Sterilizing Effect of High Temperature on
the Male Silkworm, *Bombyx mori* L.
(Lepidoptera : Bombycidae)¹

Etsuji SUGAI and Ibrahim ASHOUSH

*Faculty of Agriculture, Tokyo University of Agriculture
and Technology, Fuchu, Tokyo*

(Received May 4, 1968)

Sterility of the male silkworm, *Bombyx mori* induced by high temperature (32°C) was investigated in relation to their developmental stages. The sterilizing effect of high temperature was the strongest at the prepupal stage, from spinning to pupation, and followed by the pupal period, whereas no sterilizing effect was obtained in the fifth larval instar. In the treatment with cyclic changes of high (32°C) and moderate (23°C) temperatures for 8 days from spinning to early pupae, no sterilization occurred when the treatment was given at 12 hr intervals, but the sterility markedly increased if the time intervals were extended to 24 hr. Furthermore, a relationship between male sterilization and developmental stages of the insect was discussed, paying a particular consideration on the development of their germ cells.

INTRODUCTION

In a previous paper (SUGAI and KIGUCHI, 1967), we reported that the male silkworm, *Bombyx mori* held at a high temperature (33°C) during pupal period was almost completely sterilized and that the young pupae were apparently more sensitive to the sterilizing effect of high temperature than older ones. Furthermore, we concluded, from anatomical and cytological observations, that sterilization was due to exposure of developing spermatozoa to high temperature. These findings suggested that the most sensitive stage for the male sterilization may exist at somewhat earlier period in which sperm formation is more actively occurring.

In this paper, we demonstrated the sterilizing effect of 32°C in various stages of the silkworm development from the fifth larval instar to early pupal period and also attempted to determine the effect of the thermoperiodism on male sterilization.

MATERIAL AND METHODS

Males of Konjiki strain, one of the strains sensitive to high temperature ster-

¹ This work was supported partly by a grant from the Ministry of Education.

ilization was used. The experiments were carried out in air conditioned rooms with temperatures regulated at 23°C and 32°C and humidity at 70-80%. High temperature treatments (32°C) were performed at various stages of the insect development for the various period described in Tables 1 and 2. After the treatment, the insects were held at 23°C until emergence. Upon emergence, each adult was allowed to mate with fertile females of N. 106 × Daizo strain. Two weeks after oviposition, the percentages of unfertilized eggs were calculated in each experimental group for sterility comparison. In calculation, pigmented eggs were regarded as fertilized eggs and unpigmented and unhatched eggs as unfertilized ones after ascertaining a hatching of non-diapause eggs. Concurrently, anatomical and cytological observations were made on the treated testes and reproductive organs of the female mated with treated male.

RESULTS AND DISCUSSION

1. Difference of Male Sterility in Developmental Stages

Table 1 shows the results of investigation with larvae held at 32°C from the beginning of the fifth instar to the start of spinning. The results indicated that no sterilization of the male was obtained in the fifth larval instar. The next examination was conducted to determine the sterilizing effect of high temperature in prepupal stages from spinning to pupation. From the results shown in Table 2,

Table 1. EFFECT OF HIGH TEMPERATURE (32°C) IN FIFTH LARVAL INSTAR ON FERTILITY OF MALE SILKWORM

Days reared at 32°C	Total of moths examined	Total of eggs laid	Total of unfertilized eggs	Per cent of unfertilized eggs (%)
4 days after 4th molt ^a	21	13309	1165	8.75
3 days before spinning	24	16312	1061	6.50
Control (23°C)	11	7520	545	7.24

^a Spinning began 5 days after 4th molt at 32°C.

Table 2. EFFECT OF HIGH TEMPERATURE (32°C) IN PREPUPAL AND EARLY PUPAL STAGES ON FERTILITY OF MALE SILKWORM

Days kept at 32°C after spinning	Total of moths examined	Total of eggs laid	Total of unfertilized eggs	Percent of unfertilized eggs (%)
1	24	13271	1704	12.84
2	25	15744	4567	29.00
3	25	14416	10412	72.22
4	27	15427	13811	89.52
3 ^a	18	7700	2250	29.22
Control(23°C)	11	7520	545	7.24

^a Held at 32°C for 3 days immediately after pupation.

it was noted that the percentage of unfertilized eggs attributable to treated males increased markedly as period of the treatment after spinning extended. They were almost completely sterilized when kept at 32°C for 4 days after spinning, even though held at moderate temperature of 23°C throughout the remainder of their life cycle.

