Identifying Hybrid Genotypes of Mulberry Silkworm (*Bombyx mori*) Using Heterosis for Biological and Silk Yielding Traits

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ABSTRACT

The Mulberry Silkworm Moth is an important economic and domesticated insect species that needs continuously new genetic combinations to avoid fractioning of genetic diversity and gene erosion. In this study ten bivoltine hybrids were screened by heterosis using 11 biological and silk yielding quantitative traits. Heterosis was measured using multiple evaluation index (MEI), mid-parent heterosis (MPH) and better-parent heterosis (BPH), and hybrids were ranked using MEI and cumulative sub-ordinate function (CSF). The hybrids produced MEI >50 with the highest in $PO_{206} \times J_{101}$ (63.2). All hybrids produced evaluation index (EI) >50 for filament length, while seven hybrids produced EI >50 for fecundity, larval body weight, pupation rate, cocoon shell ratio and cocoon yield. The hybrids improved vigour of biological and silk yielding by 5.6% to 27.5% over respective mid-parents values. $PO_{206} \times J_{101}$ produced the highest MPH (66.8%) for cocoon shell weight, while $MKD_{205} \times C_{102}$ produced a negative MPH (- 0.8%) for silk productivity. A mean heterosis of 4.2% to 23.0% was found over respective better-parents. $PO_{206} \times J_{101}$ produced the highest BPH (54.8%) for cocoon shell weight. $MKD_{206} \times C_{102}$ produced a negative BPH for egg hatchability, larval body weight and pupation rate. The heterosis findings showed enough genetic divergence and variable combining ability of the parental strains. CSF varied between 0.5 and 0.79. $PO_{206} \times J_{101}$, based on MEI and SF, was ranked 1st, followed by $PO_{205} \times C_{102}$ and recommended for field rearing.

INTRODUCTION

About 125 genera of Lepidoptera order spin silk. Amongst them the mulberry silk moth, *Bombyx mori* L. (Lepidoptera: Bombycidae**)** has great economic importance. The species had completely been domesticated passing through an evolutionary process spreading over a time period of about 5,000 years (Yukuhiro *et al.*, 2002; Xiang *et al.*, 2005; Bajwa *et al.*, 2017). Currently, *B. mori* is contributing about 90% in global natural silk production (Ruiz and Almanza, 2018). The species spins white, lustrous, soft, biodegradable and highly crystalline silk. The silk fibre has magnificent mechanical properties including strength, stiffness and hardness under tensile and compressive stress (Li *et al.*, 2002; Rigueiro *et al.*, 2002;

Bajwa *et al.*, 2019). Silkworm is also a perfect model insect species for scientific studies (Meng *et al.*, 2017). The silk gland that produces natural silk is a useful and efficient bioreactor for producing many recombinant proteins (Xu *et al.*, 2019). The fibroin in natural silk is an ideal biomaterial for regenerative medicines, dental floss and pharmacological products (Pham and Tiyaboonchai, 2020). This silk is also used as surgical sutures for ligatures and cardiovascular surgery (Holland *et al.*, 2019; Sun *et al.*, 2021).

A great genetic diversity has evolved in *B. mori* during domestication and adaptability process. Currently, about 2,000 strains of the species have been maintained globally at different sericulture research and teaching organizations (Hemmatabadi *et al.*, 2016). This genetic source is classified based on either voltinism (number of lifecycles in a year), moultinism (number of moults per larval-cycle) or geographical origin (Chinese, Japanese, European and Tropical) (Furdui *et al.*, 2014). This genetic diversity is a precious source for synthesizing new strains of high productivity potential. The productivity potential can be enhanced simply by concentrating desired genes and reducing variation amongst off springs through artificial selection (Gjedrem, 2005). However, the intensive artificial

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selection increases the intra-population homogeneity and subsequently greater expression of recessive alleles called inbreeding depression or fractioning of genetic diversity (Ruiz and Almanza, 2018). To avoid fractioning of genetic diversity and loss of genes, new genotypes are evolved by hybridization of strains with interest-specific traits. This is obverse of inbreeding and effectively restore vigor and reverse the deleterious recessive alleles effect (Kang *et al*., 2004).

