3.1. BIOECOLOGY

The work on bioecology of Indian tasar silk worm, Amniadae mylitta Drury (Lepidoptera : Saturniidae), was carried out on the natural plantations of Terminalia tomentosa in Armori Taluka of District-Gadachiroli, Maharashtra during July, 1988 to January, 1989.

3.1.1. PHYSIOGRAPHY AND CLIMATE OF ARMORI

3.1.1a. Physiography of Armori Taluka

Armori Taluka lies in the latitude 20.36 degrees N to 21.38 degrees N and longitude 79.27 degrees E to 81.42 degrees E. The west bound of the taluka is formed by the river Wainganga. The approximate altitude of the taluka varies from 150 m to 803 m above the sea level. The texture of soil varies from clay to sandy-loam. However, deep black and rich soils are also found along the Wainganga river. The forest wealth of this taluka belongs to southern, tropical dry deciduous belt.

3.1.1b. Climate

The climate of the taluka is tropical monsoon type. Climatically, the year may be divided into three main seasons, viz: summer, monsoon and winter seasons.

(i) Summer Season : This season begins from March and lasts up to June when the average temperature fluctuates between 22 degrees Celsius to 36 degrees Celsius. However, the maximum temperature reaches in last May (47 degrees Celsius) to early June (46 degrees Celsius). This period is generally dry except for scantly rains.

(ii) Wet Summer/Monsoon Season : The monsoon season begins from July and lasts for about three and half months i.e., upto
3.1. BIOECOLOGY

The work on bioecology of Indian tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera : Saturniidae), was carried out on the natural plantations of *Terminalia tomentosa* in Armori Taluka of District-Gadchiroli, Maharashtra during July, 1988 to January, 1989.

3.1.1. PHYSIOGRAPHY AND CLIMATE OF ARMORI

3.1.1a. Physiography of Armori Taluka

Armori Taluka lies in the latitude 20.39 degrees N to 21.38 degrees N and longitude 79.27 degrees E to 81.42 degrees E. The west boundary of the Taluka is lined by the river Wainganga. Armori town is situated in the western side of the taluk on Gadchiroli-Wadsa road at an altitude 273 m above the sea level. The approximate altitude of taluka varies from 150 m to 803 m above the sea level. The Armori Taluka falls under the distinct tasar belt. The texture of soil varies from sandy to sandy-loam. However, deep black and rich soils are also found along the Wainganga river. The forest wealth of this taluka belongs to southern, tropical dry deciduous mixed forest.

3.1.1b. Climate

The climate of the taluka is tropical monsoon type. Climatically, the year may be divided into three main seasons, viz; summer, monsoon and winter seasons.

(i) **Summer Season**: This season begins from March and lasts up to June when the average temperature fluctuates between 22 degrees Celsius to 36 degrees Celsius. However, the maximum temperature reaches in last May (47 degrees Celsius) to early June (36 degrees Celsius). This period is generally dry except for scanty rains.

(ii) **Wet Summer/Monsoon Season**: The monsoon season begins from July and lasts for about three and half months i.e., upto
early October. Average temperature during this season ranges from 22 degrees Celsius to 34 degrees Celsius, while relative humidity sometimes reaches up to 90%. The rainfall is generally maximum during August.

(iii) **Winter Season:** There is not much cold in this area and the winter months are December and January. The average temperature range during the season is from 12 degree Celsius to 32 degree Celsius. In the month of January about 94.25 mm rainfall is also recorded.

3.1.1c. **Weather Conditions**

The weather conditions prevalent during the course of the present investigations are shown in Table-1. The average minimum temperature was recorded in the month of December (12.16 degree Celsius) while the maximum was noticed during May (47.41 degree Celsius) and June (35.58 degree Celsius). Average relative humidity was lowest in March and highest in August, 30% and 83% respectively; whereas, the lowest rainfall was nil during November, December and February and highest 318 mm during August.

3.1.2. **CENTRES FOR OBSERVATION**

Two sites in the vicinity of Armori Town were selected for conducting the rearing of tasar silkworm and for gathering data on its lifehistory in relation to climatic fluctuations during all the three crops from July to January. The sites were in the forest, 5 Km from Armori, where the farmers also conducted rearing. The farmers were involved for the watch and ward of silkworms during odd hours.

