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Breeding perspective for silk quality in tropical tasar silkworm, *Antheraea mylitta* Drury

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Abstract

Antheraea mylitta, a wild sericigenous insect is a species widely distributed in the form of various ecoraces which are contributing to the livelihood of forest based aboriginals. In the present investigation, certain tasar populations of *A.mylitta* were studied based on their environmental conditions, phenotypic variability and behavior pattern. The study revealed that some ecoraces are available in many parts of tasar producing states as robust cocoons, while others are found in limited numbers in exclusive areas of natural environs, bestowing their respective unique features. The present study is on breeding of tasar ecoraces is aimed at the improvement of to be bestowed with superior characters *i.e.,* survivability and fecundity of Daba TV and also as one of the probable strategies for enhancing the productivity and quality of tasar raw silk by breeding method.

Key words : *Antheraea mylitta*, ecoraces, phenotypic variability, breeding

INTRODUCTION

Antheraea mylitta, a wild sericigenous insect is a species widely distributed in the form of 43 ecoraces from West Bengal in the East to Karnataka in the South with its natural inhabitation in the forest areas of Andhra Pradesh, Bihar, Orissa, Madhya Pradesh, Maharashtra and Telangana. It is a polyphagous insect feeding on a number of food plants primarily on *Terminalia arjuna* and *T. tomentosa,* and a host of secondary food plants like *Ziziphus*, *Tectona, Bauhinia, Lagerstroemia, etc*. The wide range of distribution of the species has encountered diverse geographic and climatic variations of the distinct areas, leading to marked differences in not only phenotypical and physiological traits but also in the commercial and technological aspects. Among fifty races of sericigenous insects, twenty-five races of *Antheraea mylitta* widely available [1-2] *Antheraea mylitta* Drury (Andhra Local ecorace), is an exclusive ecorace of the state of Andhra Pradesh, Telangana and is well known for its superior commercial characters. However it is on the verge of extinction due to its weaknesses in voltinism, emergence, hatching, low yield *etc*. [3-6]. The ecorace conservation is must for utilizing their valuable genes in enhancing productivity and to build variation in new population through hybridization [7-8].

Andhra local ecorace of *Antheraea mylitta* D. possesses superior commercial cocoon traits, but its commercialization could not be under taken due to weak voltinism, asynchronised moth emergence (35-40%), poor fecundity (165-205), less amenable to human handling and heavy crop loss during late age silkworm rearing stages resulting in low productivity (10-12 cocoons/dfl). However, this genetic resource material is bestowed with superior commercial characters like low denier (7%), high reelability (66%) and higher silk ratio (16.8%).

Daba is an adapted ecorace of *A. mylitta* D. with commercial exploitation under tasar seed sector. This ecorace shows sustenance in cultivating breeding pocket with moderate fecundity (200-250), S.R. % (13.50-14.50%) and good survival (50-60 cocoons/dfl). Hence, the breeding programme has been formulated involving two ecoraces *viz*., Daba TV and Andhra local ecoraces. This work aims at the improvement of Andhra local ecorace with bestowed with superior characters *i.e.,* survivability and fecundity of Daba TV.

The basic information on ecology, environmental factors, climatology, flora, fauna and their inter-relationship, the life cycle, diapause, reproductive biology and voltinism and population dynamics of tasar insect reveal its critical requirements to handle for breeding. The commercial attributes of tasar insect, viability in the offered eco-climatic condition suggests their biotic and economic potential and the commercial feasibility of ecorace/ breed/ line. Hence, the coordinating of adoptable breeding strategies for tasar silk yield and quality found vital for its indispensible role in generating rural livelihood, employment and foreign revenue.

The reproductive potential of different forms of tasar ecoraces has the influence of environmental factors and physiological status of parents involved as the parental moth correlation and their origin is vital to make them commercially viable [9-11].

Earlier studies on the molecular characterization of genome extracted from silk glands of Daba TV and Andhra local ecorace of *A. mylitta* D has revealed individual and strain specificity of the ecoraces with some genetic proximity between the two ecoraces. The fact that at molecular level there is little or no difference between Daba TV and Andhra local ecoraces, it is a significant observation showing a compatibility between the ecoraces which may, in future lead to evolving a totally new strain of *A. mylitta* [12].

The selection of breeding method is to develop a breed with stability and productivity in terms of silk quantity, the method of repeated backcrossing among appropriate donor and recipient parents looks apt for attaining the quality of commercial traits was reported by [13-15].

