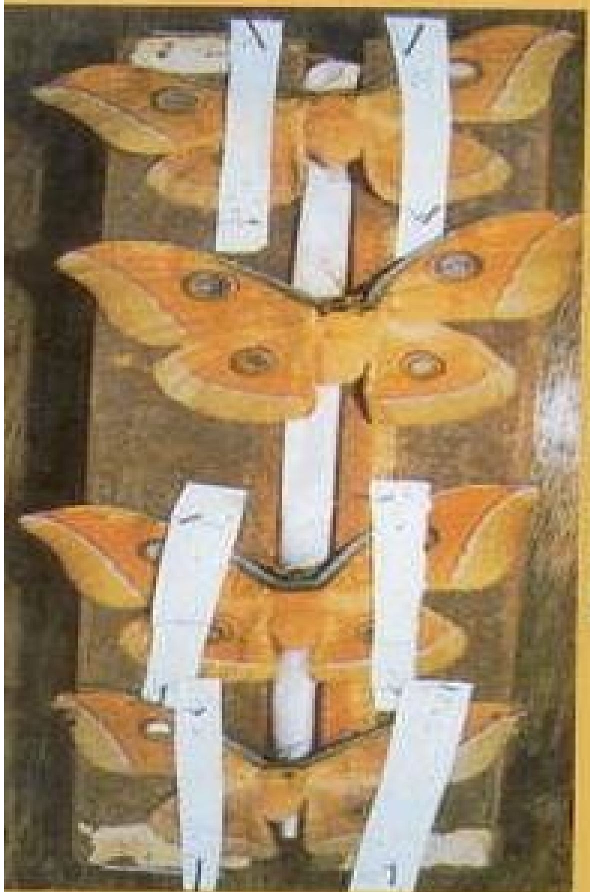
WILD
SILK TECHNOLOGY

R.P. Kavane & T.V. Sathe



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Preface

Sericulture plays a very important role in sustainable development of a country by providing valuable products. India is the second largest producer of the tasar silk in the world. Sericulture is practiced in diverse agro-climatic region across the length and breadth of the country which necessitates the evaluation of large number of region and season specific breed/hybrids. Non-mulberry sericulture known as forest or wild sericulture holds great promise for agro industry and forestry. Rearing of the wild silkworm specially, tasar silkworm provides employment and additional income to tribal families since traditional rearing method for cocoon production is less remunerative to the tribal rearers. Indoor rearing of tasar silkworms is possible up to 3rd instar but for 4th and 5th instars indoor rearing is yet to be established. In facts, rearing success rate of tasar silkworm is very less (30–35 per cent) due to number of predators, parasitoids and diseases. Hence, the present rearing technology provided in the text will be helpful for enhancement of wild sericulture activity in India. For better understanding of rearing concepts and utilization of tasar silkworm species in wild sericulture, there is urgent need of biosystematic studies of silkmoths. The present work will thus provide information of wild silkworms with respect to taxonomy, biology, and rearing potential with different food plants.

Hence, we hope that the book will be helpful to students, teachers, farmers, Sericulturists and researchers in the field of sericulture.

R.P. Kavane
T.V. Sathe

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ABOUT THE BOOK

Wild silk is very vital biological resource and has a very crucial role in economic and social development of mankind. Wild silk is better than other silks from the view point of healthcare. Therefore, the book contain taxonomy of wild silkmoths, under which 9 new subspecies of *Antheraea mylitta* have been described for avoiding confusion with ecoraces which refer to *Antheraea mylitta indica, A.m. jujubi, A.m. arjuni*, *A.m. greyi, A.m. kolhapurensis, A.m. koynei, A.m. sathei, A.m. badami* and *A.m. sahyadricus*. The book also contains biology of wild silkmoths *Attacus atlas* (Linn.), *Actias selene* Hubner and *A.m. kolhapurensis*. Rearing techniques for *A. atlas, A. selene* and *A.m. kolhapurensis* with different food plants and their assessment by food consumption potential/cocoon characterization and development rate has given special emphasis in the book. Thus, the book is unique on biodiversity of wild silkmoths and the wild silk technology which will be very helpful for students, teachers, farmers, sericulturists and researchers in India and at global scenario.

About the Author

Mr. Kavane Rangrao Pandurang (M.Sc. Sericulture, M.Sc. Zoology, Ph.D.) is working as lecturer in Zoology at Vivekanand College, Kolhapur. He has published 6 research papers in scientific journals and participated 12 conferences/workshops. He has been awarded fellowship of International Consortium of Contemporary Biologists (ICCB) Ranchi. He is also recipient of best paper presentation award and life member of ICCB, Ranchi and NESA, New Delhi respectively.

