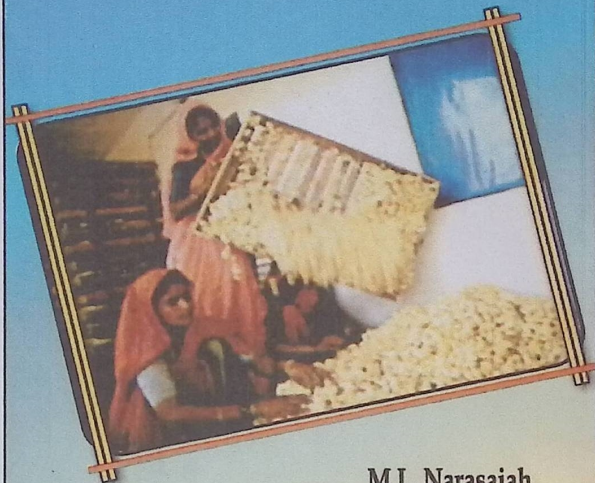


Problems and Prospects of Sericulture



M.L. Narasaiah

PROBLEMS ANA PROSPECTS OF SERICULTURE

By

Dr.Lakshmi Narasaiah

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Preface

In fact, Indian agriculture now is no longer confined to the cultivation of traditional crops. The farmers are encouraged to take up agriculture practices which are integrated with live-stock culture, animal husbandry, dairying, fisheries, poultry, horticulture and sericulture to generate more income for each household.

In spite of the limitations in agriculture, it can be said without any doubt that Indian agriculture is on the threshold of entering into a stage of development characterised by a shift from static technology to a modern technology, in which capital requirement and purchased inputs occupy a larger share. But, much of the success of new programmes will depend upon the ability of the workers who act as growth promoters.

It is in this context, sericulture with its vast potential for employment generation in rural areas plays a vital role in alleviating rural poverty. However, this is another crop enterprise which is identified as one of the most appropriate labour intensive cottage industries. This activity combines both agriculture and industry. It provides gainful employment not only at the stage of raising of mulberry plants but also at the stage of rearing of silk worms using output of the farmers as an input of the latter. Sericulture, for example, played a very important role in transforming the traditional bound Japanese agriculture into a modernised agriculture by intensive use of

MULTIMEDIA PRESENTATION

gestation period, less investment, maximum employment potential and quick turnover of the investment. Sericulture generates direct and indirect employment in various ways. First, mulberry cultivation creates employment on the farm and secondly cocoon production which uses mulberry leaves as an input creates large-scale employment for the family labour of the mulberry growers, if that operation is also undertaken by the same household to reduce their underemployment in agriculture. Further, the reeling activity is also mainly undertaken in rural areas or semi-urban areas and the employment generated there would help to reduce the rural unemployment in a significant way. In short, sericulture as a whole, by its very nature of activities creates large scale employment and income generation opportunities in the rural and nearby semi-urban areas accelerating the economic growth of these areas.

Dr. M. Lakshmi Narasaiah

Chapter—1

Introduction

Agriculture forms the backbone of the Indian economy and despite concerted industrialisation in the last four decades, agriculture occupies a place of pride. Agriculture is the source of livelihood for over 70 per cent of population in the country.¹ In a predominantly agricultural economy like India, different regions have their own contribution for an overall increase in agricultural output and employment. Though it is showing a declining trend in the planning era, the share of agriculture in the total gross national product still remains high.

India is a developing country and the striking feature of its economy is the predominance of rural sector. The prosperity of country depends on the prosperity of agriculture and allied occupations. Agriculture promotes economic development of the country. Economic development may be defined as transformation of an economy which is predominantly agriculture and traditional into largely industrial and modern. The economic history of many developed countries of the world like U.K., U.S.S.R., U.S.A., Germany, Japan etc., demonstrates that agriculture development helped and smoothened the process of industrial development by providing food raw-material and employment. Historical records also clearly indicate that no country has attained take-off stage without first achieving a substantial gain in agricultural productivity.²

Accordingly, the discovery of productive employment opportunities in the integrated rural development assumes vital

importance in the economic development of India. For proper utilisation of available manpower in rural areas, establishment of agro-industrial activities is one of the most suitable solutions. The integrated agro-industrial economy should be designed to embrace the entire development structure and process since agro-based industrial activities play vital role in providing productive employment to the rural manpower. These industries enjoy favourable linkages with agriculture and industry on the one hand and towns on the other. Hence, for rural development, small scale agro-based industries with low investment would be most beneficial compared to other schemes.

In order to promote and encourage the already established agro-based industries, the Government of India has been implementing several programmes and projects like Drought Prone Area Programme (DPAP) and Integrated Rural Development Programme (IRDP) etc.

The origin of the Drought Prone Area Programme can be traced to the Rural Works Programme (RWP) taken up during the fourth five year plan period (1969-74). The RWP in 1970-71 was started in selected areas which were identified as prone to drought on the basis of the objective criteria such as low extent of irrigated area, low and erratic distribution of rainfall and high periodicity of drought. These areas of our country periodically experience drought leading to considerable loss of agricultural production and livestock wealth, besides causing untold misery to the people inhabiting in these areas. Large sums have been spent by the government for providing relief after occurrence of drought. Such expenditure has not helped to solve the basic problem of increasing the productivity of these areas and thereby reducing the impact of the severity of the drought on the human and cattle population. Ecological deterioration because of denudation of forests and excessive grazing has led to soil erosion and decrease in the productivity of land. Because of the increasing population both human and cattle, even the marginal lands unsuitable for cultivation have been brought under the plough. The development of these areas is a challenging task and

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an attempt in this direction has been made by the government through the central scheme of Drought Prone Area Programme.

Labour intensive schemes such as medium and minor irrigation, road construction, soil conservation, afforestation, fisheries were taken up under this programme.

The important objectives of the Drought Prone Area Programme (DPAP):

1. Reducing the severity of the impact of drought;
2. Stabilizing the income of the people;
3. Restoration of ecological balance.³

The Drought Prone Area Programme as such was started on the basis of the mid-term appraisal of the fourth plan and the report of the task force on integrated rural development set up by the Planning Commission. The programme was confined to those areas which were originally taken up under Rural Works Programme. In Andhra Pradesh—Anantapur, Chittoor, Kurnool, Cuddapah and Mahaboobnagar districts are selected for the implementation of DPAP.

The main strategy of the programme is to maximise the production in good rainfall years and minimise the losses when the rainfall is not adequate. This is sought to be achieved by adopting soil and moisture conservation measures on a scientific basis, so that maximum utilisation of all available water in the area is achieved. The unit adopted for such scientific planning is the watershed. Five to ten watersheds of about 4,046 hectare each are taken up for comprehensive development. On the basis of the soil surveys and land use maps, areas suitable to various crops, pastures and afforestation are clearly indicated.

Introduction of short duration and drought resistant, varieties of crops are to be promoted to cut down losses on account of inadequate rainfall or long dry spells during the season. Since the development of agriculture has obvious limitations in the areas, the farmers are encouraged to take up subsidiary occupations like animal husbandry, sheep rearing,

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poultry, horticulture and sericulture. Sericulture assumes greater importance in drought prone areas. Among the many practices and crops suggested for commercialisation, sericulture is the most suitable one in drought prone regions since it provides more employment opportunities per acre of its cultivation and ensures greater income to the farmers.

In Indian economy, agriculture is the dominant sector in employment providing work to 70 per cent of the population and accounting for 45 per cent of the GNP.

1. Importance of the Study

Sericulture is a cottage industry par excellence with its agricultural base, industrial superstructure and labour intensive nature. It is remarkable for its low investment, and quick and high returns, which make it an ideal industry or enterprise which fits well into the socio-economic fabric of India. Sericulture is highly recommended by planners and administrators as one of the most effective tools for rural reconstruction and development of the rural society.

Sericulture is labour intensive industry in all its phases, namely mulberry cultivation, silkworm rearing, silk reeling and other post-cocoon processes such as twisting, dyeing, weaving, printing and finishing. The industry provides whole or part-time employment to more than 30 per cent are who drawn from the Scheduled Castes, Scheduled Tribes and other Backward sections. Till recently, sericulture was considered a subsidiary occupation. Introduction of new technology of sericulture has led to making the industry a highly remunerative crop,⁴ as reflected in increasing acreage being brought under mulberry cultivation and the step-up in the raw silk output being witnessed every year.

Out of the 5,76,000 villages in India, sericulture is practised in about 50,000 villages providing employment to about six million persons, most of them belonging to the weaker sections of the society *i.e.*, silkworm seed producer, farmer and rearer, reeler/spinner, twister, weaver, trader, etc.⁵

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Before implementation of Drought Prone Area Programme (DPAP) in 1975, the area under mulberry was only 1,214 hectares. It has now increased to 31,235 hectares by 1992. The financial allocation for sericulture development in the district has been increasing every year from 5.20 to 57.28 lakhs by 1988-89. After that there was backward shift in financial allocation.

As a result of this rapid investment, in sericulture practice in the district, naturally, some changes took place in the economic variables, mainly in the field of cost of production of cocoons. With the introduction of modern techniques in the sericulture, the demand for skilled labour has increased. High yielding variety of silkworm races have evolved as a result of continuous efforts of research institutions. Employment opportunities have been increasing and raising the standard of living of farmers who undertook sericulture practice a decade ago, are now in a position to maintain decent living. In addition to this, the involvement of house labour has decreased. Infrastructure facilities have increased to a greater extent. In spite of all round progress in the sericulture activity in the district, marketing facilities are inadequate. Attracted by the fair prices of cocoons, the sericulturists usually go to the cocoon markets of Karnataka State for selling cocoons.

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Chapter—2

Status of Sericulture in India

Sericulture today is a well established agro-based industry. It is an effective tool of rural development as it generates more income and employment. Sericulture industry today occupies a pride of place in the rural economy of the country. The final product of sericulture is silk. Silk can be produced and marketed where climatic conditions are favourable and labour is cheap and abundant. Sericulture, in this way, is one of the most labour-intensive activities combining both agriculture and industry. In this chapter an attempt is made to present a detailed picture of the progress of sericulture in India and Andhra Pradesh.

Sericulture is an ancient industry in India dating back at least to the second century B.C.¹ According to some historians, raw silk was exported from India to Rome during the reign of Kanishka in 58 B.C. In its long history, sericulture has passed through periods of great prosperity as well as decline.

Efforts were made in the 18th, 19th and 20th centuries, during the British rule, to patronise silk, mainly by the British traders. It actually flourished in the States of Bengal, Mysore and Kashmir. Then, sericulture suffered a series of set-backs, but briefly staged a comeback during the war period. When the Second World War ended in the mid 1940s, silk industry slumped as war related silk consumption ceased abruptly.

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Thanks to the assistance of the Government of India and the measures it took to encourage sericulture in the country after 1945. This industry once again entered a period of prosperity. Sericulture took a rapid stride towards progress especially during the last two decades. And, since then it has come of age and is now poised for a great leap forward.

Silk is a way of life in India over thousands of years, it has become an inseparable part of Indian culture and tradition. No ritual is complete without silk apparel saris, dhotis or showls being used. Silk saris from leading weaving centres and the only wear for women at any social or religious occasion.