The sustainable development of sericulture owes a lot to continuous manipulation of genetic sources of *B. mori* for hybrid vigour (Jalali *et al*., 2011). Hybridization in the Mulberry Silkworm Moth started in 19th century in China and Japan. The primary objective of silkworm hybridization is to evolve robust strains to replace the old ones. The new strains are resistant to diseases, and have high productivity potential and environment adaptive compared to earlier strains (Kang *et al*., 2004; Sahan, 2020). Scores of hybrids have been synthesized with superior biological traits like fecundity, egg hatchability, larval development, pupation rate and silk yielding traits including cocoon weight, cocoon silk percentage, silk yield, silk filament (Talebi *et al*., 2010; Ghazy, 2012; Ghazy *et al*., 2017; Fouad, 2020; Sahan, 2020).

In Pakistan, sericulture is a cottage industry and farmers traditionally rear bivoltine inbred silkworm strains. These strains are of Chinese and Japanese origin and have lost productivity and resistance against diseases due to fractioning of genetic diversity. Silkworm strains have a pivotal role in sericulture (Zhao *et al*., 2007), yet a little attention was given to evolve new genetic combinations through hybridization in the country (Bukhari *et al*., 2008). Present study was, therefore, undertaken to identify hybrid strains of superior biological and silk yielding traits for improving silkworm productivity. The superior strains will also add new productive genetic combinations in silkworm genetic source and will be handy for scientific community and sericulture development world-wide. Specifically, we screened genotypes using heterosis, a genetic parameter, based on: multiple evaluation index, mid-parent heterosis (Relative heterosis), better-parent heterosis (Heterobeltiosis) and sub-ordinate function.

MATERIALS AND METHODS

The study was conducted at Pakistan Forest Institute, Peshawar in 2019. Six inbred bivoltine strains of *B. mori* including: C_{102}, PO_{205}, PO_{206} (Chinese origin), and J_{101}, MKD_{205}, MKD_{206} (Japanese origin) were bred to synthesize ten hybrids: $MKD_{205} \times PO_{205}$, $MKD_{205} \times PO_{206}$, $MKD_{205} \times C_{102}$, $MKD_{206} \times C_{102}$, $MKD_{206} \times PO_{205}$, $J_{101} \times PO_{205}$, $PO_{205} \times C_{102}$, $PO_{206} \times J_{101}$, $C_{102} \times MKD_{206}$ and $C_{102} \times J_{101}$. The disease free layings of hybrids were surface sterilized using 2.0% formalin aqueous solution and incubated at $25 \pm 2°C$. The neonates were brushed on finely chopped leaves of *Morus alba* var. PFI-1 and reared following standard laboratory conditions as described by Bukhari *et al*. (2008). Four hundred 3rd instar larvae hybrid[-1] were separated at random from mass rearing and reared in wooden trays (30 × 20 × 2.5) cm³. The full grown 5th instar larvae were provided collapsible plastic mountages for pupation and cocoons were harvested on day-7 after pupation.

Eleven biological and silk yielding quantitative traits were recorded: fecundity (number of eggs female[-1]), egg hatchability (%), 5th instar larval body weight (g), 5th instar larval lifespan (h), pupation rate (%), cocoon weight (g), cocoon shell weight (g), cocoon shell ratio (%), filament length (m), silk productivity (cg/d) and cocoon yield 10,000[-1] larvae (kg). Pupation rate, cocoon weight, cocoon shell weight and cocoon shell ratio were estimated following Bukhari *et al*. (2008). Silk productivity was estimated according to Fouad (2020).

Evaluation index (EI), mid-parent heterosis (MPH) and better-parent heterosis (BPH) were calculated as described by Talebi *et al*. (2010) and Ghazy (2014).

Sub-ordinate function was calculated according to Gower (1971).