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Chapter 1

General Introduction

Silk not only refers to *Bombyx* silk whose insects are reared on mulberry leaves and is a high priced, valuable and delicate textile, called the "Queen of Fibres", but also to Tasar silks where tasar worms are reared on Ain, Oak, Arjun, Ber, Badam, etc. During the last century, the silk industry has greatly contributed to the foreign exchange to India. In the world, silk still offers the economic prosperity.

Mulberry silk is quite famous but other wild silks are not very popular, although Chinese tasar (*Antheraea pernyi* G.M), Indian tasar (*Antheraea mylitta* D), Eri silk (*Samia cynthia ricini* Hutt), Muga silk (*Antheraea assama* Ww) and Tensan (*Antheraea yamamai* G.M) have long been used for characteristic silk textiles, forming a small segment of the market. It has been observed through research that wild silks possess some physiological significance in activities like controlling cholesterol in blood, antibacterial functions and in UV absorption effect, etc. These newly recognized properties may greatly increase the popularity and utilization of wild silks at global scenario as world is facing the problems related to global warming. The International Society for Wild Silkmoths (ISWSM), Japan has started research work on wild silkmoths and silks. The society is established during 1980 and has used available technology to harness various means of wild silk utilization.

Based on porosity and the compactness wild cocoon filaments are classified into two types. The former has a porous structure in the cross-section of the filament, containing multiple sizes of fine tubules. The porous cocoon filament is characteristics of species of Saturniidae which includes *A. yamamai, A. pernyi*, *A. assama, A. mylitta, S.c ricini* and *Attacus atlas* L. The non-porous filament of cocoons is feature of all other families *viz.,* Bombycidae, Lasiocampidae, Thaumetopoeidae, and Psychidae. *Bombyx mori* L, *B. mandarina* M, *Gonometa postica* Walkar, *Anaphe panda* Bosid and *Cryptothelea formosicola* Strand, are very famous insects for this kind of silk from above families. Daba and Sukinda silkworms *A.mylitta* produce the thickest cocoon filaments with porous structure which is suitable for fashionable clothing like, sportswear. According to Akai (1998) porous filament maintains a constant temperature and humidity in a textile more than a compact filament. At the smallest class of porous filament, those of *Cricula trifenestrata* Helfer create a fine and soft fabric suggestive of cashmere.

The domesticated *Bombyx* silk has properties which affect cholesterol in hemolymph, alcohol metabolism, senile dementia and diabetes. In wild silks produced under environmental conditions, these factors are expected to be present even more strongly than in Bombyx silk (Akai, 1998). The antibacterial functions of *A. pernyi* and Eri silk are higher than that of *B. mori.* Similarly, the reduction of UV by *A. pernyi* is also superior.

The wild silks possess health related characteristics. Products made from these silks are popularized for these special qualities. Such added values of wild silks will stimulate their increased utilization at national and international level. Very recently, wild silk powder has been obtained (Akai, 1998). This powder too is expected to have health benefits and increase the demand for wild silks.

Until ten years ago, wild silk was related to only five species viz, *A. yamamai, A. pernyi, A. mylitta, A. assama* and *S. ricini* in the market. However, in recent years a few other wild silkmoth species have also came to front, *A. atlas, C. trifenestrata, A. panda* etc. but, there are yet no farms for these silkmoths and only cocoons collected from the field or hills are used. *Gonometa* and *Borocera* from family Lasiocampidae are also yielding interesting silks and have unique compact filaments. Establishment of the farms for these wild silkmoths is essential to introduce planned silk production in India. In the present work, *A. atlas, A. selene* and *A. mylitta indica* ssp nov have been tried for their rearing. The work will add great relevance to wild silk industry.

In India (Figure 1), Non-mulberry sericulture is an age old tradition, practiced mainly by the tribal people. When they do not have any work in agriculture and other allied pursuits non mulberry sericulture provides them moderate earnings in different lean seasons of the year. Wild sericulture remained obscure as an exclusive craft of tribal and hill folks inhabiting the forests of Central India, Sub-himalayan region and North-eastern India for long time. However, in recent years, this traditional craft of tribal has gained tremendous importance. Due to its rich production potential, eco-friendly nature of the activities and steady demand for hand made textile products within and outside the country etc, wild sericulture is commercially exploitated from traditional craft into an industry of high potential. As a industry it has an advantage of rich natural resources like food plants and tribal manpower. Utilising them to bring a balanced development without disturbing the existing ecological system is the great socioeconomic challenge in sericulture.