The Indian silks are known for their fine quality, lustrous sheen and traditional colours. The masterly designed brocades of Varanasi, the luxurious creapes, georgettes and chiffons of Karnataka, the tie and dye craft of Andhra Pradesh, Gujarat and Orissa, the delicate silks of Kashmir, sheer brilliant fabrics of Bandej and the temple silks of Kancheepuram, along with a host of other traditions of saris and other forms of appeal, are known world wide. In recent years, the tradition of woven fabrics has been joined by the more pragmatic prints from major printing centres in Bombay, Varanasi, Delhi and Bangalore.

Fruits of Sericulture

A few years ago sericulture was considered a subsidiary occupation of poor farmers, while the big landlords used to cultivate only food crops, fruits, orchards etc. Now sericulture is no longer a subsidiary occupation and more and more farmers including the big landlords are taking up. The various advantages of sericulture already identified are as follows.

This agro-based cottage industry is so labour intensive and employment creating and income generating that it is rightly called the '*kalpavriksha*' or the '*kamadhenu*' of the poor and downtrodden.² The other important feature in favour of this industry is its high profitability.

It has been found that sericulture is highly profitable as compared to many other crops. Mulberry has been replacing

important crops in many regions of India, for example sugarcane in Karnataka, horticulture in Jammu and Kashmir, Jute in West Bengal, grapes in Andhra Pradesh and several other crops.

Mulberry takes only 6 months to mature and afterwards for to six crops can be had in a year from the same plant which can stand for about 10–12 years. The leaves can be sold to silkworm rearers if the grower himself find it difficult to rear silkworms and sell the cocoons with better profits.

Sericulture involves simple technologies, easy to understand and adopt even by illiterate farmers and it gives returns in quick succession yielding income in every two or three months. Sericulture does not involve hard labour and rearing of silkworms is generally attended to by women and old people. Sericulture does not require sophisticated machinery and it involves use of simple appliances. Mulberry plants can be grown on any type of soil even in forest fringes, hill slopes and watershed areas. Sericulture ideally suits even in rainfed conditions because of its low cost of production and higher returns than any other crop. Mulberry plant withstands severe drought conditions and gives atleast some income for sustenance while other agricultural crops wither away.

Sericulture would be a more advantageous industry for improving the economy of retarded section of the society like Scheduled Caste and Scheduled Tribes. Non-mulberry sericulture is largely practised by the tribals. Sericulture ideally suit for small and marginal farmers owning less than one hectare of mulberry plantation. Sericulture provides self-employment to the educated unemployed youth in its different sectors. Sericulture also creates employment opportunities in the allied industries like manufacture of appliances, machineries etc., raw silk produced has very good demand both in domestic and international markets. Thus, sericulture is a good source of earning foreign exchange also.³

SILKS OF INDIA

In strict sense "*sericulture*" would refer to the processes involved in production of natural silk. '*Serio*' is a Latin word

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meaning silk.⁴ Silk is a natural filament created by the silkworm. Therefore, sericulture means the raising or rearing of silkworms for production of silk.

There are four kinds of silk of commercial importance in the world *i.e.*, mulberry, tasar, eri, muga. India has the unique distinction in the field of sericulture as it is the only country producing all the four varieties of silk. Silks are differentiated into these four kinds depending upon the race species of the worm and the food plants upon which they feed. The three kinds of silk tasar, eri, muga are called non-mulberry silks as mulberry is not the food plant for the worms. These are also called wild silks since the worms are not fully domesticated unlike mulberry worms and are practised in forests and hilly areas.

Tasar Silk

Tasar silk is the product of secretion from the silk glands of *Antheraea mylitta* and *Antheraea proylei*, the tropical and temperate tasar silkworms respectively. The tasar silkworms thrive on three main food plants namely *Terminalia tomentosa* (Asan), *T. Arjuna* (Arjun) and *Shorea robusta* (Sal). Tasar is copperish coloured silk and does not possess the lustre of mulberry silk. In India the principal tasar producing States are Bihar, Madhya Pradesh and Orissa. On small scale tasar culture is practised in Maharashtra, West Bengal and Andhra Pradesh. The oak tasar culture is not practised in sub-Himalayan States like Manipur, Himachal Pradesh, Uttar Pradesh, Assam, Meghalaya and Jammu and Kashmir.

Tasar silkworm rearing is practised by the adivasis and the tribals from the time immemorial in tropical belt, even much earlier to the introduction of mulberry silk in India. It is practised as a part of rich traditional culture. Unlike mulberry sericulture where 60 per cent of the cost of the cocoons accounts for growing of food plants, in tasar culture 14 million hectare of plantation in tropical and temperate zones are readily available for silkworm rearing.

Eri Silk

The Eri Silk is also known as Endi or Errandi. It ranks first non-mulberry silk production. The eri silk-worm (*Philosamia*

ricini) thrives in India and is multivoltine yielding 4 to 6 crops a year. It is domesticated and the culture extends to an altitude of 5000 ASL in the hills. Its wild counterpart is found in north-eastern India. The eri worm feeds mainly on castor. Besides Kesseru, Tapioca/Cassava, Papaya, Payam and Barkessuru are also sometimes used. Eri cocoons are white and brick red in colour with shades, varying according to the fauna and plant used. The eri cocoons and spun since they are not continuous filaments and are open mouthed. Bulk of eri silk is produced in Assam while on small scale it is also produced in Bihar, West Bengal, Orissa and Manipur.

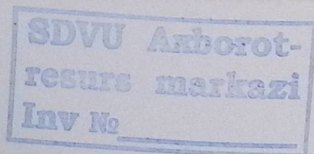
Muga Silk

Muga is obtained from the worms (*Antherea asamensis*) which is quite similar to *A. Mylitta* and *A. Prolei*. The muga silkworm is multivoltine producing 5 to 6 breeds per annum. This semi domesticated species is distributed and cultured exclusively in Assam. The cocoons of this moth are fawn coloured and hence the colour of the reeled silk is golden yellow. The caterpillars of this silkworm thrive well on aromatic leaves of plants of som (*Machilus bombycina* king) and Suolu (*Litsea polyantha* king). These are widely distributed in the valley of the river Brahmaputra.

Mulberry Silk

What is commonly called as silk is the mulberry silk. It is produced by silkworm called *Bombyx mori* and its varieties are found in all voltines. Mulberry sericulture is also known as mori culture. Mulberry silk dominates the field of sericulture in various aspects like quantity and quality of production are popularity. Mulberry sericulture is being practised in various states of India. It consists of four operations:

- (a) Mulberry cultivation;
- (b) Silkworm rearing;
- (c) Disposal of cocoons; and
- (d) Silkworm egg production.



(a) Mulberry Cultivation

Mulberry cultivation is purely an agricultural operation and a major factor determining quantity of production and hence the profitability of sericulture. It is the major cost factor and time and labour consuming activity though less skilled as compared to silkworm rearing.

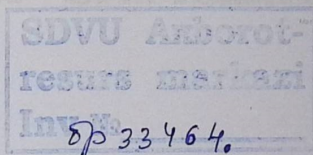
Mulberry is hard plant capable of thriving under a variety of agro-climatic conditions. It responds well to the quality and efficiency of operations and leaf yield is also very high. Mulberry can be grown as bush, high bush, medium tree or long tree. Bush type is widely grown in South India. In Andhra Pradesh, mulberry is grown in bush form upto 1.50 to 2.10 mts. and exists from 10-12 years. Mulberry plant yields leaf within six months after the saplings being planted under irrigated conditions. Bush mulberry can be grown from cuttings, (seeds) seedlings and root-stock. As it is a perennial crop, it needs manual attention throughout the year. The leaf yield of mulberry plant varies enormously basing on the inputs. It ranges from 3,000 to 10,000 kg. under rainfed conditions and from 10,000 to 40,000 kg. under irrigated conditions per hectare.⁵

(b) Silkworm Rearing

Silkworm rearing is a cottage activity carried by the sericulturists in their own house or in a separate shed built for this purpose. It is the second major activity in sericulture requiring a lot of manual attention and becomes a complicated process when various technical and scientific aspects are to be understood and implemented for high productivity. It demands a substantial amount of managerial skill on the part of the rearer and his own experience forms a good guide.

Silkworm Eggs

Rearing starts with the purchase of silkworm eggs often called Disease Free Layings (DFLs) or industrial seed. These are sold. Thick paper sheets or cards on which the silkworm lays the eggs and are adhering to it. A laying occupies an area of about 2 square inches and about 400 eggs are present. The eggs



2.3 Fertilization and Embryogenesis

The egg of the silkworm, *Bombyx mori*, is of interest to experimental embryologists; scientific studies of the eggs are of value from an embryological as well as a practical viewpoint. Silkworm eggs have several advantages: first, the number of eggs laid by one female moth is as large as about 500, though it depends on the race. Second, oviposition usually occurs in the late evening, and about 80% of the total eggs are deposited within 4 h. Third, the size of the egg is 1.20 mm in length and 0.80 mm in width, which is experimentally convenient. Fourth, a technique for artificial hatching has been established and larvae can be obtained as required. Fifth, many genetic characteristics are specific to the egg: for example, shape, shell color, yolk color, serosa color, voltinism and others.

2.3.1 Fertilization

The egg nucleus just after deposition is in metaphase of the first maturation division, which is soon terminated by elimination of the first polar body. The second maturation division takes place about 60 min after deposition and maturation of the egg nucleus is completed after the second polar body is expelled.

A sperm penetrates into the ovum and stays in the anterior part of the egg until the completion of the egg pronucleus. In the meantime, the tail of the sperm separates from the head; near the top of the egg a centrosome appears and an aster is formed. The sperm head gradually swells into a spherical body, forming the male pronucleus. As soon as the maturation division is completed and the female pronucleus is produced, the male and female pronuclei approach and finally fuse.

Sato⁵⁹ and Ohtsuki and Murakami⁶⁰ have carried out cytological observations to determine the time required for meiotic division, syngamy and cleavage in the ovum after oviposition. The durations of these phenomena were also estimated by Tazima and Onimaru;⁶¹ they treated eggs labelled with a gene marker with CO₂ through early developmental stages and observed frequent appearance of unfertilized, early-dying, and androgenic eggs. These data are summarized in Table 2.4. The duration from oviposition to syngamy showed good accord among three experiments, as shown in the table. However, the times required for each step of the first and second maturation division showed some discord. This may be due to different experi-

Table 2.4 Duration of Maturation Division, Syngamy and Cleavage in the silkworm

T	Ohtsuki and Tazima and	
Nuclear behavior	Sato ⁵⁹	— , — . . .
Murakami ⁶⁰	Omamaru ⁶¹	
Oviposition to termination of	5 min 40 min	50 min

the first maturation division

Termination of the first maturation division to

beginning of the second 55 0 —

maturation division (resting stage)

Oviposition to the second 60 40 —

maturation division

Time required for the second 20 60 60

maturation division

Oviposition to syngamy 120 140 130

Time from oviposition to

metaphase of the first 180 160 190t

cleavage division

Time from oviposition to

metaphase of the second — 220 250t

cleavage division

t Time of the termination of the first and second cleavage divisions.