RESULTS

The values of mean multiple evaluation index and evaluation index for each trait of inbred strains and hybrids are presented in Table I. MEI of inbred strains varied from 36.8 to 45.3, while MEI of hybrids varied from 51.4 to 63.2. $PO_{206} \times J_{101}$ produced the highest MEI while $MKD_{205} \times PO_{206}$ produced the lowest MEI. The difference between $PO_{206} \times J_{101}$ and $PO_{205} \times C_{102}$ was marginal. Similarly, the difference between $MKD_{205} \times PO_{206}$ and $J_{101} \times PO_{205}$ was negligible. The inbred strains C_{102}, PO_{206} and MKD_{206} produced EI >50 for pupation rate, fecundity and 5th instar larval lifespan, respectively. All the hybrids produced EI >50 for filament length, while 80% hybrids produced EI >50 for egg hatchability and 5th instar larval lifespan. Seventy percent hybrids produced EI >50 for fecundity, larval body weight, pupation rate, cocoon shell ratio and cocoon yield, while sixty percent hybrids produced EI >50 for cocoon weight and cocoon shell weight. Half of the hybrids produced EI >50 for silk productivity. $PO_{205} \times C_{102}$ produced EI >50 for 11 quantitative traits, while $MKD_{205} \times PO_{205}$, $MKD_{205} \times C_{102}$ and $C_{102} \times J_{101}$ produced EI >50 for 90.9% under study quantitative traits. $PO_{206} \times J_{101}$ and $MKD_{206} \times C_{102}$ produced EI >50 for 72.7% and 63.6% traits, respectively. $MKD_{206} \times PO_{205}$ and $J_{101} \times PO_{205}$ produced EI >50 for the least number of quantitative traits (45.5%).

The outcome of mid-parent heterosis analysis is presented in Table II. The mean MPH was positive for all the hybrids and ranged from 5.6% to 27.5%. $PO_{206} \times J_{101}$ produced the highest MPH, while $MKD_{205} \times PO_{206}$ produced the lowest MPH. All hybrids produced positive MPH for 11 quantitative traits except $MKD_{205} \times C_{102}$ that produced negative MPH for silk productivity. $C_{102} \times J_{101}$ and $J_{101} \times PO_{205}$ produced the highest MPH for fecundity and egg hatchability, respectively, while $MKD_{205} \times C_{102}$ and $PO_{205} \times C_{102}$ produced the highest MPH for 5th instar larval lifespan and pupation rate, respectively. $PO_{206} \times J_{101}$ produced the highest MPH for 63.6% quantitative traits, while four hybrids produced the highest MPH for one quantitative traits each. Trait-wise, $PO_{206} \times J_{101}$ produced the highest MPH for 5th instar larval body weight, cocoon weight, cocoon shell weight, cocoon shell ratio, filament length, silk productivity and cocoon yield. $MKD_{205} \times C_{102}$ produced the lowest MPH for silk productivity (- 0.8%), followed by $MKD_{205} \times PO_{206}$ (0.3%) also for silk productivity. Based on mean MPH, hybrids could be divided into three groups including: (i) hybrid with MPH >20% ($PO_{206} \times J_{101}$), (ii) hybrids with MPH >10% (6 hybrids), and (iii) hybrids with MPH <10% (3 hybrids).

Table I. Multiple evaluation index of bivoltine inbred and hybrid strains of *B. mori*.