India is the only country bestowed by nature to produce all the four varieties of non-mulberry silks namely tropical tasar, oak tasar, eri and muga. Of the total raw silk production of 14035 MT during the years, 1997-98, the share of non-mulberry silk was only 2.3 per cent. From non-mulberry production, eri, tasar and muga silks account for 69.78 per cent, 24.85 per cent and 5.37 per cent, respectively (Shetty and Samson, 1998).

Host food plants for wild silkworms is crucial factor for wild silk industry. There is practically no systematic plantation of food plants for rearing non-mulberry silkworms in India. Therefore, Tasar silkworms are reared on food plants available in the forest. Muga silkworm rearings is conducted on food plants in forests/village grazing reserves etc and eri silkworm rearing is largely conducted indoors with the leaves of caster (*Ricinus communis* L) collected from nature grown trees. Recently, the research institutes of Central Silk Board (CSB) have introduced the concept of systematic plantation of food plants for all non-mulberry silkworms to improve the productivity of silks in India.

Figure 1: Map of India Showing Important States Cultivating Wild Silks

According to Sathe and Jadhav (2001) tasar silkworm rearing is practiced mainly in the Central and Southern Plateau region in the humid and dense forest area covering Bihar, Madhya Pradesh, Orissa and West Bengal, extending to the fringes of Uttar Pradesh, Andhra Pradesh and Maharashtra (Figure 1). It is estimated that in India, there is 11.168 million ha of forest having different primary and secondary food plants for wild silkworms being utilised for tasar silkworm rearing. However, deep interior forests remained unexploited. In India, about 1.40 lakh tribal families have been engaged in tasar silkworm rearing and get benefited socio-economically.

During 1981 and 1986 with the financial assistance from Swiss Development Co-operation in eight tasar producing states, the CSB has implemented the Inter State Tasar Project (ISTP), under this project, 7845 ha of *Terminalia arjuna* W&A block plantation has been developed with necessary infrastructural facilities and for overall development of tasar silk industry. Under ISTP Project, efforts have been made by Rajasthan Government for involvement of Rajasthan Vidyapeeth Kul, an NGO to take up tasar culture by utilising the plantation and other Infrastructure facilities.

Eri sericulture is practiced as an indoor activity. Eri silkworms are multivoltine and reared almost throughout the year. The ericulture has a close link with the culture and tradition of the people of North-east. Rural people are involved, primarily to meet the domestic demand of warm clothing and the edible pupae as a major source of proteins. The culture is also marginally practiced in West Bengal, Bihar and Orissa, primarily for production of castor seed and its oil. In general, eri culture is considered a subsidiary source of income for meeting the domestic needs of warm clothing and pupa. The rearers can produce their own seed and conduct rearing, spinning and weaving. The pupal dish of insect diet is sold in weekly market in eastern parts of India. Similarly, the surplus quantity of Eri cut cocoons are sold openly at their door steps.

Ericulture is competting with mulberry sericulture by all means. Muga, the golden yellow silk produced by muga silkworm is unique to Assam and neighboring states of North-eastern region and also practiced in West Bengal in recent years. Muga silkworm is multivoltine in nature with six crops a year, two each of commercial, pre-seed and seed crops. Muga silkworm is semi-domesticated and rearable in the open on trees where as spinning arid seed production are indoor activities. In 3,500 ha of land muga food plants are cultivated in North-eastern region of India including West Bengal.

The drawbacks of non-mulberry sector are poor performance of seed multiplication facilities and non-availability of adequate disease free commercial seeds. There is a great need to manage seed multiplications by the Government sector for quality seed and its timely supply to the rearers in India.

Recently, a four-tier seed multiplication system is established in India. The Central Tasar Research and Training Institute (CTR&TI), Ranchi is supplying the nucleus seed to Central Tasar Silkworm Seed Station, Lakha which multiplies the same for one generation and supplies to Basic Seed Multiplication and Training Centre (BMSTCs) for further multiplication. They in turn, multiply for one generation and supply the basic seed to Pilot Production Centre (PPC) of the state government. Each BSMTC produces 40,000 dfls and supply is 32000 dfls to eight PPCs @ 4000 dfls per PPC. Eight PPCs in turn produces 3.2 lakh dfls covering 3200 rearers @ 100 dfls per rearer.