3.1.3. **THE HOST PLANT**

The primary host plant of *A. mylitta*, i.e., *Terminalia tomentosa* is naturally occurring in the forest in this area. Both the sites have abundance of *T. tomentosa* and as such the silkworms were reared on this plant only. The *T. tomentosa* plants are commonly known as *Ain* in this area.
3.1.4. OUTDOOR REARING

3.1.4.1. Plan-outlay of The Experiments

The object of the present investigation was to study the bioecology of *A. myliitta* in Armori area of Maharashtra. Since ecology is a very wide subject of study, it was thought proper to specify the scope of the work, which was confined to the study of the life-history and seasonal variations in respect of various economic characters like average fecundity, embryonic duration, hatching percentage, larval weight, larval period, single cocoon weight, pupal weight, single shell weight, shell ratio percentage, DFL/cocoon ratio, ERR% and sex ratio particularly in reference to various ecological factors.

Studies on the life-history can be undertaken by rearing silkworms either on raised plantations of *Terminalia arjuna* or naturally occurring *T. tomentosa*. In the present case the life-history and seasonal variations in respect of various characters were studied on the naturally occurring *T. tomentosa* plants. As described in the foregoing pages, two sites in the same area were selected for rearing of tasar silkworm in all the three crop seasons for collection of samples and data pertaining to various abiotic and biotic factors during the present course of study. The rearing was conducted at two sites taking into account the risk factor involved in outdoor rearing of tasar silkworm. The silkworm rearing was conducted in all the three season, as per the technique developed by Central Tasar Research and Training Institute, Ranchi. The first season of rearing was during July and August, second during September and October while third from November to January. *A. myliitta* has been divided into different ecological races as a consequence of adoption to different ecological pockets where they inhabit. However, the rearing of “Daba” ecorace, being prevalent in Vidarbha region, was selected for the present study.
3.1.4.2. Meteorological Data

While conducting rearing in the field the data for the atmospheric temperature and relative humidity were recorded with the help of a standard Maximum-Minimum thermometer (Zeal) and hygrometer (watch type) respectively. Data on rainfall, temperature and relative humidity for the remaining period of the year were obtained from Tasar Silk Centre, Armori.

3.1.4.3. Predation

While conducting outdoor rearing of *A. mylitta*, a large number of bugs, praying mantis, wasps and ants were found attacking the silkworm. Many of these animal groups are recognized for their predatory tendencies on *A. mylitta*. A heteropteran bug, *Canthecona furcellata*, found eating the silkworm, was collected for study of its neuroendocrine system. This bug along with others predators were found in abundance and associated with silkworm during first two reasons whereas, in 3rd season their population decreased to the maximum extent.

3.1.5. STUDIES ON THE LIFE HISTORY OF A. MYLITTA

The life history of *A. mylitta* was studied during the course of rearing in the field.

Outdoor Rearing : The outdoor rearing technique as developed by Central Tasar Research and Training Institute, Ranchi was followed. However, traditional methods adopted by farmers were also followed for few steps. The silkworm seed, called disease-free-layings (DFL = number of eggs laid by a single moth and found disease free after microscopic examinations), was obtained from Tasar Silk Centre, Armori for all the three seasons. Ten dfls for each site and each season were utilized. The number of eggs were counted and divided by total number of dfls of each lot to get the average fecundity. The date of egg laying was recorded on the pockets of silkworm seed by supply agency and the date of hatching
was noted. The number of days taken for the silkworm eggs to hatch, called embryonic duration, was calculated. Five hundred eggs from each lot were kept separately for calculation of hatching percentage. The hatched larvae from sample lots were counted. The hatching percentage was calculated by the following formula:

$$\text{Hatching}\% \times 100 = \frac{\text{Number of larvae brushed}}{\text{Total number of eggs}}$$

The rearing was conducted in all the three seasons.

(i) First Season:

(a) Brushing: The placing of hatching larvae on the leaves is called brushing. During the present course of study, in the first season, the hatching started on July 5, 1988 and continued for three days. The hatching was observed mainly in the first half of the day. The An leaves were placed over the newly hatched larvae in the sample lots. The larvae crawl over the leaves. The larvae were counted for calculation of hatching percentage. These leaves with larvae were tied on the An bushes in uniform distribution which ultimately crawl over the leaves of the bushes. The remaining eggs other than the sample lots kept in leaf cups on the hatching day were tied on the bushes for hatching. These cups were regularly watched for three days against ant attack and to ensure proper brushing. This is the traditional methods of brushing and practised by the farmers.

