Int. J. Indust. Entomol. Vol. 2, No. 2, 2001, pp. 123~127

International Journal of
Industrial Entomology

Studies on the Effect of High Temperature on F1 Hybrids Between Polyvoltine and Bivoltine Silkworm Races of *Bombyx mori* L.

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(Received 1 February 2001; Accepted 24 April 2001)

In order to introduce bivoltine races in a tropical country like India, it is necessary to have stability in cocoon crop under high temperature environments. Unlike any temperate country like Japan, the rearing conditions/environment, climatic conditions, quality of mulberry leaf and incidence of diseases are unpredictable in India. Geneticists and breeders of all the sericultural countries have experienced the influence of environment during the process of breeding. in order to select efficiently the breeds with high temperature tolerance, it is very important to analyse clearly the heritability nature of high temperature tolerance. In light of the above, the present study was undertaken to determine the effect of high temperature treatment of (A) 35 ± 1°C and 85 ± 5% RH for 24 hrs continuously, (B) 35 ± 1°C and 85 ± 5% RH for 48 hrs continuously and (C) the control (25 ± 1°C and 65 ± 5% RH in the normal rearing condition from the 3rd of 5th instar on the pure races such as Moria, N137 and C146 as well as their F1 hybrids. The overall performance indicate that the hybrids are more tolerant than the pure races. it was also observed that the overall performance declined in those batches where 48 hrs treatment was given. The most interesting observation noticed in this study was that there was maternal effect regarding temperature tolerance as evident from the better performance of those hybrids where the female parent used was more tolerant as pure race.

Key words : *Bombyx mori*, Bivoltine, Hybrid vigour, High temperature tolerance, Heterosis, Heterobeltiosis

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Introduction

In order to introduce bivoltine races in a tropical country like India, it is necessary to have stability in cocoon crop under high temperature environments. As such, it becomes a difficult task to breed such bivoltine silkworm races, which are suitable to high temperature environments. During the course of breeding to evolve sustainable bivoltine breeds for commercial exploitation, it will be more appropriate to evolve breeds, which can tolerate various stresses. Unlike any temperate country like Japan, the rearing conditions/environment, climatic conditions, quality of mulberry leaf and incidence of diseases are unpredictable in India. So it is necessary to keep in mind the various stresses during the course of breeding and in particular selection. It is a well-established fact that under tropical condition, unlike the polyvoltines the bivoltines are more vulnerable to various stresses. One of the important stresses faced in a tropical country like India is the high temperature environment not congenial to bivoltine rearing.

Geneticists and breeders of all the sericultural countries have experienced the influence of environment during the process of breeding. Shibukawa (1965) studied the silkworm viability and cocoon weight for 19 generations at two different temperatures and humidity. He observed that the lines selected at high temperature and high humidity perform better than the lines selected at normal temperature and humidity. The effect of high temperature higher than 30°C on silkworm larvae was reported earlier by Takeuchi *et al.* (1964), and Ohi and Yamashita (1977). Huang *et al.* (1979), and He and Oshiki (1984) used survival rate of silkworms as a main characteristic for evaluating thermotolerance. Kato *et al.* (1989) in a series of experiments observed that the resistance to high temperature is a heritable character and it may be possible to breed silkworm races tolerant high temperature. Falconer

(1990) in his review on the environmental effect on insects concluded that the performance of an insect is best improved by selection in the environment in which it is subsequently exploited. Shirota (1992), and Tazima and Ohnuma (1995) while attempting to synthesise high temperature resistant silkworm races confirmed the genetical heritability nature of thermotolerance by selection based on pupation rate of silkworms reared under high temperature conditions in fifth instar. Keeping this in view, four compatible bivoltine hybrids for rearing throughout the year were developed by utilising Japanese thermotolerant hybrids as breeding resource material (Datta *et al.*, 1997) and suggested that any study involving temperature as one of the environmental factors and viability followed by cocoon traits is a trend setter to provide basis to formulate appropriate selection policies for required environments. While studying the performance of robust and productive hybrids under two temperature conditions, Suresh Kumar *et al.* (1999) and Datta *et al.* (2000) indicated that the deleterious effect of high temperature was more pronounced in productive hybrids than the robust hybrids. In order to select efficiently the breeds with high temperature tolerance, it is very important to analyze clearly the heritability nature of high temperature tolerance. In light of the above, the present study was undertaken to determine the effect of high temperature on the pure races as well as their F1 hybrids.