Silkworm strains	Quantitative traits											Mean
	Fecun	Hatch	LBW	5th LS	PR	CW	CSW	CSR	FL	SP	CY	
C_{102}	44.6	49.3	39.3	36.2	51.7	45.2	43.7	42.4	43.5	47.3	41.7	44.1
PO_{205}	40.2	34.1	46.8	41.3	44.6	39.5	36.4	31.5	37.9	37.9	41.3	39.2
PO_{206}	59.8	46.9	39.7	41.3	34.4	45.3	46.6	49.6	44.2	49.0	41.7	45.3
J_{101}	27.7	36.1	36.2	31.0	39.3	39.9	38.9	37.7	37.7	43.4	37.2	36.8
MKD_{205}	46.3	34.0	36.2	41.3	40.4	46.5	46.7	48.0	40.8	49.1	45.1	43.1
MKD_{206}	39.9	40.3	46.4	56.9	35.5	35.3	36.5	37.9	31.7	34.6	37.5	39.3
$MKD_{205} \times PO_{205}$	47.2	60.5	63.2	62.1	58.2	54.0	54.7	55.9	55.2	51.3	54.2	56.0
$MKD_{205} \times PO_{206}$	63.9	51.4	51.8	51.7	44.9	48.4	49.7	52.3	52.6	49.2	49.2	51.4
$MKD_{205} \times C_{102}$	53.9	57.1	60.4	62.1	62.6	50.0	51.0	53.3	53.1	47.7	50.6	54.7
$MKD_{206} \times PO_{205}$	44.0	63.9	49.1	62.1	57.5	49.0	49.0	49.8	54.0	45.8	55.3	52.7
$MKD_{206} \times C_{102}$	56.1	48.2	45.2	49.1	49.9	50.9	56.5	64.9	55.9	57.0	51.4	53.2
$J_{101} \times PO_{205}$	44.3	62.9	43.9	56.9	61.3	46.8	49.0	53.2	53.3	47.1	47.8	51.5
$PO_{205} \times C_{102}$	56.0	52.3	65.4	62.1	63.0	65.9	63.6	58.8	62.9	60.0	64.8	61.4
$PO_{206} \times J_{101}$	52.0	47.3	64.1	43.9	41.5	75.2	76.0	70.6	70.2	79.4	74.5	63.2
$C_{102} \times MKD_{206}$	64.8	62.1	58.9	50.4	60.4	48.6	47.9	47.9	52.7	47.7	49.4	53.7
$C_{102} \times J_{101}$	59.3	53.5	53.3	51.7	54.8	59.6	53.8	46.4	54.4	53.4	58.1	54.4

Fecun, Fecundity; Hatch, Hatchability; LBW, Larval body weight; 5th LS, 5th instar larval lifespan; PR, Pupation rate; CW, Cocoon weight; CSW, Cocoon shell weight; CSR, Cocoon-shell ratio; FL, Filament length; SP, Silk productivity; CY, Cocoon yield $10,000^{-1}$ larvae.

Table II. Mid-parent heterosis effect (%) of bivoltine hybrids of *B. mori*.

Hybrids	Quantitative traits											Mean
	Fecun	Hatch	LBW	5th LS	PR	CW	CSW	CSR	FL	SP	CY	
$MKD_{205} \times PO_{205}$	2.2	11.7	21.7	10.5	5.8	12.8	27.1	12.9	15.1	15.0	14.6	13.6
$MKD_{205} \times PO_{206}$	5.8	4.7	14.3	5.3	2.8	2.9	5.6	2.7	9.4	0.3	7.6	5.6
$MKD_{205} \times C_{102}$	4.7	6.6	23.5	12.0	6.1	4.6	11.1	6.2	10.2	- 0.8	9.5	8.5
$MKD_{206} \times PO_{205}$	2.3	11.7	2.4	6.3	6.5	14.4	28.9	12.6	19.1	21.1	22.1	13.4
$MKD_{206} \times C_{102}$	7.9	1.4	2.3	1.3	2.4	12.8	34.9	19.8	17.7	32.3	16.4	13.6
$J_{101} \times PO_{205}$	6.1	12.3	2.5	10.8	7.2	8.6	25.5	15.6	14.9	13.1	11.9	11.7
$PO_{205} x C_{102}$	5.5	5.0	8.7	9.8	7.4	8.5	11.8	5.7	7.4	2.0	7.7	7.2
$PO_{206} \times J_{101}$	4.7	2.5	27.1	4.1	1.8	38.1	66.8	21.0	27.4	60.5	48.5	27.5
$C_{102} \times MKD_{206}$	12.7	7.3	15.8	1.9	6.2	10.0	16.6	6.1	14.5	13.7	13.5	10.8
$C_{102} \times J_{101}$	13.6	4.6	16.1	9.6	3.4	19.9	25.9	5.1	13.0	15.0	25.8	13.8
SE	1.23	1.24	2.91	1.23	0.67	3.16	5.46	2.06	1.81	5.78	3.90	1.90
CV	0.59	0.58	0.68	0.54	0.43	0.75	0.68	0.61	0.39	1.06	0.69	0.48

For abbreviations, see Table I.