In North-eastern and North-western states of India, Oak tasar culture has got good potential where abundant oak flora is available. However, Paucity of silkworm seed and lack of adequate extension support system are the main constraints for oak tasar development in this region. Therefore, a major emphasis should be given to this important basic need to ensure production and supply of quality basic silkworm seed in sufficient quantity to enhance the oak tasar silk production. CSB has established three research extension centres in order to fulfill the seed and extension requirements. These refer to REC in Assam, Nagaland and Manipur. CSB has also established the Regional Tasar Research Station (RTRS), at Bhimtal (UP) and RECS at Palampur (HP) in North-western region and these units produce and supply dfls to 35 seed farms in state sector for enhancing sericultural activities.

In India, all wild silkmoths *i.e.*, *A. mylitta, A. assama, S.c ricini,* C. *trifenestrata, A. selene, A. atlas,* etc. and many more silkmoths distributed in diverse eco climatic zones. Similarly, considering the ecological conditions, food plant distribution, presence of eco-types and species of diverse nature is in coexistence. It is speculated that North-eastern India is a possible home of origin of species of *Antheraea* from where radiation would have occurred (Nagaraju and Reddy, 1998).

A. assama is supposed to be an ancestral species from which other species would have possibly originated. There are number of reasons to accept this concept. There are a few pointers to the hypothetical evolutionary course, all starting from the unique habitat, North-eastern India, one leading to Central and South India represented by the species of *A. mylitta.* The second leading to the North-West along sub-Himalayan belt represented by *A. roylei,* the third leading to Southern China and

Japan represented by *A. pernyi* and *A. yamamai,* respectively and the fourth leading to Indo-Australian region represented by a number of tropical species. The only isolated case is that of *A. polypherous* distributed in USA. However, recent reports (Sathe, 2007) indicated that at least 15 species of wild silkmoths exists in Western Ghats of Maharashtra due to availability of rich flora of the region.

The Saturniid silkmoths have variations in chromosome number from n =15 to n = 49 with an average chromosome number of the family n = 31. Rare cases of interspecific hybrids of *Antheraea* shows diverse chromosome number of n = 31 and n = 49 are known to yield fertile and vigorous hybrids. According to Jolly *et al.* (1969) the interspecific hybrids generated using many other *Antheraea* species uncover interesting genetic relationships of the species involved. The holocentric nature of the chromosomes, presence of supernumerary chromosomes and chromosome numerical polymorphism in the species of *A. roylei* and fertility of the F_1 interspecific hybrids of *A.pernyi* and *A. roylei* despite trivalent formation show that the chromosomal fission has probably played central role in the evolution of Saturniid silk moths, (Puttaraju and Nagaraju, 1988).

Except *A. yamamai* in all the *Antheraea* moths where eggs undergo hibernation, the diapause sets in at pupal stage. Many species and eco-types have high degree of plasticity to shift from uni/ bivoltinism, trivoltinism depending on the photoperiod during larval stage and nutrition.

The biotechnological approaches such as recombinant DNA technologies, immunological methods, protoplast culture, hybridoma technology and gene transfer methods have provided solutions to many problems to conventional approaches in sericulture. The vast genetic resources from non-mulberry silk moths offer recent developments for expecting new rearable varities of wild silkmoths. In fact, the active research that is going on mulberry silkworm *B.mori* as a model genetic system has already provided important insights into the fundamental biological processess would worth implementing against wild silkmoths at global scenario.

A. mylitta as a polyphagous worm has fairly wide area of distribution in tropical belt extending from 16 to 24° N and 80 to 88° E covering both deciduous and semideciduous forests. It has 44 ecoraces distributed in India. The ecoraces such as Daba, Laria, Modal, Sukinda, Bhandara, Raily, Nalia, Sarihan and Andhra local are considered important from the commercial view point. These ecotypes differ sharply in their characteristics and offer rich genetic repertoire in terms of their qualitative and quantitative economic characters. Unfortunately, systematic use of these ecotypes is yet to be establish in tasar culture. Their diversity in voltinism, food plant preference and hybrid vigour manifestation has great practical potential value. The above study needs basic understanding of the genetic architecture of ecotypes, which would throw light on their uniqueness, genetic distances and genetic variability. Such information could be profitably used to identify the eco-types which give optimum level of heterosis in the hybrids, to preserve the genetic identity of the eco-types and to evolve breeding strategy for maintenance of genetic diversity (Puttaraju and Nagaraju, 1988).