Conditions were as follows.

Sato: Japanese and Chinese bivoltine, 26.5°C; cytologically. Ohtsuki and Murakami: Chinese 108, 25°C; cytologically. Tazima and Onimaru: red and pink egg color strain, 25°C; CO₂ treatment for 180 min (see the text and section 2.3).

mental conditions and strains used as well as differences of opinion regarding each step of maturation division in the ovum.

Polyspermy is usual in *Bombyx mori*. Kawaguchi²⁹ counted the number of sperm entering an ovum. According to his results, from 1 to 11 sperm are observed per one ovum, and 2 or 3 sperm are found most often. Among many sperm which enter the ovum, only one fertilizes the female pronucleus. However, there are exceptional cases where two or more sperm nuclei join resulting in the production of polyploids or dispermic androgenic individuals (see Section 2.4).

2.3.2 Embryogenesis

Histological studies by light and electron microscopy on the embryogenesis of the silkworm have been carried out by several authors.⁵¹⁻⁵⁸

A. Cleavage and blastoderm formation

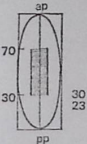
The metaphase of the first cleavage in the silkworm takes place at about 160 to 190 min as shown in Table 2.4. Cleavage proceeds through a succession of synchronous mitotic divisions of the zygote nucleus and its daughter nuclei and by the spread of the resulting cleavage energids through the yolk mass. Early divisions occur almost synchronously but the synchronization is lost before the formation of the blastoderm. The mitotic spindles of each synchronous division are randomly orientated and a number of nuclei progressively approach the periplasm. The rate at which synchronous divisions proceed within the yolk mass is one mitotic cycle per 60 min.⁶⁰⁾ Murakami⁶³⁾ estimated that the number of synchronous mitoses in the early cleavage stage is 11 times greater than those of *Anagcista kiihniella*⁶⁴⁾ and *Chilo suppressalis*.⁶⁵⁾

When the cleavage nuclei migrate to the periplasm surrounding the yolk mass and initiate the formation of the syncytial blastoderm, a small number of nuclei remain within the yolk masses, functioning as vitellogophages.

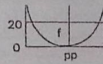
Mitotic divisions occur successively in the syncytial blastoderm and cell nuclei are packed in close array within the surface layer of the cytoplasm. The nuclei then change shape from round to oblong, and the syncytial blastoderm is transformed into a uniform cellular blastoderm. Blastoderm formation occurs about 12 h after oviposition.

B. Germ band formation and presumptive area of the silkworm egg

As soon as the blastoderm is completed, formation of a dense embryonic primordium follows. At about 15 h after oviposition, the cells inside the presumptive embryonic primordium area increase in thickness and become concentrated, still increasing in number through cell divisions. This area in the blastoderm extends broadly on the ventral to ventrolateral side of the egg and is called the germ band. The cells existing outside this area become flat and connected with each other. The area where the germ band is formed is roughly predetermined in the silkworm. The egg is symmetrical only with respect to the median plane. A longitudinal section through it shows an elliptic shape. The germ band is formed along the periphery on the side of shorter radius. If a germ band of regulative type were cut through anterior to the posterior direction at the dorsal line, two complete embryos could differentiate and develop in an egg. However, the silkworm egg, which is a non-regulative type, cannot differentiate and develop after cutting. Therefore, by means of cauterisation, presumptive areas for the embryonic ectoderm, mesoderm, nerve cord and germ cell have been determined.^{53 66 67)}



10
0
10
0
85
80
75
65



ap
PP

A

Fig. 2.4. Presumptive regions of the embryonic primodium in the egg of *B. mori*. A: Lateral view, B and C: ventral view, ap: anterior pole; pp: posterior pole; a and f: extraembryonic area; b: cephalic segment; c: gnathal segment; d: thoracic segment; e: abdominal segment.

The stippled area in B indicates the predetermined region of mesoderm formation. The two lines both sides of the stippled area in B indicated the predetermined region of the nervous system. The solid black square in C shows the presumptive region of germ cell differentiation.

(Adapted from Kuwana and Takami;66> reproduced by kind permission of Baifukan K. K., Tokyo.)

As shown in the figure, the presumptive area of the mesoderm lies within 30 to 70 % of the area from anterior to posterior through the ventral center line and 20% on both sides of the line. The presumptive area of germ cell lies in a limited region on the ventral center line, and the nerve cell area lies at the site on the border line of both sides of the presumptive mesodermal region (Fig. 2.4).

C. Growth of the embryo

B

The germ band grows by cell multiplication and differentiation, and the band differentiates into the three germinal layers; ectoderm, mesoderm and

endoderm. These are followed rapidly by a surface partitioning whereby the body segmentation and appendages are set out. After about 10 days from the time of oviposition, the formation of the embryo is completed, as shown in Fig. 2.5.

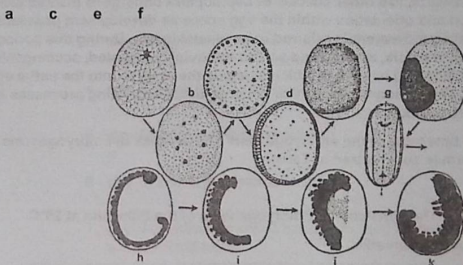


Fig. 2.5. Diagrammatic representation of embryonic development in the silkworm, a: Fertilization, 2 h after oviposition; b: early cleavage, 8 h after oviposition; c: late cleavage; d: formation of blastoderm; e: formation of germ band, 15 h after oviposition; f: germ band, 24 h after oviposition; g: gastrulation; h: formation of stomodeum and proctodeum, 2 days after oviposition; i: formation of appendages, 3 days after oviposition; j: blastokinesis, 4 days after oviposition; k: completion of embryo, 8-10 days after oviposition. (Source: ref. 39. Reproduced by kind permission of Logos Press Ltd., England.)

From the ectoderm are formed the epidermis and its appendages, ocellus, salivary gland, prothoracic gland, corpora allata, molting gland, oenocyte, silk gland, fore-gut and hind-gut, Malpighian tubules, trachea, nervous organ, and outer reproductive organ.

From the mesoderm are formed the inner part of the reproductive organ, muscles, dorsal vessel, subesophageal ganglion, fat body, blood cells and pericardial glands. From the endoderm, only the mid-gut is formed.

D. Differentiation of germ cells

Cytological observations on the differentiation of the germ cells of *Bombyx* have been reported by Miya.^{54'68*} The germ cells differentiate from the blastoderm cells of a predetermined region on the ventral side of the egg. The first multiplication of the primordial germ cells probably occurs during the blastoderm formation. Kawaguchi^{69'} suggested that the germ cells do not

originate from one primordial germ cell, but are of multi-cellular origin, and this was confirmed by Miya.⁵⁴)

E. Blastokinesis

The silkworm, like other species of Lepidoptera undergoes marked changes in position and orientation within the egg space as development proceeds. This embryonic movement is referred to as blastokinesis. During this period of reversal, the yolk, amnion and serosa are quickly ingested, accompanied by a further increase in volume which expands the embryo into the entire egg space. The reason why embryos undergo these surprising processes is not yet fully clarified.

F. The time table of the embryogenesis The process of embryogenesis of the silkworm is summarized in Table

2.5.

Table 2.5 The Timetable of Embryogenesis of the Silkworm at 25°C

Time period Remarks

0- 2 h	The second maturation division and syngamy occur.
2-10 h	Progress of synchronous cleavage mitosis is followed by uniform invasion of the periplasm. The remainder persist within the yolk mass as primary vitellophages.
12 h	The syncytial blastoderm is established.
20 h	The germ band is formed. Suitable for acid treatment for artificial hatching.
25 h	Gastrulation takes place, accompanied by elongation of the germ band.
35 h	Segmentation of the mesoderm is completed and 17 segments can be clearly observed.
40 h	The appendages at the head and thoracic region develop. At this stage, the egg enters diapause in univoltines.
48 h	Differentiation of the appendages at the abdominal region occurs.

2. day Invagination of the proctodeum takes place and
5 s reaches the borderline between 18th and 19th
segment.
 - 3 day Invagination of the trachea is initiated.
s
 3. day Invagination of the proctodeum proceeds and
5 s reaches the 16th segment.
 - 4 day Blastokinesis has begun.
s
 - 5 day Blastokinesis is completed.
s
 - 6 day External processes formed.
s
 - 7 day Taenidium in spiral band is formed in the
s tracheal tube.
 - 8 day Pigmentation begins at the head.
s
 - 9 day Pigments formed at seta and epithelium.
s
 9. day Hatching.
5 s
-

Л

2.4 Parthenogenesis and Polyploidy

Parthenogenesis and polyploidy in the silkworm have been extensively reviewed by Tazima³⁹ and Astaurov.⁴⁰ Only the principal features will be described below.

Method for Artificial Parthenogenesis

The first study of artificial parthenogenesis was reported by Sato.⁴¹⁻⁴⁴ who raised parthenogenetic silkworms by HC1 treatment, analyzed them genetically and cytologically, and suggested the possibility of obtaining homozygous individuals.⁴³ Kawaguchi⁴⁵ and Astaurov⁴⁰ also carried out extensive studies.

The method for inducing parthenogenesis was developed by Astaurov.⁴⁰ The procedure consisted of heat activation of unfertilized eggs extirpated from ovarian tubules. The best results were obtained by immersing these unfertilized

eggs in hot water for 18 min at 46° C. The procedure can be adapted not only to *Bombyx mori* but also to other Lepidoptera species. According to Astaurov, treated eggs could undergo parthenogenetic development to the extent of up to 82 %. Some attained sexual maturity. The most striking feature of these experiments was that parthenogenetic individuals arising in this way were all females, resembling the maternal type. In this respect his results sharply differed from those of Sato and Kawaguchi, who obtained both sexes.

Polyploidy

Very often artificial as well as facultative parthenogenesis is followed by polyploidy. Primarily tetraploid nuclei occur at the early stage of parthenogenetic development of the diploid eggs either through fusion of identical diploid cleavage nuclei or through chromosome duplication without subsequent cell division.

Crossing of tetraploid females with normal diploid males results in the production of triploid offspring of both sexes. Because of the irregular distribution of three chromosome sets in the meiosis of these triploids, 75% both females and males are absolutely sterile when crossed with one another or with normal diploid partners.

On the other hand, triploid parthenogenetic clones (exclusively females) were obtained by successive thermoactivation of the triploid oocytes.^{40'} Instead of two successive maturation divisions, only one division takes place and this corresponds to the second maturation division. An explanation is that the thermal stimuli hinder the normal course of maturation divisions by destroying the spindle at the first metaphase. Next, although the triploid oocytes usually show abnormal maturation divisions, the three homologous chromosomal sets divide equationally and regularly in some oocytes. These equational chromosomal sets then fuse due to the thermal stimulation, resulting in complete triploid parthenogenesis.