Materials and Methods

The polyvoltine race, Moria and the bivoltines, N137 and C146 and their F1 hybrids including reciprocals were used as materials for the study. Composite layings consisting of about 100 eggs from each of the 5 layings were prepared for brushing. Rearing was conducted in an environmentally controlled rearing house at National Institute of Sericultural and Entomological Sciences, Matsumoto, Japan. All the batches were reared upto the 2nd day of 5th instar in the normal rearing condition. On the 3rd day of 5th instar, 100 larvae each of these breeds/hybrids were subjected for high temperature treatment of (A) 35 ± 1°C and 85 ± 5% RH for 24 hrs continuously, (B) 35 ± 1°C and 85 ± 5% for 48 hrs continuously and (C) the control (25 ± 1°C and 65 ± 5% RH) in the normal rearing condition. The larvae were kept in plastic trays (size 65.5 cm× 44.5 cm× 10.8 cm) covered with a lid. The temperature treatment was given a separate chamber where temperature and humidity to the desired level was set. Various parameters like daily mortality, survival rate, cocoon weight, shell weight, shell ratio and cocoon size measurements were recorded. For the cocoon size measurement, the length and breadth of 50 cocoons of each treatments were measured using vernier calipers and the standard deviation was calculated for assessing the cocoon uniformity with respect to all the temperature treatments. Besides, heterosis and heterobeltiosis were also calculated with respect to all the temperature treatments. The results obtained were also subjected for ANOVA for finding the significance of treatments.

Results and Discussion

The overall performance indicate that the hybrids are more tolerant than the pure races. In the case of Moria, no mortality was observed before spinning in all the treatments. But in all the other cases more mortality was observed in those batches where 48 hours treatment was given. Maximum mortality before spinning was observed in N137 where 48 hrs treatment was given. It was also observed that the overall performance declined in those batches where 48 hrs treatment was given. The performance of both the pure races and their hybrids at the three different temperature treatments is given in Table 1 and 2. Perusal of the data clearly indicate that the deleterious effect of temperature with regard to pupation, cocoon weight, shell weight and shell ratio on the pure races as well as their hybrids was more pronounced in the treatment B (48 hrs), followed by B (24 hrs) and C (Room temperature).

With regard to the pupation rate in pure races, the highest pupation was recorded by Moria and the least by N137

Table 1. Performance of pure races at different temperature treatments*

Breeds	Pupation (%)			Cocoon weight (g)			Shell weight (mg)			Shell ratio (%)		
	A	B	C	A	B	C	A	B	C	A	B	C
Moria	94.3	93.3	96.6	1.03	0.80	1.11	14.2	10.8	16.0	13.8	13.5	14.4
N137	64.7	12.7	83.3	1.20	1.02	1.40	25.3	18.3	32.1	21.2	17.9	23.0
C146	78.3	42.0	83.7	1.49	1.37	1.61	33.6	30.0	39.0	22.6	21.9	24.2
CD at 5%(temp.)		102.25			0.035			0.69			0.56	
CD at 5%(breeds)		95.30			0.027			0.51			0.44	
CD at 5% (temp×breeds)		165.07			0.046			0.88			0.76	

Table 2. Performance of hybrids at different temperature treatments*

Hybrids	Pupation (%)			Cocoon weight (g)			Shell weight (mg)			Shell ratio (%)		
	A	B	C	A	B	C	A	B	C	A	B	C
Moria×N137	97.0	89.7	97.7	1.60	1.39	1.84	30.8	25.4	36.3	19.2	18.3	19.7
N137×Moria	88.3	79.3	97.3	1.35	1.19	1.48	25.5	21.8	28.9	18.9	18.3	19.5
Moria×C146	96.0	89.7	97.7	1.57	1.42	1.93	30.5	26.4	39.5	19.5	18.6	20.4
C146×Moria	82.3	74.0	92.7	1.38	1.30	1.81	26.9	24.4	39.5	19.5	18.8	21.8
N137×C146	82.0	66.7	88.3	1.64	1.45	2.18	37.4	31.5	54.8	22.8	21.7	25.1
C146×N137	96.0	83.7	98.3	1.78	1.81	2.14	42.5	42.7	53.6	23.9	23.5	25.1
CD at 5 % (temp)		110.47			0.031			0.75			0.17	
CD at 5% (hybrids)		78.11			0.022			0.53			0.12	
CD at 5%(temp.×hybrids)		191.33			0.053			1.31			0.29	

*High temperature treatment was performed from 48 hrs after 4th moult.
A = At 35 ± 1°C and 85 ± 5% RH for 24 hrs continuously.
B = At 35 ± 1°C and 85 ± 5% RH for 48 hours continuously.
C = At 25 ± 1°C and 65 ± 5% RH continuously.