Ecoraces have not studied with line of worth while economic interest. There is pronounced phenotypic and behavioral plasticity between ecoraces which would worth establishing their rearing relationships. The breeding programmes related to different eco-types conducted earlier could not succeed to the desired level. The reasons for such failures might be absence of well defined genetic markers. The breeding plans fail to reach the desired levels due to the fact of absence of genetic identity and genetic distance of the parents. Therefore, Tasar culture still waits for ways and means for rational characterization and utilization of ecotypes to arrive at high yielding, disease resistant and amenable hybrid combinations of silkworms. There are several recently introduced molecular marker technologies such as random amplified polymorphic DNA, intersimple sequence repeat polymorphisms, simple sequence repeats based polymerase chain reaction, restriction fragment length polymorphisms etc. Such techniques are being intensively used in crop and animal genetics for protection of breeders' rights, conservation of genetic diversity, incorporation of desired traits through molecular marker assisted selection, germplasm characterization, genome mapping and map based cloning (Shi *et al.,* 1995; Nagaraja and Nagaraju, 1995; Nagaraju *et al.,* 1995).

The fertile interspecific hybridization is a rare phenomenon in insects. Genetic analysis of such fertile interspecific hybrids will throw light on the phylogenitic relationships of the wild silkworm species. Jolly *et al*. (1969) reported the cases of fully and partially fertile interspecific hybrids in the genus *Antheraea*. Nagaraju and Jolly (1986) studied the species of *A. pernyi* (Chinese oak silk moth) (n = 49) and *A. roylei* (Indian silk moth) (n = 30, 31), followed by the studies of Kobayashi and Tanaka (1988) and Shimada and Kobayashi (1992) involving *A. yamamai* (n = 31) and *A. pernyi* (n=49).The hybrids have quite interesting features in one full and the other partial fertile interspecific. The interspecific hybrid involving *A. pernyi* (n = 49) and *A. roylei* (n = 31) produces fully fertile F1 hybrids and could be inbred and backcrossed as well to *A. pernyi*. The chromosome pairing pattern in the fertile F_1 hybrid revealed 18 trivalent and 13 bivalents. Wherein stabilized chromosome number of n = 49 in the interspecific hybrid population inbred was found for 49 generations. It is very likely that the chromosomes which involved in trivalent formation were gradually excluded during in breeding and only those zygotic combinations which contain bivalents would have been retained in the population. Thus, the present day inbred hybrid of *A. pernyi* x *A. roylei*, referred to as *A. proylei* has only n = 49(Nagaraju, 1998). However, the fact is unknown as to what extent the parental genomes are represented in the stabilized inbred populations of *A. pernyi* and *A. roylei* (*A. proylei*). According to some workers many silk yield attributes and the vigour have substantially declined in the present inbred populations of *A. proylei*. The molecular genetic analysis of the interspecific hybrid and descendant populations, using DNA markers and chromosome painting, probes could explain the status of *A. roylei* chromosomes, which involved in the trivalents during the course of interbreeding of the hybrid.

The work on retaining and breeding a large number of inbred derivatives from backcross and F_2 populations of the interspecific hybrid, which are likely to carry a different of chromosomal complements to track the parental chromosome, by using mapped DNA markers and chromosome painting probes have great importance in wild sericulture development.

According to Kobayashi *et al*. (1982) there are conflicting observations with regard to the fertile nature of the *A. yamamai* and *A. pernyi* interspecific hybrids. Shimada and Kobayashi (1992) have not been able to advance the interspecific hybrid beyond F_1 generation. But, Kobayashi *et al*. (1982) have advanced the interspecific hybrid to F_2 generation. Shimada and Kobayashi (1992) have successfully obtained progenies from the backcross of F_1 male (*A. yamamai* female X *A. pernyi* male) X female of *A.yamamai*. Such success opens up the possibilities of raising new breeds that combine the high quality silk of *A.yamamai* and bivoltinism of *A. pernyi*.

Biotechnological advances have made it possible to express foreign genes in heterologous organisms. The main goal of such work should be to develop methods for efficient production of a large amount of purified, biologically active proteins to study the basic mechanism of gene expression and the biological effects of products in cells and organisms and their possible pharmaceutical use.