It may seem strange that the thermoactivated triploid females, when crossed with diploid males, produce male and female tetraploid individuals among the progeny, although the production of the progeny occurs in low frequency. The explanation of this unexpected fertility of the triploid females is as follows: in the course of triploid parthenogenetic development a somatic polyploidization occurs frequently during the early stage of cleavage. This results in the appearance of mixoploid females with the two different chromosomal sets ($3n + 6n$). Such mixoploid females produce oocytes of which about 0.5 % are hexaploid. These females also produce triploid nuclei. Now, the triploid female nuclei acquire the ability for fertilization with haploid sperms and give rise to tetraploid zygotes (both male and female) (see Fig. 2.6). In this way, tetraploids of both sexes can be obtained.

Since autotetraploid males are almost sterile, no tetraploid bisexual strain has ever been reared. However, if amphidiploid animals are produced, as in plants, fertilization can occur. Astaurov⁴⁰ obtained an allotetraploid bisexual race from 4n "amphidiploid" (In *Bombyx mori*, $n = 28 + 2n$ B. mandarina, $n = 28$, i.e., Ussuri or Shanghai race).

2.4.3 Androgenesis, Gynogenesis and Polyploidy in the Silkworm

The artificial induction of androgenesis, gynogenesis and polyploidy of silkworm eggs has been investigated by many researchers. When a heat stimulus is administered to newly deposited eggs, the female pronucleus is often incapacitated and a substitutional development takes place by the conjugation either of two sperm nuclei or of surviving polar nuclei.³⁴ 40,42,43,45,47,48)

Hasimoto⁴⁷ unexpectedly found many mosaics, individuals due to di-spermic androgenesis, triploids and tetraploids among larvae hatched from eggs treated at high temperature (40° C for 60 min) just after oviposition. The genetic constitution of parents was $+/+ 55 \times od/od$ Tazima⁴⁸ also obtained mosaics with very high frequency by high temperature treatment of Fi eggs of a cross $+ / + 55 \times relre \<^>$ In this case eggs about one hour old were treated at 30° C for 135 min.

Tazima and Onuma⁴⁹ discovered that CO₂ was also a very effective agent for the induction of androgenesis, gynogenesis and polyploidy. Eggs were collected from cross combinations as follows; $++/++ 55 \times Pe\ re/pe\ re$ (for androgenesis), $re/re 55 \times +/+ dd$ (for gynogenesis) and $pe +/+ re 55 \times pe\ re/pe\ re$ (for "ameiotics"). These eggs were put into a CO₂ chamber and allowed to stand for 30, 60, 120, 180 or 240 min at various egg ages from

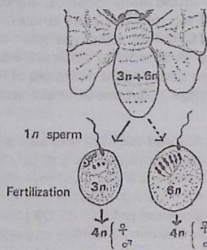
3n unfertilized egg extirpated from ovarian tubules



Equational maturation Heating division

46°C.18min.

3n. 6n Somatic polyploidization



Larva ($3n + 6n$) Mixoploid
Pupa ($3n + 6n$) Mixoploid
Mixoploid female

$1n$ sperm

Fig. 2.6. Schematic representation of the appearance of mixoploid females ($3n + 6n$) by thermal stimulation of the unfertilized $3n$ egg and reproduction of $3n$ and $6n$ gametes which can be fertilized by normal males.

(Kawaguchi obtained $4n$ females and males in the progenies from $6n$ female crossed with $2n$ male.⁷⁶)

Fertilization

0 to 280 min after deposition. They found that androgenetic individuals appeared at 45 % on average at an egg age of 70 min. The incidence of gynogenetic individuals was very low in comparison with androgenetic ones. The maximum incidence was about 10% and it was observed at an age of 50 min. The "ameiotics" category includes exceptionals developed from a nucleus that failed to undergo reduction division. Observationally, exceptions were detected as wild-type eggs among pink and red segregates in the cross of $pe/+ re \$5 \times pe re/per$. They were all females and comprised 3.2% diploids, 87.8 % triploids and 9.0% tetraploids. Since all of them carried both homologous chromosomes in the mother ($pe/+$ and $+1 re$), they were treated as "non-reductionals" or "ameiotics" regardless of the ploidy. The incidence of this type fell almost immediately after deposition.

Hasimoto⁷⁷ was the first to induce mosaics and gynandromorphs by low-temperature treatment at very early stages of the eggs. Later, Tamazawa⁵⁰ also succeeded in the induction of androgenesis, gynogenesis and polyploidy by means of exposure to a very low temperature ($-10^{\circ}C$) for 20 to 24 h at ages of 0 to 180 min (150 min was the best) after oviposition. The incidence of abnormal development was very high, and many gynandromorphs were found due to combination with dispermic androgenesis and normal binucleated syngamy.

2.4.4 Significance of Polyploidy

Polyploid silkworms have been compared with diploid ones from an economic point of view.⁷⁸ The sizes of eggs, silk gland cells and the first spermatocytes are significantly larger in the polyploids than in the diploids. However, they do not necessarily show advantages, for instance, in the weight of cocoons and the number of eggs laid per female. The number of silk gland cells is rather smaller in the polyploids than in the diploids. In addition, the maintenance of a polyploid strain is difficult in general, because of the irregular chromosomal behavior in meiotic division. For these reasons, polyploids are not economically useful in sericulture.

2.5 Diapause and Artificial Hatching

2.5.1 Diapause

The number of generations in a year is mainly determined by hereditary factors. Non-diapause eggs of the silkworm hatch out at $25^{\circ}C$ in about 10 days after deposition, while in diapause eggs, the embryos cease development two or three days after oviposition and acquire developmental capacity only after exposure to a low temperature around $5^{\circ}C$ for several months. Among nondiapause strains, there are bi- and multivoltine types. The most popular types for commercial rearing are bivoltines. The number of generations per year

in bivoltines can be altered by varying conditions such as temperature and lighting. It was known empirically by egg-producers that incubation of bivoltine eggs at low temperature yields female moths which lay non-diapausing eggs. Watanabe⁷⁰* confirmed this experimentally: incubation at a temperature of 15° C gave nondiapause eggs and incubation at over 25° C gave diapause eggs. Moths incubated at an intermediate temperature, 20° C, deposited a mixture of diapause and nondiapause eggs. The temperature during the larval stages also influences the mode of diapause. The effects of temperature during earlier stages of the larva are the same as at the embryonic incubation stage. The effects of temperature at the late stage of the larva were opposite to that at the earlier stage, and high temperature tended to produce nondiapause egg layers.

Illumination of bivoltine eggs also tended to produce diapausing egg layers. Light has almost the same effects as high temperature and darkness has results similar to those produced by low temperature. Eggs of bivoltines incubated in complete darkness at 25° C produced moths that did not always lay diapause eggs. Illumination for 15 h per day is necessary for the eggs to produce moths that lay 100% diapause eggs⁷¹ (see section 3.4.4.)

The nondiapause eggs in usual Japanese types are characterized by serosa cells without pigmentation. However, Katsumata⁷¹> reported that in an Indonesian multivoltine strain the serosa pigments formed even in nondiapausing eggs incubated at 21 to 26° C. Pigmentation and capacity for producing a nondiapause egg layer are controlled by a single, recessive, autosomal gene.

Extensive endocrinological and physiological studies on the diapause have been carried out (see Chapter 8).

2.5.2 Artificial Hatching

Silkworm eggs can be converted to nondiapausing by treatment with hot hydrochloric acid just before or shortly after the onset of diapause.

A. Acid treatment

The time suitable for this treatment is about 20 h after oviposition, when eggs have been preserved at 25° C. However, the development of the embryos varies depending on the preservation temperature after oviposition, and at a constant temperature it varies with the strain: bivoltines develop fast, while univoltines develop rather slowly.

Hydrochloric acid of specific gravity 1.075 at 15°C, or about 15% concentration is heated to 46° C before use. The eggs are then dipped into the hot solution. The standard duration of dipping is 5 min, but varies depending on the strain

and age of the egg: for Japanese strains 5 to 6 min; for Chinese strains 4 to 5 min; for European strains 6 to 7 min.

When the treatment is to be postponed, eggs can be cold-stored at 5° C at an age of 20 h. The duration of the cold-storage must be less than a week; the shorter the better. Before acid treatment the eggs should be brought up to 25° C for 2 to 3 h, to avoid a sudden change in temperature. Before dipping in the hot acid, presoaking of egg-cards in 2% formaldehyde solution for about 2 min is effective to prevent the eggs from falling off the paper. After the acid treatment eggs are washed in running water to remove hydrochloric acid, and dried in an air current as soon as possible.

Acid-treated eggs can be stored in a cold room at 2.5 to 5.0° C if necessary, for 20 days. The egg age suitable for cold storage is 20-30 h at 25° C after acid treatment.

B. Acid treatment after cold storage of the eggs

For the postponement of hatching by more than 40 days, acid treatment is performed after cold storage of the eggs. The best time for preservation of eggs in this way is 42 to 50 h after oviposition at 25° C. The eggs are transferred from 25° C to 5° C via an intermediate temperature of 15° C for about 6 h. High humidity of 75 % to 80 % is necessary. After taking eggs from the cold room, they can be kept at room temperature for 3 to 6 h before acid treatment.

The concentration of hydrochloric acid in this case is 1.100 specific gravity at 15° C (about 20% concentration). The acid is pre-heated to 48° C. The time of dipping in the hot acid solution varies depending on the silkworm strain and the length of cold-storage; for European and Japanese univoltines, 6 to 7 min; for Chinese uni- and bivoltines and Japanese bivoltines, 5 to 6 min. The eggs of hybrids follow the standard for the mother races.

2.5.3 Preservation of Hibernating Eggs until the Next Spring Season

Univoltine or bivoltine eggs are generally laid in June through August. Although the procedure for preservation varies with the season of oviposition, a standard procedure is presented in Fig. 2.7.

As shown in the figure, for the first 50 to 60 days, in particular, the eggs are kept at 23 to 25° C. In September through October, the eggs are still in diapause, so a slight variation in temperature does no harm, but in November through December, the eggs enter the pre-termination of diapause, and high temperature should be avoided.

In order to secure simultaneous hatching, it is necessary to expose the eggs to a low temperature of 7.5 to 5°C or below for more than 50 days, as shown in step 3 in Fig. 2.7.

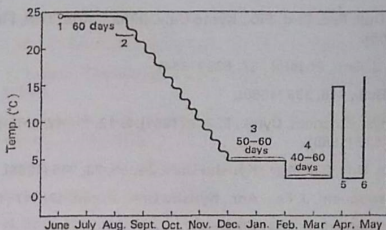


Fig. 2.7. Preservation of silkworm eggs deposited in June. 1, Oviposition; 2, in warm regions, a decrease of the temperature may be necessary; 3, exposure to low temperature; 4, cold storage; 5, intermediate care; 6, removal from cold storage for incubation

(After Ayuzawa et al. ;73) reproduced by kind permission of Fuji Publishing Co. Ltd., Tokyo.)