Results of better-parent heterosis for hybrids and traits are presented in Table III. Mean BPH effect was positive in all tested hybrids and ranged between 4.2% and 23.0%. $PO_{206} \times J_{101}$ and $MKD_{205} \times PO_{206}$ produced the highest and the lowest mean BPH, respectively. $PO_{206} \times J_{101}$ produced the highest BPH for 5th instar larval body weight, cocoon weight, cocoon shell weight, silk productivity and cocoon yield. $J_{101} \times PO_{205}$ produced the highest BPH for egg hatchability, 5th instar larval lifespan and pupation rate, while $C_{102} \times MKD_{206}$ and $MKD_{206} \times C_{102}$ produced the highest BPH for fecundity and cocoon shell ratio, respectively. All hybrids produced positive BPH for 54.5% traits, while four hybrids produced BPH for 90.9% traits. $MKD_{206} \times C_{102}$ produced positive BPH for 72.7% traits. $MKD_{205} \times C_{102}$ produced negative BPH for silk productivity and rest of hybrids produced positive BPH for all silk yielding parameters. Based on the outcome of mean MPH, the hybrids could be divided into three groups including: (i) hybrid with BPH >20% ($PO_{206} \times J_{101}$), (ii) hybrids with BPH >10% (3 hybrids), and hybrids with BPH <10% (6 hybrids).

The outcome of cumulative sub-ordinate function (CSF) and sub-ordinate function of traits is presented in Table IV. CSF varied between 0.5 and 0.79. $PO_{206} \times J_{101}$ and $PO_{205} \times C_{102}$ resulted in the highest CSF, while $MKD_{205} \times PO_{206}$ resulted in the lowest CSF. $PO_{206} \times J_{101}$ resulted in the lowest sub-ordinate function for 27.3% traits: egg hatchability, 5th instar larval lifespan and pupation rate, while $J_{101} \times PO_{205}$ resulted in the lowest SF for larval body weight, cocoon weight and cocoon yield. $PO_{206} \times J_{101}$ and $PO_{205} \times C_{102}$, based on MEI and CSF, were ranked 1st and 2nd, respectively, while $MKD_{205} \times PO_{206}$ was at the bottom of the ranking list.

Table III. Better-parent heterosis effect (%) for bivoltine hybrids of *B. mori*.

Hybrid	Quantitative traits											Mean
	Fecun	Hatch	LBW	5th LS	PR	CW	CSW	CSR	FL	SP	CY	
$MKD_{205} \times PO_{205}$	0.5	11.7	15.6	10.5	5.0	8.4	14.8	6.0	13.5	3.9	11.8	9.2
$MKD_{205} \times PO_{206}$	2.1	1.9	12.3	5.3	1.7	2.1	5.5	2.0	7.7	0.2	5.3	4.2
$MKD_{205} \times C_{102}$	3.7	3.3	21.6	13.5	3.9	3.9	8.0	4.0	8.8	- 2.3	7.1	6.9
$MKD_{206} \times PO_{205}$	2.2	5.1	2.3	10.5	4.7	11.5	29.0	9.7	15.5	16.7	19.0	11.5
$MKD_{206} \times C_{102}$	6.5	- 0.5	- 1.2	6.8	- 0.6	6.4	25.3	17.7	11.4	17.3	16.1	9.6
$J_{101} \times PO_{205}$	2.3	11.8	- 2.7	13.9	6.1	8.3	22.1	12.7	14.8	7.2	8.9	9.6
$PO_{205} \times C_{102}$	6.4	1.3	17.7	13.5	4.1	23.4	39.3	12.9	17.8	22.7	31.1	17.3
$PO_{206} \times J_{101}$	- 4.0	0.2	24.8	6.9	0.8	33.9	54.8	15.6	23.7	52.8	44.0	23.0
$C_{102} \times MKD_{206}$	11.3	5.3	19.5	7.4	3.1	3.8	8.3	4.3	8.4	0.8	10.3	7.5
$C_{102} \times J_{101}$	8.2	1.8	14.3	11.1	1.1	16.3	19.9	3.1	10.0	11.0	22.1	10.8
SE	1.4	1.4	3.1	1.0	0.7	3.2	4.9	1.8	1.6	5.1	3.8	1.7
CV	1.1	1.1	0.8	0.3	0.7	0.9	0.7	0.6	0.4	1.2	0.7	0.5

For abbreviations, see Table I.

