Int. J. Indust. Entomol. Vol. 3, No. 2, 2001, pp. 109~112

[Review]

International Journal of *Industrial Entomology*

Androgenesis in Mulberry Silkworm *Bombyx mori* L. : A Review

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(Received 11 October 2001; Accepted 2 November 2001)

Androgenesis in silkworm acquires a special significance as along with combined applications of other breeding strategies like parthenogenesis and cloning, it may serve as a valuable tool for sex control in sericulture as well as selection and production of bisexual homozygous androgenetic lines. Production of hybrid silkworm yielding high proportion of male larvae is of immense use to silk industry (Strunnikov, 1975, 1983). In this review, an attempt has been made to assimilate the works carried out on androgenesis, different techniques of induction towards androgenetic development and its role in silkworm breeding.

Key words : Androgenesis, *Bombyx mori* L., Breeding

Introduction

Androgenesis is a form of parthenogenetic development in which the androgenetic organism develops at the expense of the cytoplasm of the mother egg cell and the male nuclear material (Astaurov, 1957). Androgenesis has been induced in a wide group of animals through various inducing agents such as gamma rays (Ye *et al.,* 1989; Nagoya *et al.*, 1996), X-rays (Whiting, 1955), spermatozoa (Thorgaard *et al.*, 1990; Arai *et al.*, 1995; Onozato, 2001) and shaking of eggs (Bovery, 1989). Androgenesis may prove to be a powerful tool in animal breeding for the production of completely homozygous individuals within a short period of time (Parsans and Thorgaard, 1984, 1985).

Studies on androgenesis in the mulberry silkworm, *Bombyx mori* have been extensively reviewed by many workers (Astaurov, 1957; Strunnikov, 1975; Chowdhury, 1989). Several agents like low and high temperature (Strunnikov, 1983; Sugai; 1987), CO_2 gas and dry ice (Tazima and Onuma, 1967; Li, 1983; Li *et al.*, 1988), gamma rays (Xu *et al.*, 1997 and ultraviolet light (Strunnikov, 1983) have been used to induce androgenetic development in silkworm. Anying *et al.* (1996) have induced bipaternal androgenesis in silkworm.Studies on androgenesis in silkworm is of great interest in view of the development and selection of bisexual homozygous androgenetic lines in early generations with small populations as compared to huge populations that are needed in conventional breeding (Malinova *et al* ., 1996; Xu *et al.*, 1997; Nacheva *et al.*, 1998, 1999). Some important studies carried out on androgenesis in mulberry silkworm, *B. mori* have been presented in Table 1.

Table 1. List of important agents inducing androgenesis in the mulberry silkworm, *Bombyx mori* L.

Inducing agents	% of androgenesis	References
A) Low temperature:		
i) - 11°C (48 hours)	7 8	Strunnikov, 1983
ii) 0°C (4 – 9 days)	80	Sugai *et al.*, 1987
B) High temperature:		
i) 40°C (60 – 135 minutes)	70	Sugai *et al.*, 1987
ii) 40°C (60 minutes)	8 - 10	Strunnikov, 1983
iii) 46°C (30 seconds – 2 minutes)	90	Hirokawa, 1993
C) CO_2 gas (90 – 240 minutes)	70	Li *et al.*, 1988
D) Gamma rays & thermal stimulation	74	Strunnikov, 1983
i) 20 – 80 KR (40°C, 40 minutes)		
ii) 80 KR (38°C, 200 minutes)	76	Xu *et al.*, 1997
E) Ultraviolet light (1 – 2 minutes)	0.4	Strunnikov, 1983

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Methods for induction of androgenesis in the silkworm

Various methods have been tried to induce androgenesis in silkworm. In Japan, Hashimoto (1934) first induced androgenesis in the silkworm with complete development up to adult stage and almost simultaneously in USSR in 1936, it was induced by Astaurov by means of high temperature (40°C air) for 135 minutes, 18 minutes at (46°C in water) and a combination of heat shock with heavy doses of X-rays. It was proved that the nuclei of diploid androgenetic individuals induced by heat shock treatment, were due to fusion of two sperm nuclei. Treatment of 60-80 minutes old eggs with hot water (40°C) treatment for 60-135 minutes produced 70% androgenetic individuals (Sugai *et al.*, 1987). Ravindra Singh *et al.* (1991) obtained several androgenetic larvae in F1 hybrids between multivoltine Pure Mysore Chocolate and bivoltine NB7 following subjecting the eggs to hot air (40°C) for 135 minutes. Comparative performance of normal and androgenetic males demonstrated that the quantitative characters in androgenetic individuals were inferior as compared to normal males (Table 2). Males were induced from meiotic parthenogenesis by exposing silkworm eggs at high temperature (46°C) for 30 seconds to 2 minutes (Hirokawa, 1993).