At the end of January or beginning of February, diapause will be almost terminated at 5 to 7.5° C. At this time the embryos increase in length and the head lobe is wide open. If kept at 5° C for a long time at this stage, the embryos that have terminated diapause become unviable. Therefore, within about 60 days they must be transferred to 2.5° C, as shown in step 4 (Fig. 2.7). Thereafter, the eggs can safely be stored for 40 to 60 days at 2.5° C. To ensure survival, eggs are allowed to develop one step by conditioning at 15° C for a few days (step 5). Thus the embryos reach the stage of "the longest embryo", just one day before appearance of the neural furrow. The eggs are then kept at 2.5° C until the time of incubation.

Other procedures for the preservation of silkworm eggs are described in Handbook of silkworm rearing™).

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CHAPTER

Hiroshi Doira.

Institute of Silkworm Genetics, Faculty of Agriculture, Kyushu University,
Fukuoka 812, Japan

A large number of inbred and characteristic stocks are maintained in several Research Institutions in Japan. Closed line culture system is usually employed in stock maintenance, because difficulties due to inbreeding depression are frequently encountered. An exhaustive compilation of the silkworm stocks cultured in Japan was published by Chikushi.¹ It should be emphasized that this chapter is not intended as an extensive review of the subject. Rather, it is a compendium of Bombyx mutant stocks potentially suitable, if not unique, as laboratory tools. Most of the genetic stocks described are available upon request. Information concerning the availability of various strains of silkworm can be obtained by writing to the scientists in charge of the four stock centers listed in Table 4.1. Readers should consult the comprehensive review of Tanaka,² Kikkawa,³ Tazima,⁴ and Tazima et al.,⁵ For illustrations of the mutants, see Chikushi¹ and Tazima.⁴

Table 4.1 Bombyx Stock Centers in Japan

T. Fukuda Y. Tazima

Tokyo Misima

H. Doira

Fukuoka

N. Yoshitake

Tokyo

National Sericultural Experiment Station National Institute of Genetics Institute of Silkworm Genetics, Faculty of Agriculture, Kyushu University Faculty of Agriculture, University of Tokyo 4.1 Morphological Traits

More than 320 hereditary traits of the silkworm have so far been analyzed. Most of them are related to morphologically recognizable characteristics. The morphological mutants comprise the basic tools for genetic analysis and are easy to isolate and study. Here the silkworm genes are classified into groups according to the different developmental stages they affect. The format is; gene name (gene symbol, linkage group-locus): description.

4.1.1 Serosa Colors

Normally the silkworm egg is short-elliptic, slightly narrowed at the anterior end where the micropyle is situated, and has dark brown pigment in a single-layer membrane of serosa. The egg shape is entirely dependent on the shape of the chorion which is formed in the maternal body before fertilization. Hence, it is inherited pseudomaternally as described in section 4.3.5. The serosa, lying underneath the chorion and covering the yolk and embryo, consists of cells which are derived from cleavage nuclei. Hence, unlike the chorion, its genotype coincides with that of the embryo. The abundance of egg color mutants due to serosa pigment is a unique feature of silkworm genetics. The normal egg is dark brown, while mutants are white, pink, red, brown, and so on. Usually the serosa color coincides with the eye color of the imago, but not always. Chemical steps involved in the formation of brown pigments are assumed to be: tryptophan —> formylkynurenine —> kynurenine —> 3-hydroxykynurenine —> xanthommatin (pigment). As in the "specific loci method" developed by Tazima, ordinary inherited egg color mutants comprise effective tools in mutation research.

Pink-eyed white egg (pe, 5-0.0)

Pigment in serosa cells is light orange; the compound eyes of the moth are pink.

Red egg (re, 5-31.7)

The serosa of the red egg contains as many pigment granules as the serosa of the normal egg, but the pigment is reddish-brown. The compound eyes are dark red. Low hatchability.

White egg 1 (w-1, 10-12.7)

Serosa contains no pigment, compound eyes are also white; lacking the enzyme which converts kynurenine into 3-hydroxykynurenine. The w-1 gene shows maternal inheritance for the egg color but ordinary mode of inheritance for the eye color.

White egg 2 (w-2, 10-16.1)

Freshly laid eggs are yellowish white, gradually becoming red-tinted;

compound eyes are white. In the pupa or egg, 3-hydroxykynurenine is accumulated due to the absence of the enzyme. Ordinary inheritance.

White egg 3 (w-3, 10-19.6)

Egg color is light yellowish-brown, and the eye color of the moth is black. The w-3 gene also causes a weak translucency of the larval skin. Locus 19.6 of the 10th chromosome is the seat of alleles which cause white egg and translucent larval skin to various degrees. Genes so far known at this locus are Aojuku white egg translucent (w^ol), white-egg translucent (oew), new white egg translucent (wox), brown egg-3 (b-3), melamine-induced oily skin and white egg (oml), and Matamukashi translucent (ot). All but b-3 are inherited ordinarily.

Brown egg 1 (b-1, 6-8.0)

Serosa cells are brown, compound eyes are black. Inheritance of b-1 is a typical case of maternal determination. The transmission of 3-hydroxykynurenine from hemolymph to the ovum is somehow prevented. Selective permeability of ovarian cells of b-1 mutant may be involved.

Brown egg 2 (b-2, 6-8.0)

Serosa cells are light brown, compound eyes are black. In this mutant a peculiar combined type of maternal and ordinary inheritance is seen. Fi egg of + / + \$ x b-2/b-2 £ is normal black, whereas that of the reciprocal cross is similar to the recessive b-2 mother. In F2 segregation occurs within a batch laid by a single female with a ratio of 3 black (+) to 1 light brown (b-2). In BFi of the cross +/b-2 \$ x b-2/b-2 segregation also takes place in eggs of a single batch with the ratio of 1 black to 1 light brown. The mechanism is rather complicated. Selective permeability of ovarian cells was assumed to occur in this strain. However, this can only account for the phenotypic difference between the reciprocal Fi eggs. To explain the mechanism of segregation in F2, Kikkawa⁶ proposed that enzyme action in serosa cells of b-2/b-2 egg is too weak to convert 3-hydroxykynurenine into pigments.

Brown egg 4 (b-4, 20-21.9)

Serosa cells are reddish-brown, compound eyes are black with reddish tint. Ordinary inheritance.

4.1.2 Larval Characteristics

A. Newly hatched larvae

The larvae soon after hatching are normally dark brown and are covered with black, fairly long setae on all segments. Mutations that manifest themselves in newly hatched larvae are also a characteristic feature of silkworm genetics.

a. Chocolate (c/r, 13-9.6)

The newly hatched larva is reddish brown. The characteristic becomes less evident with larval growth, but the eye-spots, the crescents, the star-spots and the sieve plates of spiracles remain reddish brown in full-grown larvae, other parts being white. In combination with Moricaud (pM) or Striped (ps), ch manifests a beautiful reddish color in grown larva, while in pip^{ch} grown larvae, chocolate character is recognizable only by the reddish color of the spiracles.

Dominant chocolate (l-a, 9-5.9)

This is phenotypically like the chocolate, but the head is black as compared with the reddish brown of ch. Spiracles are not red-tinted but normal dark-brown.

Sex-linked chocolate (sch, 1-21.5)

The newly hatched larva is reddish brown, like the chocolate. However, the grown larva is white, showing no departure from the normal. Black color of ps or brown of pM is not affected by sch gene.

B. Larval markings

Larval color patterns depend chiefly on the nature and distribution of pigments in the hypodermal cells and the epicuticle. The markings are not clear in younger larvae before the third molt, after the 4th instar they are clearly recognizable. The normal pattern is composed of three kinds of spots; i.e., 1) eye spots on the 2nd thoracic segment, 2) crescents (or lunules) on the 2nd abdominal segment, and 3) star-spots on the 5th abdominal segment.

/r-alleles (2-0.0)

Plain (p) and normal pattern (+) are the most widespread markings among silkworm varieties. Conventionally, the plain is considered as the basic mutant allele. When grown, the pjp larva is white all over the body, except for some linear traces of crescents and star-spots which are often recognized. The larval marking of the wild silkworm, *Bombyx mandarina*, is moricaud. The Moricaud (pM) marking of *B. mori* also consists of innumerable dark grayish brown lines and dots, though somewhat darker than that of the wild silkworm. In the full-grown Striped (ps) larva, the body surface is solid black except for the posterior margin of each segment, where a white "stripe" is left. Several other genes are known at this locus; e.g., Black (pB), Ventral striped (p^v), Whitish striped (pSw), Sable (pSa), and Sable-2 (pSa-2). The dominance relation between the major alleles of this group is $pB > ps$ ^

$pM > + > P > p_-$

S-alleles (2-6.1)

As described above, the 0.0 locus of the second chromosome is assigned to the ji-allelic genes. Locus 6.1 of the same chromosome is occupied by the S-allelic group, New striped (S), Dilute striped (Sd) and New striped-2 (S2), all of which resemble the Striped group of the $\wedge$ -alleles and cannot be discriminated phenotypically. It is not known whether a shift of the ps to 6.1 locus may have occurred.

Zebra (Ze, 3-20.8)

A narrow black band appears on the anterior portion of each larval segment, and dark-brown cheek spots can be seen on both sides of head cuticle. In Zebra faded (ZeF), outlines of the zebra markings are indistinct.

Speckled (Spc, 4-33.1)

Dark brown small spots are scattered on the whole body surface. Female moths hardly lay eggs. X-ray-induced and homo-lethal.

Multilunar (L, 4-0.0)

A pair of large brownish or yellowish spots appears on the dorsal side of each segment. Many modifying genes have been found to affect the number of spot pairs. Incubation temperature during embryonic development also affects the spot number. Marking of the caltrop-type multilunar (Xe) is similar to that of L until the 4th instar, then changing to a caltrop-shaped pattern in the 5th instar. Homozygotes for LP die as embryos.

$\wedge$ -alleles (6-0.0)

Thus far 24 genes are known in this group, comprising one of the most interesting experimental tools from a developmental point of view. For details, see section 4.3.1.

No crescents (Nc, 6-1.4)

A similar gene to $\wedge$ -alleles. Crescents are absent, star-spots and eye-spots are pigmented lightly; homo-lethal.

Quail (q, 7-0.0)

The body is tinted reddish purple, and is covered with shred-like lines. The posterior outline of the crescent is loosened and looks as if pigments are lost.

Multistars (ms, 12-15.5)

When well developed, additional pairs of star-spots are seen on the dorsal side from the 3rd to the 7th abdominal segments. The number of spots depends on the genetic background and incubation temperature, j. M-group (14—)

In the 14th chromosome three different deficiencies, all including oa locus at 42.2 position, are known. They have similar phenotypic effects, lack of

crescents and star-spots in spite of the presence of eye-spots. Homozygotes or heterozygotes between them die as embryos.

No lunule (N1): left end of the deletion 35.2 No-lunule 1 (N11): left end of the deletion 37.7. No-lunule 2 (N12): left end of the deletion 39.7. k. Ursa (U, 14-40.5)

Dorsal and lateral sides of the body are covered by dark brown pigments, leaving a white longitudinal band above the dorsal vessel. When combined with p, only lateral sides of anterior segments are pigmented dark. A compound nature of the locus was suggested. 1. Dirty (Di, 14-43.2)

Irregular black lines and dots cover the body surface, and the regions

of crescents and star-spots lack pigment when homozygous. In ms/ms; DijDi, the multistars are represented as white areas in the dark ground color; in plp/Di/Di, the body is as white as in pip; +/ + . m. Blind (bl, 15-?)