(b) Supervision and Maintenance of Larval Population: The tasar silkworm rearing, being outdoor, is exposed to various vagaries of nature, besides pests and predators. The An bushes, with larvae on it, were covered with bamboo mats during early stages as a protection against sun and rain. The strict vigil was kept from dawn to dusk against their enemies with the help of farmers practising tasar culture in the same area. The larval population on a particular bush was maintained as per the availability of leaf. After about 80%
consumption of leaves of a particular plant the larvae were transferred by cutting off the small branches and attaching them to new food plants. This process of transfer continued till the spinning of cocoon. The observations on the larvae characters were recorded. The larvae were kept undisturbed during moult’ng. The weight of 20 numbers of mature larvae in fifth instar was taken together with the help of a counter balance. The average weight of a single larva was calculated as follows:

\[
\text{Single larva weight} = \frac{\text{Total weight of 20 numbers of larvae in g}}{\text{Total number of larvae weighed i.e. 20}}
\]

The date of spinning by the first and last larvae was recorded to calculate the larval period. The number of days required for the larvae from the date of hatching to spin cocoons is called larval period. The mature larvae before spinning discharge last excreta which is semi-solid. Discharging of last excreta is the beginning of spinning. The spinning worms require enough foliage to form the hammock properly.

(c) **Harvesting** : After the spinning is completed and pupae are formed, the cocoons are harvested. The branches are cut and the cocoons are pulled off the twigs by breaking it near the ring. The leaves attached to cocoons were removed. The cocoons harvested from the two sites were mixed and counted. Cocoons numbering one hundred were cut open for the analysis of following characters:

**Single Cocoon Weight** : Weight of 100 cocoons in two lots of 50 numbers each was taken with the help of counter balance. Average weight of a single cocoon was calculated as under:

\[
\text{Single cocoon weight} = \frac{\text{Weight of 100 cocoons in g}}{\text{Total number of cocoons weighed i.e. 100}}
\]

**Single Pupa Weight** : All the pupae from 100 cocoons were separated from their shell and weighed in two lots of 50 each. The
weight of 100 pupae in g divided by 100 gives the weight of a single pupa.

**Single Shell Weight**: All the shells of 100 pupae were weighed in two lots of 50 each. The weight of 100 shell in g divided by 100 gives the weight of a single shell.

**Sex Ratio**: The pupae of 100 cocoons were sex separated and counted for determination of sex ratio. The male and female pupae have distinct genital markings on the ventral side of eighth and ninth abdominal segments.

**Shell Ratio Percentage (SR%)**: The shell ratio percentage was calculated by the following formula:

\[
SR\% = \frac{\text{Total weight of cocoon shell (g)}}{\text{Total weight of cocoons (g)}} \times 100
\]

**DFL/Cocoon Ratio**: The ratio of total numbers of cocoons harvested out of total numbers of dfls brushed is called DFL/Cocoon ratio and calculated as under:

\[
\text{DFL/Cocoon Ratio} = \frac{\text{Total number of cocoons harvested}}{\text{Total number of dfls brushed}}
\]

**Effective Rate of Rearing (ERR%)**: The ERR% is calculated by the following formula:

\[
\text{ERR}\% = \frac{\text{Number of cocoons harvested}}{\text{Number of larvae brushed}} \times 100
\]

(ii) **Second Season**: The second season silkworm rearing was undertaken during September-October. The required silkworm seed (20 dfls; 10 for each site) was obtained from Tasar Silk Centre, Armori. The hatching was observed on September 17, 1988. The hatching worms were brushed and the rearing conducted as per the methods described for first season. All the above data were recorded for the second season also.
(iii) **Third Season**: During third season also ten dfls for each of the two sites were obtained from Tasar Silk Centre, Armori. The hatching started on November 5, 1988 and the rearing completed during January 1989. The rearing methods as described in foregoing pages for the first two crops were adopted except that the *Ain* bushes were not covered during early stages. All the data was recorded.

Observations on the adult stages were made during moth emergence at Tasar Silk Centre, Armori.