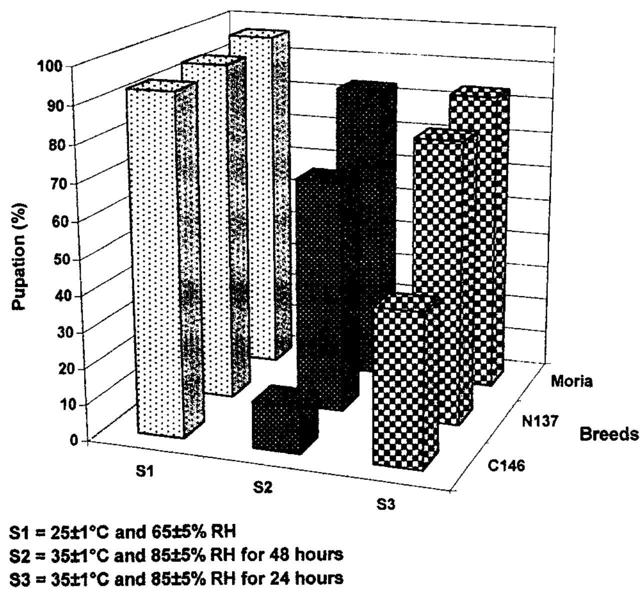

Fig. 1. Comparative performance of pure breeds at different temperature treatments.

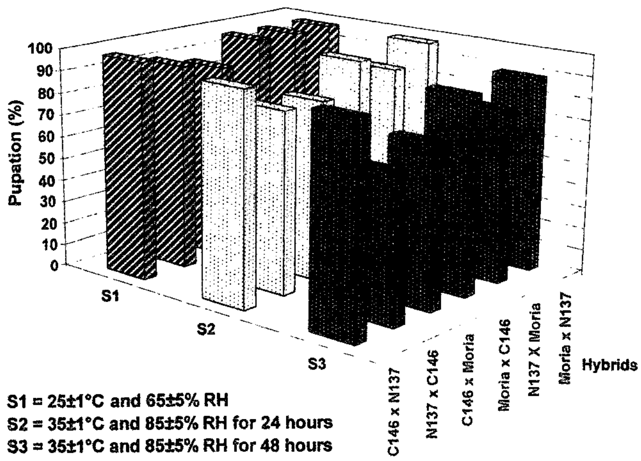

Fig. 2. Comparative performance of hybrids at different temperature treatments.

in all the three temperature treatments (Fig. 1). Similarly, among the hybrids, the highest pupation rate was recorded by Moria× N137 and the least by N137× C146 in all the temperature treatments (Fig. 2). Comparatively higher survival noticed in Moria could be attributed to the fact that this breed is from a tropical country and well acclimatised with high temperature environment. The low survival of N137 indicates that it is very difficult to rear bivoltine pure races under high temperature environments. The higher survival of the hybrids than the pure temperature under high temperature conditions noticed in the present study is in concurrence with the earlier observations of Kato *et al.* (1989), Suresh Kumar *et al.* (1999) and Datta *et al.* (2001). The cocoon weight, shell weight and shell ratio were also low in the high temperature treated batches when compared to the batches reared under normal condition.

The most interesting observation noticed in this study was that there was maternal effect regarding temperature tolerance as evident from the better performance of those hybrids where the female parent used was more tolerant as pure race. It was also observed that in all the hybrid combinations there was no maternal effect under normal rearing condition and it was observed only in temperature treated batches with the maximum effect noticed in 48 hrs treatment.