Baculo viruses are extremely useful vectors for their successful expression of biologically active proteins. For expressing proteins of biomedical importance, a good progress have been made in *B. mori* nuclear polyhedrosis virus (BmNpV). Zhang *et al*. (1992) developed the recombinant expression vector of *A. pernyi* nuclear polyhedrosis virus (ApNPV) successfully and used for the expression of DE protein in *A. pernyi* pupae. *A. pernyi* can be considered as an ideal host to express the foreign proteins using ApNPV due to its advantages such as pupal diapause when pupa sleeps for several months, its sensitivity to infection and cheap cost of production.

Insects are capable of detecting foreign cells and foreign molecules of eliciting effective and specific defensive response. Among the components of immune response, wound healing and haemocytes and synthesis of a battery of defense proteins have been investigated. The synthesis of bacteria elicited proteins has been reported in *A. pernyi* (Qu *et al.,* 1982), *Philosamia cynthia* D (Boman and Hultmark, 1987) and *A. mylitta* (Nagaraju *et al.,* 1992). But, the novel antibacterial proteins, their regulation of expression and their precise role in immune response in Saturniid silk moths is to be detected in near future.

Silk proteins, particularly, fibroin, small fibroin, and P_{25} which constitute actual silk fibre are produced only in the posterior silk gland and sericin protein which cements the silk fibre is produced only in the middle silk gland in a highly tissue specific manner is demonstrated in *B.mori*. The genes coding for all these proteins have been cloned and characterized. However, *Bombyx* and *Yamamai* fibroin genes are quite different in their structure but their regulatory regions are conserved.

According to Kundu *et al*. (Personal communication) and Syed (personal communication) in *A. mylitta* and *A. assama* the amino acid sequence and composition are quite different from *Bombyx*. Silkmoths are holometabolous insects and they manifest robust circadian behaviours that involve in the timing of the photoperiodic termination of pupal diapause, adult eclosion and egg hatching behaviour.

The discovery of period gene that codes for period protein revealed that period gene expresses early in four cells of each of brain hemispher, with one lateral pair and one medial pair in the dorsolateral region (Sauman and Rappert, 1996). The period gene has important role in the circadian system. Extension of such studies to Saturniid silk moths on circadian behavioural pattern would be helpful for managing wild silk in better way in sericulture business.

Wild silkworms exhibit well defined taxonomical diversity. Their biodiversity is well known in all aspects of their life from egg to moth. Diversity in food habit and physiological constitution has been illustrated in *A. mylitta* (Indian tasar) *A. pernyi* (Oak tasar), *A. roylei* (Wild oak tasar), *A. Assama* (Muga), *Attacus synthia* (Wild eri) and *A. ricini.*

The tasar silkworm *A. mylitta* in Central India is being fed on Sal *Shorea robusta* Roxb and its “Daba” variety in northern Bihar (India) feeds on Asan *Terminalia tomentosa* W&A and *T. arjuna*. Attempts to rear Raily, the ecorace of tasar on *T. tomentosa* and *T. arjuna* resulted in high mortality with poor specimens of cocoons. While, Sal based raily are characterized by black coloured short peduncle and deep brownish grey to blackish cocoons. They are hard as stones, rich in silk content and the silk thread is thick with high denier. The raily silkworms are predominantly green with or without lateral shining spots. They camouflage perfectly with the back ground of Sal leaves. The race goes in diapause in pupal stage and complete one life cycle in a year showing univoltinism. The male moths are dark brown to deep brick red colour and females are deep yellow to brownish grey. Males have wings well adopted for long flights before mating. The eggs are different from Daba variety by a

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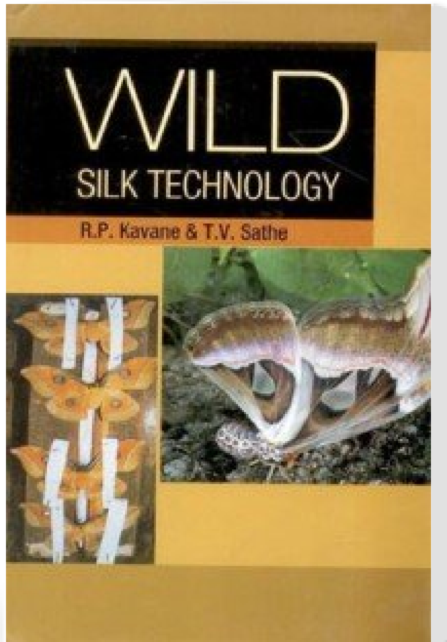

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