### 3.2. NEUROENDOCRINE ORGANS AND NEUROSECRETION

The present study was carried out in the following two insects –

(i) *Antheraea mylitta* Drury (Lepidoptera : Saturniidae)

(ii) *Canthecona furcellata* Wolff (Heteroptera: Pentatomidae)

#### 3.2.1. COLLECTION AND REARING OF INSECTS

During the course of bioecological studies, the rearing of *A. mylitta* was done and both these insects were collected at that time.

#### 3.2.2. MORPHOLOGICAL AND HISTOLOGICAL STUDIES

The insects of the present study were dissected under a stereoscopic binocular microscope. Aquous Bouin’s fixative was injected prior to the dissections to avoid post mortal changes. The brain, retrocerebral endocrine aortal complex and nerve cord were dissected out and fixed for 18-24 h in the fixative. Some of the fixed materials were used for making bulk preparations while other were used for making paraffin blocks. These paraffin blocks were cut 6-8 μm thick.

#### 3.2.2a. Histological Preparations

GOMORI’S aldehyde fuchsin technique modified by EWEN (1962a).
Paraldehyde fuchsin (PF) was prepared by the following method.

(i) 1 g basic fuchsin was dissolved in 200 ml boiling distilled water. It was boiled further for one minute, cooled and filtered.

(ii) To the filtrate added 2 ml of each of concentrated hydrochloric acid and paraldehyde.

(iii) Left stoppered at room temperature.

(iv) The solution was checked at intervals by placing a drop of it on a filter paper. When the red colour of fuchsin disappeared (after about 4 days), the solution was filtered and the precipitate was dried in an oven on filter paper and stored. The yield was about 1 g.

(v) Made a stock solution of 0.75 g of dried precipitate in 100 ml of 70% ethanol. This solution (solution A) keeps for one year without any apparent change in staining characteristics. The solution used for staining the insect material was prepared by adding 75 ml of 70% ethanol in 25 ml of stock solution (sol. A) and 1 ml of acetic acid.

**Staining Procedure:**

(i) The sections, after removal of paraffin and proper hydration, were oxidized in Gomori’s fluid for one minute.

(ii) Rinsed in distilled water and decolourized in 2.5% solution of sodium metabisulphite.

(iii) Rinsed in distilled water and transferred through 30% to 70% alcohols.

(iv) Stained in paraldehyde fuchsin for about 10 minutes.

(v) Washed quickly in 95% ethanol and differentiated in acid alcohol.
(vi) Sections were hydrated through 70%, 50%, 30% alcohol and distilled water and transferred in mordant solution for 10 minutes containing -
Phosphotungustic acid - 4.0 g
Phosphomolybdic acid - 1.0 g
Distilled water - 100 ml

(vii) Rinsed in distilled water and transferred in Halmi's counter stain for one hour. It had
Distilled water - 100 ml
Light green SF Yellowish - 0.4 gm
Orange G - 1.0 gm
Chromotrope 2R - 0.5 gm
Glacial acetic acid - 1.0 ml

(viii) Sections were rinsed in 95% ethanol acidified with 0.2% acetic acid.

(ix) Dehydrated in absolute alcohol, cleared in Xylol and mounted in DPX.

The ordinary neurons and B type NSC stain green in colour whereas A cells dark purple colour; nuclei orange colour and neuropile light green colour.

3.2.2b. Bulk Preparations

Gomori's aldehyde-fuchsin technique (modified by Cameron and Steele, 1959 and further modified by Dogra and Tandon, 1964).

(i) In this technique the fixation time was 18-24 h. Fixed material was washed thoroughly in 70% alcohol and brought down to distilled water.

(ii) The material was oxidised for 2-3 m in oxidant.

(iii) Excess oxidant was blotted off with filter paper.
(iv) The material was bleached with 4% sodium bisulphite solution till it decolourised.

(v) Washed in distilled water for 5 to 10 m and dehydrated through 30% to 70% alcohol for 5 m each.

(vi) Stained in staining solution for about 1 h.

(vii) Quickly blotted off excess stain with filter paper.

(viii) Differentiated in 95% alcohol until no more superfluous stain was given off, changed alcohol once or more.

(ix) Dehydrated, cleaned and mounted as usual.

Figures: The line drawings are diagrammatic and the various neurosecretory cells shown in these figures are not visible in any particular section because of their locations at different planes in the tissue; these are reconstruction diagram of serial sections and are not to the scale. Photomicrographs were taken with Nikon and Wild Leitz Biomed Photoautomat. The films were processed at Eden Colour Lab, Meerut.