The problems of asynchronised moth emergence and poor mating compatability of Andhra ecorace were answered to some extent through *in situ* grain age model. As a result natural mating has shown improvement from 79.5 to 96.00% against 5-6% in captive conditions. Pilot studies on mating compatibility and survival rate of Andhra ecorace was conducted by utilizing Daba ecorace. More than 77% of emerged Andhra ecorace females mated naturally with Daba ecorace males. However, in reciprocal crosses 57% of natural mating (Daba TV × Andhra) was obtained under in situ conditions. The evolved hybrid combinations exhibited 32-45% of effective rate of rearing. All hybrids of diallel analysis of Daba mutant lines were better in respect of hatching [16].

Hence the present investigation is taken up to study the genetic proximity of Andhra local ecorace with DabaTV ecoraces of *Antheraea mylitta,* by comparing their geographic distribution, morphological and ecological parameters and bring about an idea of evolving a new breed without losing its beneficial commercial characters and suggest methods to overcome its weaknesses.

MATERIALS AND METHODS

VOLTINISM

The tasar silkworm, *Antheraea mylitta* Drury, is trivoltine in nature, i.e. three generations in a year during the months of June and December *i.e*., from monsoon to mid winter; termed as seed crops (June-August), second (September-October) and third (November-June) or commercial crop, respectively. Each generation has 4 stages in its life cycle i.e., egg, larva, pupa and adult/moth. Unlike *Bombyx mori*, where the egg stage is in diapause in *Antheraea mylitta*, the tasar pupae after third crop (mid winter season) remain under diapause and continue till the onset of monsoon (May/June).

MOTH EMERGENCE AND COUPLING

The emergence of moth occurs in the night from 18.00 to 22.00 hr. coupling of moths just after emergence is not allowed by keeping male and female moths separately in the cages for about 2-3 hr so as to from 19.00 hr and continued u to 23.00 hr. Coupling (45-60%) takes place in the captive condition inside cages and the coupling of the remaining moths was induced mechanically. The optimum temperature and relative humidity for moth emergence and coupling are 22-32ºC and 75-85%, while the average rainfall recorded during July-August was 132.5 mm.

On reaching the female moth, the male moth starts courtship behavior with raised antennae, fluttering wings around the female followed by mating. Mating lasts for 10-12 hr but it continues up to 24 hr of the next day if not disturbed. Similar finding of mating on *A. pernyi* was reported by some workers [17]. Copulation by one male moth is enough for complete fertility of the female moth. Male moths are utilized for second time mating when there is shortage. In the natural condition the male moth flies long distances in search of females and the female moth also flies particularly after mating to lay the eggs on the leaves and branches of the food plants. However, they usually do not fly at day time. The moths do not lay all eggs at one place only but in a scattered way. The coupled moths detach at the slight mechanical disturbance. The life span of the adult moths is 7-10 days.

Trials on hybridization were made during breeding season, during 2 consecutive years, for Andhra local ecorace with six other ecoraces. However, it was observed that hybridization was possible only between Andhra local and Daba TV ecoraces because of their synchronization in emergence pattern and variation in emergence pattern of other ecoraces.

The cocoons of selected ecoraces of Tasar silkworm, *Antheraea mylitta* D., were collected from various ecopockets and were maintained in well ventilated grainage chambers in the laboratory. The date of emergence of each of the ecorace (male/female) was noted. From the data collected, it is observed that there is a chance of coupling between Andhra local and Daba TV ecoraces due to corresponding/ synchronising dates of emergence, seasonal coincidence and physiological compatibility towards breeding (Fig 1; Table 1).

HYBRIDISATION

- Trials on hybridization were made during breeding season, during the three years, for Andhra local ecorace with six other ecoraces.

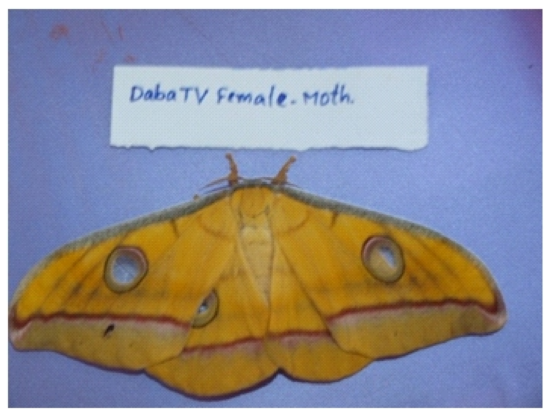

Fig 1: AThe newly emerged adult moths of Tasar silkworm, Antheraea mylitta D., Andhra local and Daba TV

Table 2: Emergence pattern of various ecoraces of Tasar Silkworm, Antheraea mylitta Drury in various seasons (based on the dates in table 1a-g)

Summer		Rainy			Winter			
March	**April**	**June**	**July**	**Aug.**	**Sept.**	**Oct.**	**Nov.**	**Dec.**
Bhandara Raily	Raily Daba BV	Sukinda Modal	Sukinda Modal Daba TV	Daba TV Andhra local	Daba BV	Daba TV Andhra local	Daba BV Daba TV	Bhandara

Fig 2: Hybridisation of tasar silkworm, *A. mylitta* D. Andhra local (male) and Daba TV (female).