In a previous paper (SUGAI and KIGUCHI, 1967), we reported that immediately after pupation 3 days of continuous 33°C treatment were required for the complete sterility. For the comparison with present results, the next experiment was undertaken to determine the effect of 32°C on the sterilization of young pupae. As shown in Table 2 (a), approximately 70% of the eggs fertilized in the present experiment. Accordingly, it can be safely said that the prepupal period is apparently more sensitive to sterility induced by high temperature than that of pupal period. Furthermore, this relation was reconfirmed using other strains such as C. 25 and Kp.

2. Effect of Thermoperiodism on Male Sterility

The temperature of 32°C used in this experiment is not exceedingly high as it is naturally encountered in the summer season. Under natural conditions, however, the sterilization of the male does not occur as frequently. To investigate this problem, the effect of thermoperiodism on male sterility was examined. Namely, the insects at the period from spinning to early pupae were treated with cyclic changes of temperature (32°C and 23°C) for 8 days. As seen from Table 3, no sterilization occurred when the treatment was given at 12 hr intervals. The

Table 3. EFFECT OF CYCLIC CHANGES OF TEMPERATURE (32°C and 23°C) FOR 8 DAYS AFTER SPINNING ON FERTILITY OF MALE SILKWORM

Time intervals	Total of moths examined	Total of eggs laid	Total of unfertilized eggs	Per cent of unfertilized eggs(%)
12 hr	23	10840	860	7.93
24 hr	19	8211	3254	39.62
4 days ^a	34	15192	14589	96.03
Control(23°C)	19	9205	891	9.65

^a Held at 32°C continuously for 4 days after spinning.

sterilization markedly increased when the time intervals were extended to 24 hr, although they received same doses of high temperature during the treatment. From these result, it was confirmed that continuous high temperature was required for the induction of male sterility.

In parallel with experiment mentioned above, we conducted anatomical examination on sterilized males held at 32°C for 4 days after spinning. The result obtained indicated that copulatory organs exhibited no visible abnormality. Cytological observations of the treated testes also revealed no morphological difference in either eupyrene and apyrene spermatozoa between treated and control. However, when the spermatozoa penetrated from testicular follicles into vas efferens and ejaculated into female reproductive organs, a marked reduction in the amount

and motility of spermatozoa was observed as we reported in previous paper (SUGAI and KIGUCHI, 1967). From these findings, it is presumed that spermatozoa may be affected qualitatively to some extent by the high temperature treatment during spermatogenesis even in the testes. It seems quite possible that effectiveness of high temperature for the male sterilization is stronger at prepupae than pupae, because spermatogenesis is occurring more actively in the former compared to the latter in which the spermatozoa are fully matured. Extending this concept further, however, a problem has arisen as to why no effect of high temperature was revealed when the treatment was carried out in the fifth larval instar in which the division and differentiation of germ cells are occurring most actively. Though the reason involved is not yet clear, it may be closely related with the state of nutritional physiology, because we observed in the previous experiment (SUGAI, 1966) that even in the fifth larval instar a marked degeneration of germ cells occurred when the larvae were starved. In other words, sufficient supply of nutrition during the feeding may reduce the effect of high temperature for the male sterilization.

ACKNOWLEDGEMENT

Authors wish to express their sincere thanks to Dr. G. C. LABRECQUE, Entomology Research Division, Agric. Res. Serv. U.S.D.A., Florida who read this manuscript and gave valuable suggestions for preparing this paper.

REFERENCES

- SUGAI, E. (1966) The effect of starvation on silkworm, *Bombyx mori* L. spermatogenesis. *Zool. Magazine*. **75**: 34-39.
- SUGAI, E. and K. KIGUCHI (1967) Male sterility of the silkworm, *Bombyx mori* L. induced by high temperature during pupal period. *J. seric. Sci. Japan* **36**: 491-496.