this character was determined in $T_{15/4} \times TBV_{2/24}$ and $XT_{215/38} \times TV_{3/2}$ hybrids and their reciprocal crosses (53.5 – 55.7 cg) which exceeded significantly the control values with 0.5 – 0.7 cg (9.63 – 14.14%). Comparative high shell weight in newly evolved silkworm hybrids determines high values of shell ratio, that characterized the relatively silk content in cocoons, 22.69 – 23.2%, respectively, compared with 22.2% for the control. The highest value of this character was determined in $T_{15/4} \times TBV_{2/24}$ and $XT_{215/38} \times TV_{3/2}$ hybrids and their reciprocal crosses (22.9 – 23.1%) and their superiority over the control (3.2 – 4.3 points) was proved.

The newly evolved bivoltine hybrids exhibit high degree of phenotypic variability in connection to main technological characters of the filament (Table 4). It is evident, that comparative high values of main cocoon technological characters (shell weight and shell ratio) in new hybrids, created with participation of sex-limited parent lines lead to higher values of filament length, 1,281 – 1,326 m, respectively, compared to 1,280 m for the control. $T_{15/4} \times TBV_{2/24}$ and $XT_{215/38} \times TV_{3/2}$ hybrids and their reciprocal crosses was manifested pointedly high filament length, 1,307 – 1,326 m, which exceeded the $Super_1 \times Hessa_2$ control hybrid significantly with 27 – 46 m (2.11 – 3.59%). In respect to filament weight and silk ratio characters, analogically to filament length character, all new hybrids were demonstrated with 1.6 – 3.6 cg (3.71 – 8.35%) and 0.35 – 1.55% (0.33 – 1.46 points) higher values. They were characterized with comparatively thinly filament, 3.08 – 3.17 denier, compared with 3.04 denier for the control.

Data for the main silkworm moth reproductive characters, that take part in productivity of silkworm eggs from unit of seed cocoons put into grenage production form up show that almost all new hybrids are characterized with genetically determined values, smaller with 12 – 38 number and with 5 – 18 mg weight of normal eggs per lying, compared to the $Super_1 \times Hessa_2$ control hybrid (Table 5). It was established, that when sex-limited were used as female parent, these hybrids had with 15 – 17 and 7 – 11 mg, respectively lower number and weight of normal eggs per lying. Irrespective of this fact, newly evolved hybrids of silkworm were characterized with comparative high values of number and weight of normal eggs per lying, 567 – 593 and 345 – 364 mg, respectively. It was not observed significant differences between tested hybrids and control in connection to physiological waste (parched, sterile, dried, etc. eggs) in lying.

According to Petkov (1995) silkworm productivity of cocoons and raw silk are complex quantitative traits, that integrate silkworm biological and cocoon and filament technological characters. From the data in Table 6 it is evident that all new hybrids of silkworm were demonstrated

Table 5. Reproductive characters of moths

Hybrids	Number of normal eggs per lying		Weight of normal eggs per lying (mg)		Physiological waste in lying (%)	
	$\bar{x} \pm Sx$	± D	$\bar{x} \pm Sx$	± D	$\bar{x} \pm Sx$	± D
$Super_1 \times Hessa_2$ (Control)	605 ± 10.5		363 ± 4.4		2.86 ± 0.05	
$T_{15/4} \times TBV_{2/24}$	569 ± 7.8	-36***	347 ± 4.2	-16***	3.56 ± 0.05	0.70***
$TBV_{2/24} \times T_{15/4}$	586 ± 11.2	-19**	358 ± 3.7	- 5*	3.49 ± 0.04	0.63***
$XT_{215/38} \times TV_{3/2}$	578 ± 9.9	-27***	355 ± 3.3	- 8**	2.84 ± 0.06	-0.02
$TV_{3/2} \times XT_{215/38}$	593 ± 6.3	-12*	364 ± 4.1	1	2.63 ± 0.07	-0.23
$XT_{215/38} \times B_{2/6}$	567 ± 7.7	-38***	345 ± 3.5	-18***	3.04 ± 0.05	0.18
$B_{2/6} \times XT_{215/38}$	582 ± 8.4	-23**	354 ± 2.9	- 9	2.99 ± 0.04	0.13
GD at P = 0.5		12		5		0.24
P = 0.1		17		7		0.35
P = 0.01		27		11		0.54

*Significant at 5%, **Significant at 1% and ***Significant at 0.1%.

Table 6. Yield of cocoons and raw silk per 1 box of silkworms

Hybrids	Cocoon yield per 1 box (kg)		Raw silk yield (kg)	
	$\bar{x} \pm Sx$	± D	$\bar{x} \pm Sx$	± D
$Super_1 \times Hessa_2$ (Control)	38.0 ± 0.60		6.62 ± 0.15	
$T_{15/4} \times TBV_{2/24}$	41.0 ± 1.01	2.90***	7.33 ± 0.22	0.72**
$TBV_{2/24} \times T_{15/4}$	43.2 ± 0.90	5.10***	7.78 ± 0.23	1.17***
$XT_{215/38} \times TV_{3/2}$	42.3 ± 0.99	4.15***	7.61 ± 0.20	1.00**
$TV_{3/2} \times XT_{215/38}$	43.6 ± 0.78	5.49***	7.74 ± 0.18	1.12***
$XT_{215/38} \times B_{2/6}$	39.3 ± 0.91	1.20**	6.92 ± 0.21	0.30
$B_{2/6} \times XT_{215/38}$	40.5 ± 0.63	2.43***	7.04 ± 0.17	0.43
GD at P = 0.5		0.59		0.47
P = 0.1		0.75		0.70
P = 0.01		1.35		1.08

*Significant at 5%, **Significant at 1% and ***Significant at 0.1%.

comparatively high yield of cocoons and raw silk per one box of silkworms, 39.3 – 43.6 kg and 6.9 – 7.8 kg, respectively, compared to 38.1 kg and 6.6 kg for the control. Once again $T_{15/4} \times TBV_{2/24}$, $XT_{215/38} \times TV_{3/2}$ hybrids and their reciprocal crosses were manifested the highest values, which surpass the control $Super_1 \times Hessa_2$ hybrid with 2.9 – 5.5 kg (7.62 – 14.42%) and 0.7 – 1.17 kg (10.84 – 17.61%), respectively.

Based on the performance of the hybrids at the laboratory, these hybrids can be recommended for spring rearing.

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