- It was observed that hybridization was possible only between Andhra local and Daba TV ecoraces.
- Breed options were less due to varied behaviour of diapause and non-diapause and varied climatic conditions
- Parental stocks of Andhra local and Daba TV were maintained in different gardens to avoid intermixing during breeding in three seasons.
- The breeding activity was done based on the synchronisation of emergence and mating compatibility.
- F1 hybrid was obtained by crossing male Andhra local and female Daba TV

During hybridization, Daba TV (female) was crossed with Andhra local (male) to produce F1 hybrids, in which Daba TV (female) will be donor parent whereas male counterpart of Andhra local acts as recipient parent. This method is aimed at introgression of survivability traits (Fig. 2).

OVIPOSITION OF HYBRIDIZED FEMALE MOTH

After decoupling, the individual female moths are kept for egg lying in nylon net bags (15 × 20 cm) or mud cups kept in dark condition at $22+2^{\circ}$C and 75+5 % R.H. Eggs are collected after 72 hr of oviposition but egg lying continues up to 4-5 days. The average fecundity ranges from 185-230 eggs in both the cropping seasons (Fig.3).

Fig 2: Oviposition of hybridized female tasar silkworm, *A. mylitta* (Daba TV).

INCUBATION AND HATCHING OF HYBRIDIZED EGGS

After proper disinfection the eggs are incubated at $22+2^{\circ}$C and 70-80% R.H. Hatching takes place after 9-10 days of incubation. Profuse hatching does not occur initially and it continues up to late hours of the day. The average hatching varies from 60-75% (Fig.4).

Fig 4: Newly hatched larvae of F1 hybrids of Tasar silkworm, *A. mylitta* D.

Fig 5: Hatching and early larval development of F1 hybrid tasar silkworm, *A. mylitta* D., maintained under indoor conditions.

F1 HYBRID *LARVAL DEVELOPMENT*

The brushing and rearing of tasar silkworm need special care. The duration of development of first, second third and fourth instars took 6 days, 5 days, 7 days and 8 days respectively while the fifth instar took prolonged time (19 days) where the temperature and relative humidity ranges from 23-32°C and 64-78 % R.H., however during some days an R.H. of 29-35% was also recorded. The larva spins the cocoon after about 35 days of active feeding through five larval instars but it is prolonged up to 44 days depending upon the climatic condition. In the present studies, the hybrid stocks were maintained under indoor conditions (Fig. 5) in first and second instars, while late age larvae were reared in the field of *Terminalia* Plantation at Kakatiya University, by careful observation of larval growth, ecdysis and moulting (Fig.6 & 7).

Laboratory studies have shown that the optimum favourable temperature and relative humidity for larval development is 28°C and 80 % for first and second instars, 27°C and 75 % for third instar and 28°C and 80 % for fourth and fifth instars respectively (Fig.6).

FEEDING BEHAVIOUR OF F1 HYDRIDS

The larvae prefer tender leaves to mature and hard leaves irrespective of the instars. Just after hatching the larvae crawl in search of food and are in the habit of eating bits of egg shell when hatched. It is observed that the worms crawl up to the tip of the branches and start eating tender leaves. It is desirable to provide tender leaves to the chawki worms (1^{st} to 3^{rd} instars) while semi-mature and mature leaves should be provided to the 4^{th} and 5^{th} instars respectively for healthy growth of the larvae. The larvae feed on the entire leaves including midrib. It stops feeding at the slight disturbance. A larva consumes about 75-80 g of leave during the entire larval development (Fig 8).

PUPATION AND ADULT DEVELOPMENT OF F1 HYDRIDS

The mature larva completes the pupation inside the cocoon within 5-7 days. The pupa develops into adult moth after about 20-35 days. After second crop the pupa undergoes diapause for 5-6 months. However, in the present study a few stray emergences of moths was observed (Fig 9).

TEMPERATURE (°C) AND RELATIVE HUMIDITY (%)

The optimum temperature and relative humidity for the tasar silkworm rearing are 25-30°C and 60-70% respectively. In the present study, the temperature and relative humidity were recorded with the help of lab thermometer and hygrometer respectively. The average of lowest and highest values taken and noted them instar-wise.