Eye-spots on the 2nd thoracic segment are solid black, n. Cheek and tail spots (cts, 16-4.6)

Chocolate-colored spots are present on both sides of the head cuticle,, and there is a pair of large spots of the same color on the anal plate, o. Brown head and tail spots (bts, 17-36.4)

The head is uniformly pigmented reddish brown, the tail spots being more reddish than those in cts, and the thoracic legs and the spiracle sieve plate are brown, p. Melanism (mln, 18-21.3)

Head cuticle, anal plate, thoracic legs and sieve plate of the spiracle are tinged very dark brown. The mln gene also manifests itself in the adult; moths are black on both the body and wings.

C. Translucent skins

The integument of the silkworm larva is usually opaque, containing whitish crystals of urate. Several mutants are known which have translucent skin. The translucent character is unique in silkworm genetics in that it is caused by many different genes. So far, more than 35 different kinds of genes, located at different loci on different chromosomes, are known to cause translucency of larval skin. The translucency varies according to the mutant strain, depending mainly on the quantity of uric acid in the hypodermal cells. The more translucent the skin becomes, the less uric acid the larva accumulates. The amount of uric acid in the hypodermis of translucent mutants- ranges from 3% to 80% of that of normal larvae. Several mottled translucent mutants are known in which translucent and opaque areas are intermingled in the skin. Translucent genes manifest themselves not only in the skin but also in the serosa of the egg and in various organs and tissues of ectodermal origin. Some are lethal in various degrees, and some are sterile. Translucent genes were all recessive until Yoshimura and Shinkura (unpublished data) found a dominant Obs mutation.

Similar translucent phenotype of the larval skin can also be induced artificially in genetically normal larvae which have been fed for 2-3 days with mulberry

leaves painted with melamine (2,4,6-triamino- Δ -triazine) solution. Small patches of translucent areas appear at first, then extend, and at last melamine-fed larvae become highly translucent all over the skin.^{7*} Another chemical that can induce translucent phenocopy is allopurinol, a inhibitor of xanthine dehydrogenase, which converts xanthine into uric acid (Tamura and Sakate, unpublished data). The following are major translucent genes, a. Sex-linked translucent (os, 1-0.0)

Weakly translucent, antimorphic.

Distinct translucent (od, 1-49.6)

Highly translucent, development retarded, mortality high, antimorphic. At this locus, there is a mutable gene, orf-mottled translucent (odm).

«al-mottled translucent (oal, 2-26J)

Mottled skin with highly translucent and opaque areas, conditioned by i-oal on the 13 th chromosome.

Chinese translucent (oc, 5-40.8)

Moderately translucent.

Giallo Ascolli translucent (og, 9-7.4)

Extremely high translucency, mortality high especially in the pupa, female sterile; activity of xanthine dehydrogenase low. Phenotype of og of Tanaka (og*) is similar to og, but moths of both sexes are infertile.

w-3 group (10-19.6, see also section 4.1.1)

Translucent skin to various degrees, inseparably combined with white eggs. By use of this white egg translucent gene group, interactions between different translucent genes were investigated. Uric acid content in the hypo- dermis is in the order: 1) w-3⁺od>od; w-3>oew>od; oew,2) w-3>oa> oew>oa;w-3>oa;oew, and 3) w-3>os>os;w-3 = os;oew>oew.8)

Aojuku translucent (oa, 14-42.2)

Moderately translucent, antimorphic. There are two different alleles, Aojuku translucent 2 (oa2) and variegated oa (oav).

Waxy translucent (ow, 17-30.1)

Moderately translucent.

Hoarfrost translucent (oh, 20-0.0)

Mottled translucent with a number of indistinct fine opaque dots, j. r- Translucent (or, 22-0.0)

Fairly high translucency. k. Dominant obese translucent (Obs, 18-?)

Translucency moderate, glossy larval cuticle, body stout and spindle- shaped, difficult ecdysis especially at the 4th molt and metamorphosis. Thoracic legs of adult are poorly developed, making copulation difficult.

D. Body colorsLemon (lem, 3-0.0)

Larvae are distinctly yellow due to the presence of a large amount of 7,8-dihydropteridine (sepiapterin) in their hypodermal cells. The chromo- granules in the hypodermis contain sepiapterin-protein complex, sepiapterin combining with a specific protein which is necessary for maintaining the pigment in the hypodermal cells.^{9'}

Inhibitor of lemon (i-lem, 2-29.5)Yellow color of lem larva becomes extremely light in the presence of i-lem.Dilute black (bd, 9-6.7)

Larval trunk is grayish black. The female moth is completely sterile

producing eggs whose micropilar structures are abnormal. The male moth is fertile but cannot copulate unaided, d. Sooty (so, 26-?)

The head is colored dark, the thorax and abdomen are smoky in the larva as well as in the moth. The so/so pupa is black in contrast to yellowish-brown of the normal.

Blood colors

Normal hemolymph is "white" (light yellow or colorless), and readily turns black when exposed to air due to strong activity of phenol oxidase.

Yellow blood (Y, 2-25.6)

Hemolymph is deep yellow, since carotenoids present in mulberry leaves, pass through the digestive organs of the larva. Usually yellow-blooded larvae are also yellow cocoon spinners but not always.

Yellow inhibitor (I, 9-0.0)

This gene completely suppresses Y, hence it produces a dominant white cocoon. Sooty plain yellow inhibitor (I/s), a recessive allele, has a similar effect.

Red hemolymph (rb, 21-0.0)

Hemolymph of the rb/rb silkworm shows a faint reddish coloration after exposure to air. This mutant lacks the enzyme which converts 3-hydroxykynurenine into anthranilic acid in tryptophan metabolism, and thus accumulates a large amount of 3-hydroxykynurenine in the hemolymph.^{10*}

Body shapes

Elongated (e, 1-36.4)

The 1st and 2nd abdominal segments of the larva are unusually elongated, and the intersegmental folds are fully stretched in that region.

Stick (sk, 4-25.8)

Reminiscent of a withered twig, the larval body is extremely slender, firm to the touch and less flexible.

Stony (st, 8-0.0)

The larval body is compact and hard, and bulges appear at the posterior margin of each segment, giving a bamboo effect.

Gooseneck (gn, 9-5.8)

The larval body is slender and constricted between the segments.

Tsujita's lanky (Lcm, 21-17.9)

The larval body is extremely slender, and larval development is slightly retarded. This is not a gene mutation but a chromosomal deletion including rb (Blood color) locus; homo-lethal.

Narrow breast (nb, 19-0.0)

The larval body is nearly spindle-shaped with a narrowed thorax and stout abdomen, flabby to the touch.

Knobbed (K, 11-0.0)

Dermal protuberances appear on the sites of crescents and star-spots as well as on spots manifested additionally by L, ms, and so on. The size of knobs is markedly reduced by ps. h. Extra spiracles (es, 12-16.9)

A pair of small spiracles appears on the 9th abdominal segment.

4.1.3 Cocoon Characteristics

A. Cocoon colors

Almost all of the silkworms reared in Japan for industrial purposes are white cocoon varieties. Many cocoon color mutants, however, have been maintained, possibly since the beginning of silkworm culture. These include yellow, golden yellow, pinkish and green. Yellow and pinkish colors are due to carotenoids, carotenes and xanthophylls, derived from mulberry leaves, and appear only in sericin which coats the fibroin base. Green color is due to flavonoids, and unlike carotenoids the green pigment is distributed in both sericin and fibroin. White (colorless) is conventionally considered to be normal.

C-alleles (12-14.2)

Presence of the yellow blood gene, Y, is a prerequisite for the manifestation of this group of cocoon color genes. They are concerned with the uptake of carotenoids present in larval hemolymph into the middle silk gland. The +c allele does not allow transmission of the pigment into any part of the middle silk gland, hence producing a white cocoon.¹¹

Golden yellow (C): The cocoon is golden yellow on the outside and nearly white inside.

Inner layer yellow (C1): Inner layer of a cocoon shell is deep yellow, while the outer layer is faintly pigmented. Straw color (Cst): Both outer and inner layers of the cocoon shell are faint yellow.

These differences in cocoon color are correlated to the differences in pigment permeability between the parts of the middle silk gland.

Flesh (F, 6-13.6)

In combination with Y and + 0 (or C1), this produces a reddish-yellow color on the outer layer of a cocoon.

Pink cocoon (Pk, locus unknown)

The cocoon is pink on the outside. Coexists with Y; +c (or C1); F is needed for the manifestation of Pk.

Green color genes

At least three independent genes are known concerning the flavonoids of the cocoon. They are; Green a (Ga, locus unknown), Green b (Gb, 7-7.0) and Green c (Gc, 15-?).

B. Cocoon shapes and structures

The most economically important cocoon characteristics are shape, size and structure, and these are also interesting from the viewpoint of behavioral genetics. These characteristics are determined by the spinning behavior of the larva, but it is very difficult to correlate the characteristics to a limited number of genes.

Elliptic shape is most general, but in the Japanese types the cocoon may be constricted deeply in the middle, while most tropical varieties spin peaked cocoons attenuated at one or both ends. Among Chinese types there are many varieties which spin spherical cocoons. There is also a wide range of variation in the structure of the cocoon, some having smooth, and others rough surfaces; some have a tight, while others have a loose texture; some are perforated at one or both ends, others are sparse in the middle. Variations are also seen in the size. In some varieties, two or more larvae spin a cocoon together (double cocoon). A variety indigenous to Okinawa tends to spin "polypupal cocoons," usually about 5, rarely exceeding 20, animals get together and spin a large and irregularly shaped cocoon.

C. Silk production

Naked pupa (Nd, 23-1)

The Nd larva spins a very thin cocoon consisting only of sericin. They frequently fail in spinning, thus becoming naked pupae. The posterior silk gland, site of fibroin synthesis, is markedly degenerated, and subcellular organelles are poor and/or defective.^{12*} Under a light microscope, Machida^{13*} has observed that fibroin-like masses are abnormally distributed in the cytoplasm of the Nd posterior silk gland cell. As an independent mutational event, Naked pupa of Hasimoto (Nd11) has been obtained.

Sericin cocoon (Nd-s, 14-19.2)

Very similar to Nd.

Flimsy cocoon (flc, 3-49.0)

The larva of flimsy cocoon mutant tends to spin an extremely thin-shelled cocoon. In flc cocoon, the amount of fibroin per cocoon was less than 40 % of the normal amount, while the amount of sericin was as much as 70 %.^{14*}

4.1.4 Pupal and Adult Characteristics

The silkworm pupa has an elliptic shape and is yellowish-brown. The imaginal buds of wings protrude from the thorax, extending down to the 2nd abdominal segment on the ventral side. Moths are covered with scales, both on the body and wings. The moth is nearly white, while the wild moth is dark brown.

A. Wing shapes

Mutants for wing shape of the moth are also recognizable in the pupal stage.