The cocoon characters were also poor in all the temperature treated batches as evident from the measurements of cocoon size (Table 7). It was also evident that the variation of cocoon shape was more in those batches where 48

Table 3. Heterosis and heterobeltiosis% of pupation rate in hybrids at three different temperature treatments*

Hybrids	Heterosis %			Heterobeltiosis %		
	A	B	C	A	B	C
Moria× N137	26.3	66.4	8.4	8.5	-5.7	1.4
N137× Moria	14.2	48.3	8.8	-1.9	-15.9	1.7
Moria× C146	14.1	31.5	8.9	6.7	-4.9	2.1
C146× Moria	-2.6	8.6	-1.5	-8.9	-21.6	-7.7
N137× C146	15.2	143.9	11.0	27.3	426.2	11.2
C146× N137	34.3	242.7	17.8	48.4	639.3	18.0

Table 4. Heterosis and heterobeltiosis% of cocoon weight in hybrids at three different temperature treatments*

Hybrids	Heterosis %			Heterobeltiosis %		
	A	B	C	A	B	C
Moria×N137	43.71	52.10	46.61	51.03	602.63	16.40
N137×Moria	21.62	31.14	17.93	36.59	526.15	16.80
Moria×C146	24.34	30.88	41.98	22.55	113.49	16.73
C146×Moria	9.52	19.82	33.09	4.69	76.19	5.57
N137×C146	21.93	21.34	45.07	5.11	58.73	10.75
C146×N137	32.09	51.74	41.97	22.56	123.02	17.53

Table 5. Heterosis and heterobeltiosis% of shell weight in hybrids at three different temperature treatments*

Hybrids	Heterosis %			Heterobeltiosis %		
	A	B	C	A	B	C
Moria× N137	55.8	74.7	50.5	33.7	35.5	31.7
N137× Moria	29.0	50.1	20.0	12.5	17.0	5.7
Moria× C146	27.5	29.2	43.5	5.1	3.6	19.8
C146× Moria	12.4	19.6	43.6	-7.4	-5.1	12.4
N137× C146	27.0	30.5	54.2	10.1	5.8	35.6
C146× N137	44.4	76.7	50.9	19.2	32.4	32.7

hrs treatment was given as indicated by the higher standard deviation values and least was observed in those batches reared in normal room temperature as indicated by the low standard deviation values.

Besides, the hybrid vigour (heterosis and heterobeltiosis) calculated for pupation rate, cocoon weight, shell weight and shell ratio indicated that high degree of heterosis and heterobeltiosis is recorded in all the hybrids taken for the study and the values are more where 48 hrs treatment was given as compared to other treatments (Table 3, 4, 5 and 6). According to Gamo and Hirabayashi (1983), the F1 hybrids performance were always superior than the parents. Influence of environment during the process of breeding have been experienced by many breeders and geneticists. The concept of genotype environemnt interaction influencing the level of heterosis has been dealt with by different authors for different animal species (Knight, 1973; Orozco, 1976; Ehiobu and Goddard, 1989).

Table 6. Heterosis and heterobeltiosis of shell ratio in hybrids at three different temperature treatments*

Hybrids	Heterosis %			Heterobeltiosis %		
	A	B	C	A	B	C
Moria× N137	10.1	16.8	5.3	21.5	38.9	12.8
N137× Moria	7.8	16.6	4.3	0.7	19.3	-10.1
Moria× C146	7.1	4.9	5.9	-9.3	-12.1	1.2
C146× Moria	7.0	6.2	13.0	-20.0	18.7	1.3
N137× C146	4.1	9.2	6.4	11.3	5.1	40.5
C146× N137	9.3	18.3	6.4	26.5	42.2	37.5

*High temperature treatment was performed from 48 hrs after 4th moult.
A = At 35 ± 1°C and 85 ± 5% RH for 24 hrs continuously.
B = At 35 ± 1°C and 85 ± 5% RH for 48 hours continuously.
C = At 25 ± 1°C and 65 ± 5% RH continuously.

Table 7. Cocoon uniformity of pure races and their hybrids at different temperature treatments*

Breeds/Hybrids	Standard deviation of length/breadth× 100		
	A	B	C
Moria	14.96	50.37	18.83
N137	10.58	29.92	12.14
C146	8.97	21.30	6.14
Moria× N137	10.58	13.99	2.96
N137× Moria	8.02	22.32	4.44
Moria× C146	15.38	22.32	4.44
C146× Moria	6.96	28.19	2.93
N137× C146	8.78	55.16	8.16
C146× N137	10.16	23.76	6.05

The results of the present study is also in concurrence in these observations.

Lerner (1954) reported that according to the concept of homeostasis, heterozygotes are likely to be better buffered than homozygotes against environmental variation. The results from the present study indicate that the expression of hybrid vigour is different in hybrids at the three different temperature treatments. It is also clear form this study that the level of heterosis present in the hybrids can be influenced by the environment factor.

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