Androgenetic individuals have been produced following treatment of silkworm eggs with CO_2 gas at various developmental stages (Tazima and Onuma, 1967; Li, 1983; Li *et al.*, 1988), X-ray treatment of females fertilized by normal males and thereafter heat treatment of eggs after 90 minutes of oviposition (Chowdhury, 1989), by laser microbeam irradiation (Xu *et al.*,1990), gamma irradiation (Xu *et al.*, 1997) and supercooling of eggs at -11°C (Tamazawa, 1977a ; 1977b). More than 80% androgenetic larvae were obtained when 20-40 minutes old eggs were exposed to 0°C for 4-9 days (Sugai *et al.*, 1987). When freshly laid silkworm eggs were cooled at -11°C, 7% androgenetic larvae were observed (Astaurov, 1967).

Role of androgenesis in silkworm breeding

During the last 3, 4 years, there have been two remarkable developments in the field of silkworm breeding by using androgenesis. One of these reported by Xu *et al.* (1997) from Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang, China isolated self bred line of silkworm by means of dispermic androgenesis using marker genes for eggs and newly hatched larval colour. Female pupae were irradiated with 80 KR gamma rays 2-3 days before emergence. Female moths were crossed with normal males of test silkworm breeds. Eggs were treated with hot water at 38°C for 200 minutes. It was observed that male silkworms of 54A and Daizo self bred lines were similar to males of normal 54A and Daizo. Characteristics of some silkworm self bred lines were presented in Table 3.

The other reported by Nacheva *et al.* (1999) from Sericultural Experiment Station, Vratza, Bulgaria, Forest University, Sofia, Bulgaria and Sericultural Institute, Ton Rin, North Korea is that they bred bisexual lines of silkworm

Table 2. Performance of androgenitic and normal silkworm larvae (Ravindra Singh *et al.*, 1991)

Combination	Normal/Androgenetic male	larval Span D : H	Cocoon Weight (g)	Shell Weight (g)	SR%	Filament Length (m)	Denier
NB7 × PM (Chocolate)	Normal	26:06	1.405	0.288	20.49	804	2.40
	Androgenetic	29:00	0.900	0.153	17.00	375	2.20
PM (Chocolate) × NB7	Normal	26:06	1.346	0.258	19.16	712	2.30
	Androgenetic	26:06	1.046	0.145	13.86	460	2.10

D, Denotes Days; H, Denotes Hours.

Table 3. Characteristics of self- bred lines of silkworm (Xu *et al.*, 1997)

Breed	Larval Span D : H	V instar Larval Span D : H	Cocoon Weight (g)	Shell Weight (g)	SR%
W313 (Sbl)	23:06	706	1.536	0.397	24.67
W317 (N)	23:06	706	1.777	0.443	24.91
54A (Sbl)	23:12	706	1.637	0.397	24.25
54A (N)	24:00	712	1.721	0.415	24.13
Daizo (Sbl)	22:00	612	0.916	0.130	14.19
Daizo (N)	21:23	610	0.977	0.143	14.64

Sbl, Denotes Self bred lines; N, Denotes Normal silkworm breed.

Table 4. Comparative performance of bisexual androgenetic lines of silkworm (Nacheva *et al.*, 1999)

Lines	Hatching %	Pupation Rate	Cocoon Weight (g)	Shell Weight (g)	SR%	Filament Length (m)	Denier	Reela-bility %	Raw Silk %
4C	99.18	98.67	2.000	0.495	24.90	1281	2.99	86.97	42.62
5C	97.83	98.50	2.154	0.531	24.87	1225	2.57	87.92	42.18
12C	98.27	99.00	1.978	0.470	23.99	1445	3.28	87.68	43.57
Super	98.78	98.00	2.076	0.484	23.50	1251	3.01	85.30	42.60
7P	98.72	98.66	1.979	0.470	23.92	1231	2.80	89.84	44.35
Hessa	98.01	98.66	2.018	0.476	23.80	1277	2.88	88.90	43.55

by utilizing a race as genetic marker to identify androgenetic individuals. Ps allele from P multiallele series responsible for larval marking was used as genetic marker. Female moths of marker P race were crossed with male moths of F2 Japanese hybrid Shunei x Shogetsu. Eggs were treated at 42°C for 210 minutes. The androgenetic lines were used as donors for transmission of their homozygosity into promising bisexual lines by series of backcrosses. This method appears to be suitable for development of bisexual androgenetic lines. Four lines bred through this method manifested productivity similar to those of Bulgarian pure lines Super and Hessa. Characteristics of bisexual lines produced with androgenetic origin were presented in Table 4.

The success in activating androgenetic development in silkworm eggs depends upon the combined effect of different temperatures (Strunnikov *et al.*, 1996). Malinova *et al.* (1996) have developed bisexual homozygous silkworm lines through androgenesis and they found that thermal treatment of eggs at 42°C for 210 minutes was ideal for produciton of androgenetic individuals. Low phenotypic variability in quantitative characters in isogenic hybrids obtained from homozygous individuals has been observed (Strunnikov *et al.*, 1982).

Studies on androgenesis in silkworm have distinct advantages like production of homozygous lines within short period of time, development of outstanding genotypes with less phenotypic variability exhibiting more hybrid vigour and combining ability. It can prove to be an important tool in silkworm breeding as maximum hybrid vigour is obtained from F1 hybrid between homogygous lines. As estimated by Huang (1980), 10 to 15% more silk can be produced by rearing only male silkworms.

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