Wingless (Fligellos) (Δ 10-13.0)

Both anterior and posterior wings are absent in the pupa as well as in the moth: often dies of bleeding from the uncovered border area between the thorax and abdomen. The 2nd and 3rd legs of the moth are poorly developed, which makes copulation difficult for the male.

Vestigial (Vg, 1-38.7)

Both fore and hind wings are poorly developed, mostly club-shaped and sometimes cut. X-ray-induced and accompanied by a deficiency including the *od* locus at 49.6 position. Homo- and hemi-zygotes die as embryos.

Micropterous (mp, 11-24.0)

The wing buds cover only the thoracic segment in the pupa, The emergence wing size is about 80 % of normal.

Minute wing (mw, 22-16.3)

Similar to mp, but wings are shorter.

Wrinkled wing (wri, 14-0.0)

Wings are poorly developed, not extending fully.

Crayfish (cf, 13-20.9)

Wing buds, both anterior and posterior, are swollen and protrude laterally from the body, producing the chela-like appearance of the crayfish. The swollen buds are fragile, and liable to bleed.

Crayfish of Eguchi (cf-e, 4-?)

Very similar to cf.

B. Body and wing colors

Smoky larvae homozygous for *so* turn black after pupation in contrast to the amber color of the normal pupa. Black moth color is also brought about by a larval marking gene *min*.

Black pupa (bp, 11-17.1)

The pupal skin is tinged with black. Manifestation depends largely on the temperature during several hours pre-pupation. The higher the temperature, the lighter is the body color. All bp/bp animals become black pupae when kept at 20°C during the critical period, but if kept at 30° C they form a normal amber color. The manifestation of bp is controlled by the protei- naceous hormonal system.^{15'}

White-winged pupa (Wp, locus unknown) „

Wings of pupa are very light exhibiting a creamy white color. This white-wing character is most marked in the copresence of bp or so.

Black moth (Bt, 17-0.0)

Both body and wings of the moth are black, though the color intensity is very variable.

White-banded black wing (wb, 5-35.8)

Both the fore and hind wings of the moth are black at the outer margin and in the proximal part of the wing, leaving a wide white band in between. The body is white.

e. Wild wing spot (tVs, locus unknown)

This was originally a characteristic of the wild moth *B. mandarina*. A black spot appears on the apex of the wing, but is less conspicuous in females especially when heterozygous.

C. Eye characteristics

Eye colors are closely related to egg colors. The normal egg color gene makes the eyes black, the red-egg gene gives dark-red eyes, and the white-egg genes give white eyes as a rule, though not always (see section 4.1.1).

When an egg color gene such as pe, w-1 or w-2 is present in combination with the larval pteridine pigment gene, lem, the eye exhibits a greenish- yellow color due to the presence of a large amount of sepiapterin. Either the lem gene or the egg color mutation alone does not lead to accumulation of sepiapterin in the adult eye.

Lustrous (lu, 16-0.0)

Compound eyes have a luster, reflecting light on the eye surfaces. Arrangement of facets is somehow irregular as compared with the normal.

Varnished eye (ve, 6-11.1)

Compound eyes are extremely lustrous, and the number

of facets is only a few percent of that of the normal eye.^{16'}

4.2 Physiological and Biochemical Traits

4.2.1 Embryonic Diapause

Generally speaking, voltinism in the silkworm is maternally determined. Moths lay either pigmented diapause eggs or unpigmented non-diapause eggs. However, a type of voltinism that is not maternally determined has also been reported.^{17'}

A. Maternal inheritance of voltinism

It has been assumed that the diapause hormone is responsible not only for the determination of embryonic diapause but also for the pigmentation of the egg. This "brain—subesophageal ganglion—diapause hormone" system is controlled by multiple gene series. Bivoltine is assumed to be normal.

N₂-alleles (1-?)

According to Nagatomo,^{18'} voltinism is determined by a series of sex-linked multiple alleles, Sex-linked univoltine (Hs) and Sex-linked multivoltine (Hsm), modified by a number of autosomal genes, each of which has only a small effect.

F-alleles (6-21.5)

In contrast, Morohoshi^{19'} believes that the major gene determining voltinism is a series of Γ -multiple alleles, Univoltine (V) and Multivoltine (Vs), located on the 6th chromosome, although their action is affected to a great extent by the sex-linked maturity genes, Lw-alleles (see section 4.2.3).

B. Ordinary inheritance of voltinism

Pigmented non-diapausing egg (pnd, 11-?)

Moths of an Indonesian multivoltine silkworm strain, homozygous for pnd, lay pigmented and non-diapausing eggs when they are incubated as embryos at 25 °C. This characteristic is inherited ordinarily, and pnd gene comes into action after fertilization of the eggs through some unknown mechanism, preventing the embryos from entering diapause.

Non-colored egg lethal (l-n, 12-21.0)

Two ordinarily inherited lethal genes are known to constitute a multiple allelic series causing unpigmented serosa and non-diapausing lethal embryos. The embryos within unpigmented eggs do not enter diapause but continue to develop until blastokinesis (l-n/l-n) or bristle formation stage (l-nh/l-nh) and then die. Non-colored egg lethal of Hasimoto (l-nh) gene was formerly referred to as lethal early (symbol, 1-ey) and was located at 14.2 position on the 12th chromosome.

Molting Characteristics

There are at least 5 different types of molting in the silkworm, namely di-, tri-, tetra-, penta-, and hexamolters. The most common type in the primitive domestic silkworm varieties and in the wild moths are trimolters, whereas almost all present-day commercial strains are tetramolters. Tetramolting character has been considered as the standard (normal) type in silkworm genetics. The expression of molting character is also affected partly by the sex-linked maturity genes (Lin).

A/-alleles (6-3.0)

Trimolting (M3) and Pentamolting (Ms). The dominance relations are $M3 > +M > M5$.

Recessive trimolting (rt, 7-9.0)

Feeding cycle of rt larva in each instar is different from that of M3 larva, rather resembling that of tetramolters eliminating the 5th instar. The M3; rt usually gives rise to trimolters, but occasionally to dimolters.

Dimolting (mod, 11-?)

Larva matures after two molts, and tends to produce trimolters and prothetely (rarely metathetely).

Maturity

A major gene controlling the onset of maturity is located on the Z (or X) chromosome.

a. Zm-alleles (1-2.0)

This gene produces sex-linked inheritance of the life span and growth rate. The Lm alleles also affect, to a marked extent, voltinism, molting and many other economic characteristics such as cocoon weight, cocoon-shell weight, body weight of the pupa and so on.

Late maturity (Lm): Larval maturity is deferred, thus producing a larger body size.

Early maturity (Lme): Larval maturity is promoted, thus producing a smaller body size.

The Lm allele acts to increase the number of molts and to diapause the embryo, Lme allele acts oppositely.

Food Habits

The silkworm is usually monophagous, depending on mulberry leaves, but some mutant stocks have been induced and selected which cannot discriminate mulberry leaves from others.

Non-preference (Np, 11-7.3)

X-ray-induced and fed on beet leaves. This mutation appears to be concerned with the loss of function of the sensory hairs on the maxillary lobes.²⁰ It is accompanied by a recessive lethal effect, and the locus was determined by lethality.

Yokoyama's Sawa-J stock

Selected from a mixed population of 29 Japanese and 37 Chinese strains by feeding as 4th instar larvae with cabbage leaves. Though genetic analysis has not yet been completed, 5th instar larvae of this peculiar stock can survive and mature on fruits such as persimmon or apple as well as on cabbage leaves (Yokoyama, unpublished).

Adaptabilities to artificial diets

Adaptability of various silkworm stocks to artificial diets has been tested by following the growth rate of newly hatched larvae fed for 24 h with an artificial diet. The mode of inheritance of adaptability is seemingly rather simple. High adaptability, with growth comparable to mulberry feeding, is inherited as a single dominant character over low adaptability, which shows almost no growth on artificial diet (Yamamoto, unpublished).

Enzymes

Investigations on naturally occurring polymorphisms of various enzymes have been carried out mainly by means of gel electrophoresis. A considerable number of biochemical genetic markers has become available.

A. Amylases

Matsumura²¹ found that the amylase activities in digestive and body fluids are controlled by two closely linked genes. Isozyme studies are now in progress.

Digestive juice amylase negative (ae, 8-2.8)

Amylase activity in digestive juice is weak. Amylase zymograms of the digestive juice of 10 larvae each from 360 strains, which have been maintained at Kyushu University, were compared. A few very weak amylase isozyme bands, migrating toward the anode, were detected in the digestive juice of ae animals on gel electrophoresis. Those weak anodal isozymes were also detected in + ae larvae. In addition, + ae digestive amylase zymograms showed the presence of 4 or 5 highly active bands migrating toward the cathode. The most rapidly moving of the 5 cathodal amylase isozymes was missing in the 4-isozyme type. Among the 360 strains, 201 were of ae-type, while 3 were mixed with ae and + ae (4-type). In 134 strains, all the larvae showed 4 cathodal amylases, 2 were mixed and in 20 strains all the larvae had 5 cathodal amylases. Mating experiments involving ae alleles and st, a larval shape gene linked with ae, revealed that the presence or absence of cathodal amylase isozymes is controlled by the gene on the 8th chromosome. Larvae of the wild moth showed 4 highly active cathodal amylases and several less active anodal ones (Hara, unpublished).

Body fluid amylase negative (be, 8-4.2)

Amylase activity in the hemolymph is weak.

B. Esterases

Blood esterases

Esterases of the hemolymph have the properties of both cholinesterases and aliesterases, being controlled codominantly by ites-alkles (11-?). Blood esterase A (BesA): The most rapidly moving isozyme. Blood esterase B (BesB): Rapidly moving isozyme. Blood esterase C (BesC): Slowly moving isozyme. Blood esterase O (Bes^o): Isozyme band lacking.

Silk gland esterases

The esterase activity of the silk gland is very high at the anterior portion of the middle silk gland compared to other parts of the gland. Two types of silk gland esterases are controlled by a pair of codominant Ses-allels (locus unknown).

Silk gland esterase A (SesA): Rapidly moving isozyme. Silk gland esterase B (SesB): Slowly moving isozyme. Silk gland esterase O (Ses^o): Isozyme band lacking.

Larval integument esterases

Controlled by codominant /«-alleles (locus unknown). Integument esterase A (lesA): Rapidly moving isozyme. Integument esterase B (lesB): Slowly moving isozyme. Integument esterase C (lesC): The most slowly moving isozyme. Integument esterase O (lesO): Esterase isozyme lacking.

d. Egg esterases

Esterases in the egg are classified into 5 groups of active bands. These are controlled by different genes located at separate loci, but presumably on the same chromosome.

Egg esterase-1 (Eesi, locus unknown): Cholinesterase.

Egg esterase-2 (Eesz, locus unknown): Cholinesterase.

Egg esterase-3 (Eesa, locus unknown): Aliesterase.

Egg esterase-4 (Ees4, locus unknown): Aliesterase.

Egg esterase-5 (Eess, locus unknown): Aliesterase.

C. Alkaline phosphatases

Alkaline phosphatases of midgut tissue can be resolved into active bands, F and S, where F is a soluble enzyme and S is a membrane-bound enzyme.