Table IV. Sub-ordinate Function for bivoltine hybrids of *B. mori*.

Hybrid	Quantitative traits											CSF
	Fecun	Hatch	LBW	5th LS	PR	CW	SW	CSR	FL	SP	CY	
$MKD_{205} \times PO_{205}$	0.53	0.88	0.93	1.00	0.83	0.47	0.46	0.62	0.61	0.37	0.46	0.65
$MKD_{205} \times PO_{206}$	0.98	0.58	0.53	0.67	0.37	0.33	0.34	0.53	0.54	0.33	0.32	0.50
$MKD_{205} \times C_{102}$	0.71	0.77	0.83	1.00	0.98	0.37	0.37	0.56	0.56	0.29	0.36	0.62
$MKD_{206} \times PO_{205}$	0.44	1.00	0.44	1.00	0.81	0.34	0.32	0.47	0.58	0.25	0.48	0.56
$MKD_{206} \times C_{102}$	0.77	0.47	0.31	0.58	0.54	0.39	0.51	0.85	0.63	0.50	0.38	0.54
$J_{101} \times PO_{205}$	0.45	0.97	0.26	0.83	0.94	0.29	0.32	0.55	0.56	0.28	0.28	0.52
$PO_{205} \times C_{102}$	0.76	0.61	1.00	1.00	1.00	0.77	0.69	0.70	0.81	0.57	0.74	0.79
$PO_{206} \times J_{101}$	0.66	0.44	0.96	0.42	0.25	1.00	1.00	1.00	1.00	1.00	1.00	0.79
$C_{102} \times MKD_{206}$	1.00	0.94	0.78	0.63	0.91	0.33	0.29	0.42	0.54	0.29	0.32	0.59
$C_{102} \times J_{101}$	0.85	0.65	0.59	0.67	0.71	0.61	0.44	0.38	0.59	0.42	0.56	0.59
SE	0.06	0.07	0.09	0.07	0.08	0.07	0.07	0.06	0.05	0.07	0.07	0.03
CV	0.28	0.28	0.41	0.28	0.36	0.48	0.47	0.32	0.23	0.52	0.46	0.17

For abbreviations, see Table I.

DISCUSSION

The superior silkworm strains that can withstand adverse environmental conditions and produce greater silk are evolved through artificial selection and hybridization or both. Apart from improving productivity, hybridization is necessary to set aside the degenerating effect of recurrent rearing and protecting gene erosion. The superior strains/ hybrids are identified using either of the three genetic parameters: general combining ability, special combining ability or heterosis for important biological and silk yielding traits (Sahan, 2020). Biological and silk yielding traits are under multiple gene control and are also affected by nutrition and rearing conditions (Mirhosseini et al., 2005; Zhao et al., 2007; Hemmatabadi et al., 2016). In present study both, parental strains and hybrids were subjected to same environmental and nutrition conditions, hence any difference in biological and silk yielding traits is assigned to their genetic constitution.

Many methods are applied to assess heterosis including: multiple evaluation index, mid-parent heterosis and better-parent heterosis (Ghazy, 2012; Fouad, 2020). Among these, MEI is a simple statistical application that identifies important economic and robust strains by giving equal weightage to the quantitative traits. According to Ghazy (2014), strains that produce MEI >50 are considered of high economic importance. The under study hybrids produce MEI >50, thus indicate that all hybrids have high economic importance. MEI of hybrids varies from 51.4 to 63.2 and that of parental strains from 36.8 to 45.3. Similarly, EI of different quantitative traits varies from 41.5 to 76.0. EI is relatively greater for silk yielding traits compared to biological traits. The present MEI values are broadly comparable with earlier reported by Bhat et al. (2018); Sajgotra and Gupta (2018), and Alam et al. (2020). Bhat et al. (2018) reported MEI between 45.16 and 56.86 for 18 pure and hybrid strains. Sajgotra and Gupta reported MEI from 31.38 to 57.51 for ten bivoltine hybrids, while Alam and co-workers found MEI between 42.98 and 61.66. All these researchers found variably higher EI values for pupation rate, cocoon weight, cocoon shell weight, cocoon shell ratio, cocoon yield and filament length. The difference between present MEI values and that earlier reported by different researchers may be explained in terms of different genetic constitutions and rearing conditions. Because biological and silk yielding traits are influenced, inter alia by environmental conditions, rearing season, nutrition and genetic constitution of the strains (Rahmathulla, 2012). The present findings further reveal that Chinese parental strains (PO$_{205}$, PO$_{206}$ and C$_{102}$) give relatively more productive hybrid combinations either with Japanese parental strains or among themselves.