Alkaline phosphatase-/ (Aphi, locus unknown)

Isozyme F lacking.

Alkaline phosphatase-2 (Ap/12, locus unknown)

Isozyme S lacking, linked with Aphi gene with a crossing over value of about 30%.

D. Acid phosphatases

The acid phosphatase isozymes of larval hemolymph are controlled by the codominant S^+ -alleles, which are located on an unknown autosome independent of Aph genes.

Blood acid phosphatase A (BphA): The most slowly moving isozyme.

Blood acid phosphatase B (BphB): Slowly moving isozyme.

Blood acid phosphatase C (Bphc): Rapidly moving isozyme.

Blood acid phosphatase D (BpliD): The most rapidly moving isozyme.

Blood acid phosphatase O (Bph^o): Isozyme lacking.

4.2.6 Proteins and Blood Cells

A. Hemolymph proteins

The hemolymph proteins of the silkworm undergo a complex sequence of changes which are synchronized to the morphological alterations which take place during metamorphosis. Heritable changes in the nonenzymic proteins have also been reported, a Π/Π -alleles (19-6.2)

An albumin fraction migrating more rapidly to the anode than other proteins on polyacrylamide gel electrophoresis is controlled by a pair of co- dominant alleles.

Albumin F (AlbF): Rapidly moving albumin.

Albumin S (Albs): Slowly moving albumin.

Pt-3 alleles (14-?)

A protein which shows the solubility characteristic of an albumin and migrates next to the Alb zone is controlled by codominant alleles on the 14th chromosome.

Blood protein-3 F (Pt-3F): Rapidly moving Pt-3 fraction.

Blood protein-3 S (Pt-3S): Slowly moving Pt-3 fraction.

Lp-alleles (20-6.2)

Lipoprotein fractions of pupal hemolymph migrating towards the anode on gel electrophoresis are controlled by Lp complex alleles (Gamo, unpublished).

Slow-moving lipoprotein (Lps): Further divided into three sites, Lps-A, Lps-B and Lps'c.

Moderately moving lipoprotein (LpM): Presence or absence of LpM fraction.

Fast-moving lipoprotein (LpF): This region also consists of three components.

Hemocytes

The blood cells of the silkworm are classified into five types; proleuco- cytes, plasmacytes, granular cells, spherule cells, and oenocytoids. Nittono22' has shown that the presence or absence of spherule cells is genetically controlled. Absence of spherule cells may be inherited as single dominant or as single recessive, depending on the spherule-cell minus strain used in mating experiments. Linkage studies have shown that the dominant spherule-cell minus (Sph) gene is linked with the red hemolymph (rb) located on the 21st chromosome (Ninaki and Doira, unpublished).

Silk protein

A mutation specifying the mobility of sericin-2 subfraction, which is synthesized in the middle part of the middle silk gland, has been discovered in Nd-s strain.23' This structural gene of the sericin-2 component, band No. 6 on the electropherogram, is linked with the Knobbed (K) on the 11th chromosome (Gamo, unpublished).

D. Glutinous protein of the mucous gland

Normally silkworm eggs are firmly attached to paper or cloth by glutinous proteide substances secreted from the mucous glands connected with the oviduct, a. No glue (Ng, 12-0.0)

Eggs of Ng mutant are only lightly attached to the substrate, and easily become detached. In mucous glands of Ng, proteide substances are scarcely synthesized due to a reduced amount of r-RNA (Kamijo et al., unpublished).

4.3 Differentiation and Development

Many genes have been analyzed concerning developmental disorders and/or distorted differentiations.

4.3.1 Distortions in Segment Differentiation

Disorder in segment differentiation is frequently encountered in stocks having most members of X-alleles and some other genes, a. [^]-alleles (6-0.0)

From the developmental point of view, genes of the iT-group are among the most interesting in silkworm genetics. They act in very early stages of embryonic development involving differentiation of body segments, frequently causing asymmetric fusion of the segments. All are concerned not only with repetition and/or disappearance of markings and appendages, but also with abnormalities of reproductive organs. In addition, most of them are lethal as embryos in the homozygous state. Heterozygotes between two different alleles tend to show additive, exaggerated or diminished manifestations. Advances in the study of the fine structure of the gene revealed that genes of the E- group are located in the same region but at separate sites in close proximity, thus constituting a pseudo-allelic complex region.²⁴ The distribution of the sites may cover the whole region from 0.0 to 1.4, where a similar gene, Nc, is located. Major alleles of this group are listed. Abdominal segments are referred to as A1 for the first, A2 for the second, A3 for the third, and so on.

Plain extra legs (E): Rudimentary crescents and extra abdominal legs on A1 and A2.

Additional crescents (ECa): Additional crescents on A3, star-spots absent. Homozygotes lack abdominal legs and die as embryos.

Extra crescents and degenerated abdominal legs (EDl): Extra crescents on A3, star-spots absent; first abdominal legs on A3 rudimentary.

Deformed gonad (EGd): Additional crescents on A1, supernumerary legs on A1 and A2; gonads abnormal.

H-extra crescents (EH): Extra crescents on A1; semi-lethal.

Kp-supernumerary legs (Ekp)\ In homozygote, extra crescents on A3, extra legs on A2; heterozygote lacks extra crescents.

New multistar (EMs): Additional star-spots on A4 and A6; homozygote dies as embryo with extra legs on A7 and A8.

Mu-supernumerary legs (EMu): Additional star-spots on A6, extra legs on A7.

New additional crescents (EN): Extra crescents on A3, star-spots lacking, homozygote dies as embryo with thoracic legs on all segments.

Extra crescents and short wing (ESw): Additional crescents on A1, small

abdominal legs on A1 and A2, hind wings of moth stunted. Tanaka's double crescents and supernumerary legs (ET): Extra crescents on A1; in homozygote, supernumerary legs on A1 and A2; only A2 extra legs in heterozygote; homozygous female cannot lay eggs because two oviductus gemini fuse together at their proximal ends and are not connected with the oviductus communis. Triple crescents (ETc)\ Additional crescents on A1 and A3, star-spots absent; in homozygote abdominal legs are entirely missing. Highly lethal.

Malformation (mal, 4-14.8)

Larval body is twisted mostly around the 5th abdominal segment, penetrance varies with incubation temperature during the embryonic stage.

Burnt (Bit, 11-5.5)

Along the dorsal midline of the 2nd to the 5th abdominal segments, the skin shows a burn-like scar; the dorsal vessel of that region is expanded. Homozygous lethal.

Abnormalities in Appendages

Most members of if-alleles have extra or degenerated legs.

Apodal (ap, 3-22.3)

Thoracic legs are degenerated, abdominal legs are normal. Larvae have difficulty in creeping and feeding, moths have no legs and cannot walk. The female moth is sterile; the male is fertile but must be held in an appropriate mating position to achieve copulation.

V-supernumerary legs (Sl-v, 21-19.4)

There are extra legs on the 2nd abdominal segment. Homozygotes die as embryos having supernumerary legs on the 2nd and the 7th abdominal segments.

Dwarfs

Retarding (Rt, 2-?)

Due to deficiency in the 2nd chromosome, including Gr (Chorion color) locus, homozygotes die as embryos. Heterozygotes are small in size, and slow in development.

Retarded growth (Rg, 3-24.9)

This is not a gene mutation but a chromosomal deletion ranging from 24.9 to 41.8 in the 3rd chromosome, homo-lethal. Larval growth is distinctly retarded in the heterozygote.

Slow growing (Slg, 15-?)

Larval growth is retarded by about one week as compared to normal, homo-lethal.

Tsujita's dwarf (Df-t, 20-11.0)

Development is retarded, the larva is small in size. Homozygotes die as embryos.

Dwarf-k (dw-k, 10-0.0)

Body size is very small, larval development is retarded, viability of homozygote is good. The number of eggs laid by a single female is one-third of normal at most.

4.3.4 Lethals

Lethal mutations occur very frequently in the silkworm, as in other experimental organisms. Lethal mutations in the silkworm so far reported are mostly concerned with embryonic death. A few lethals manifest themselves during post-embryonic development. Some of the morphological as well as physiological mutations are also accompanied by lethal action in homozygous states. In this section they are not repeated.

A. Embryonic lethals

In the silkworm, embryonic lethals can easily be detected because all eggs are attached to an egg-card and dying embryos are readily distinguishable from those going to hatch and from hatched ones. However, the occurrence of lethals is very frequent, and readers having special interests should consult Tazima et al.b) Maternally conditioned lethals:

ki (see section 4.3.5A), lem1 (see section 4.3.4B (below)) Lethals accompanied by serosa color alterations: Lethal brown (l-br, 9-18.1)

Homozygote is lethal, manifesting brown pigmentation in serosa. White lethal egg (pe1, 5-0.0)

Multiple allele of pe. Eggs homozygous for pe1 colored yellow and lethal.

The pel/pe is light orange in serosa, deep pink in compound eye.

B. Post-embryonic lethals

Lethal mutations that act during post-embryonic development are concerned with defective hormonal and/or metabolic systems as well as abnormalities in the larval cuticle.

Newly hatched larva lethal (l-nl, 15-?)

Homozygotes hatch normally but die with shrinking within a few hours after hatching.

Non-molting (nm, locus unknown)

Homozygotes hatch normally, survive about two weeks without molting as 1st instar larvae and then die. The omission of molting is related to a structural and perhaps functional abnormality of the oenocytes.²⁵ Ibino (al, 5-37.9)

Homozygotes for al hatch and develop normally, exhibiting no departure from normals during the 1st instar. Distinct albino features appear soon after the 1st molt. Due to insufficient differentiation and incomplete hardening of the cuticle,²⁶ albino larvae can neither retain water in their body cavities nor chew mulberry leaves. They die with shrinking within 30 h after ecdysis. A different allele, albino-2 (al2), has been obtained as an independent spontaneous phenomenon.

Lemon lethal (lem1, 3-0.0)

This is a multiple allele of the lemon (Larval body color), inherited as a maternally conditioned embryonic lethal. Embryos homozygous for the lem1 gene obtained from sibmating moths having + /lem1 genotype develop normally to young blackish larvae which can hatch and grow normally until the 1st molt. Immediately after ecdysis, lemon lethal larvae become distinctly yellow due to the presence of a large amount of sepiapterin in the hypodermis. This cannot chew mulberry leaves, and die. Yellow lethal larvae at the beginning of the 2nd instar can also be obtained from the cross + /lem12 x lem/lem. However, from the cross lem/lem12 x + /lem or lem/lem1 x lem/lem1⁺, all homozygous lem1/lem1 embryos develop normally but manifesting yellowish body color. They cannot gnaw off the egg chorion and die inside the egg.²⁷

Skunk (sku, 22-?)

Homozygous larvae as well as their feces stink terribly. The malodorous larvae mature, spin poor cocoons and die before pupation.

4.3.5 Defect in the Function of Ovarian Follicle Cells

The main products of the ovarian follicles are the chorion substances. The arrangement and function of ovarian follicle cells determine the shape and structure of the egg shell. Thus abnormalities in the egg shape, which is entirely dependent on the shape of the chorion, reflect defects in the functional activities of the follicle cells.

A. Egg shapes