The poor performance of Japanese strains and their combinations may be due to unfavourable environmental conditions. Previously, Rahmathulla (2012) reported poor performance of Japanese bivoltine strains under tropical conditions.

The present hybrids produce positive mean MPH and BPH. The MPH was positive for all under study traits except for silk productivity in MKD$_{205}$×C$_{102}$. Whereas, six hybrids produce positive BPH for all quantitative traits. BPH was positive for 5th instar larval lifespan, cocoon weight, cocoon shell weight, cocoon shell ratio, filament length and cocoon yield. MKD$_{206}$×C$_{102}$ produces a negative BPH for 27.3% traits, i.e. the egg hatchability, larval body weight and pupation rate. These findings are broadly in corroboration with Talebi and Subramanya (2009); Ghazy (2012) and Fouad (2020). Talebi and Subramanya (2009) found the highest heterotic effect on cocoon weight, cocoon shell weight, cocoon shell ratio and filament length, while Ghazy (2012) found positive MPH in 14 hybrids for biological and silk yielding traits and positive BPH in majority of the hybrids for economic cocoon characters. Similarly, Fouad (2020) reported positive heterosis over respective mid-parent and better-parent values in six hybrids for cocoon weight, cocoon shell weight, cocoon shell ratio, silk productivity and pupal weight. He could not, however, find a hybrid with positive BPH for all tested 14 biological and silk yielding traits. The negative heterotic effect may be assigned to higher concentration of nonadditive genes in these genetic combinations as the magnitude of the heterotic effect is regulated by the amount of genes with nonadditive expression (Mai et al., 2021). The magnitude of positive heterotic response is inversely related to the amount of nonadditive genes. The present findings and previously reported too show inter-trait large variation in heterosis. This highlights the importance of using maximum quantitative traits preferably widely related for assessing heterosis.

Heterosis is a genetic parameter used to measure vigour of hybrid genotypes. Theoretically the level of heterosis is directly proportional to the scale of genetic dissimilarity or genetic divergence in parental strains. The inter-specific hybrids produce greater heterosis compared to intra-specific hybrids (Mai et al., 2021). Magnitude of heterosis in present hybrids, specifically in PO$_{206}$×J$_{101}$ may be attributed to different levels of genetic divergence in parents. Earlier Bajwa et al. (2017), using five random amplified polymorphic DNA primers, found a mean genetic similarity distance from 0.124 to 0.943 in current parental strains. They found further that at 66% similarity level, J$_{101}$, C$_{102}$ and PO$_{205}$ were clustered in one group, while MKD$_{205}$ and PO$_{206}$ in another group. The current variation in heterosis is further explained in

terms of combining ability of different parental strains that varies with parents, as well as, with quantitative trait. Previously, Talebi *et al*. (2010) recorded different levels of heterosis for larval weight, cocoon weight, cocoon shell weight and shell percentage due to genetic divergence and combining ability in parental lines. Greater genetic divergence and higher combining ability in parents derives greater heterosis.

CONCLUSION

Our findings show a significant heterosis in the hybrids. Hybrid $PO_{206} \times J_{101}$ increases mean vigour by 27.5% and 23.0% over the mid-parent value and better-parent (PO_{206}), respectively. Moreover, $PO_{206} \times J_{101}$ improved cocoon shell weight by 66.8% and 54.8% over mid-parents and better parent, respectively. Broadly, heterosis effect was greater in silk yielding traits compared to biological traits. The intra and inter hybrid variation in MEI, MPH, and BPH for different traits shows enough genetic diversity and different levels of combining ability in the parental strains. Based on present findings of MEI, MPH, BPH and CSF, $PO_{206} \times J_{101}$ is ranked 1st and recommended for field rearing.

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Statement of conflict of interest
The authors have declared no conflict of interest.

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