MORTALITY

The mortality of F1 hybrids was calculated by counting the number of dead worms and removal of diseased worms during

Fig 6: Third and Fifth instar F1 hybrids of Tasar silkworm, *A. mylitta* D., feeding on *Terminalia arjuna* in outdoor conditions.

Fig 7: Ecdysis in third and fourth instar of F1 hybrid of tasar silkworm, *Antheraea mylitta D.*

Fig 8: Feeding behaviour of F1 hybrid of tasar silkworm, *Antheraea mylitta D.*

each instar by observing the symptoms (Fig. 13, 15). The mortality percent was calculated for the DFLs(Fig.10).

RESULTS

The instar wise average maximum temperature and its standard deviation of Tasar silkworm *Antheraea mylitta* (Daba TV) in July August were 25.16 ± 1.47 (S.D.), 28.86 ± 0.62 (S.D.), 31.67 ± 1.84 (S.D.), 32.25 ± 4.62 (S.D.) and 27.31 ± 2.47 (S.D.) in I, II, III, IV and V instars respectively. The instar wise average minimum temperature and its Standard Deviation of Tasar silkworm *Antheraea mylitta* (Daba TV) in July August were 19.01 ± 0.93 (S.D.), 21.38 ± 0.51 (S.D.), 22.71 ± 0.80 (S.D.), 26.25 ± 5.67 (S.D.) and 21.31 ± 3.95 (S.D.) in I, II, III, IV and V instars respectively (Table 2).

The instar wise average maximum Relative Humidity and its Standard Deviation of *Antheraea mylitta* (Daba TV) in July August were 73.16 ± 2.31 (S.D.), 77.8 ± 1.92 (S.D.), 89.85 ± 4.22 (S.D.), 94.37 ± 6.78 (S.D.) and 87.47 ± 7.05 (S.D.) in the I, II, III, IV and V instars respectively. The instar wise average minimum Relative Humidity and its Standard Deviation of *Antheraea mylitta* (Daba TV) in July August were 51.33 ± 2.42 (S.D.), 59.6 ± 3.36 (S.D.), 75.71 ± 6.52 (S.D.), 83.37±13.55 (S.D.) and 66.84 ± 9.00 (S.D.) in the I, II, III, IV and V instars respectively (Table 2).

The instar-wise average mortality of *Antheraea mylitta* (Daba TV) in July August were 11, 13, 16, 20, 26 in the I, II, III, IV and V instars respectively . The instar-wise % loss of Tasar silkworm, *Antheraea mylitta* (Daba TV) in July August were 7.3%, 8.6%, 10.6%, 13.3%, 17.3% in the I, II, III, IV and V instars respectively. The instar-wise larval life span of Tasar silkworm, *Antheraea mylitta* (Daba TV) in July August were 6, 5, 7, 8, 19 in the I, II, III, IV and V instars respectively (Table 2).

Table 2: Instar-wise Average Temperature (°C), Average Relative Humidity (%) and Mortality due to Breeding (Andhra Local vs. Daba TV) of Tasar silkworm, *Antheraea mylitta* in various seasons (July-August). [for 1 DFL = 150 eggs].

Instar	Temperature (°C)		Humidity (%)		Mortality	% of Loss	Instar-wise larval life span
	Max.	Min.	Max.	Min.			
I	25.16 ± 1.47	19.01 ± 0.93	73.16 ± 2.31	51.33 ± 2.42	11	7.3	6 days
II	28.86 ± 0.62	21.38 ± 0.51	77.8 ± 1.92	59.6 ± 3.36	13	8.6	5 days
III	31.67 ± 1.84	22.71 ± 0.80	89.85 ± 4.22	75.71 ± 6.52	16	10.6	7 days
IV	32.25 ± 4.62	26.25 ± 5.67	94.37 ± 6.78	83.37±13.55	20	13.3	8 days
V	27.31 ± 2.47	21.31 ± 3.95	87.47 ± 7.05	66.84 ± 9.00	26	17.3	19 days

Fig 9: Pupal and cocoon formation of F1 hybrid tasar silkworm, *Antheraea mylitta* D.

Fig 10: The F1 hybrid tasar silkworm, *Antheraea mylitta* D., attacked by viral disease.

Table 3: Instar-wise Average Temperature (°C), Average Relative Humidity (%) and Mortality due to Breeding (Andhra Local vs. Daba TV) of Tasar silkworm, *Antheraea mylitta* D., in various seasons (September-October). [for 2 DFLs = 220 eggs].

Instar	Temperature (°C)	Humidity (%)	Mortality	% of Loss	Larval life span/days F1	Larval life span/ days Control
I	28.65 ± 1.14	35.5 ± 2.25	16	7.27	6	3-5
II	28.92 ± 0.66	48.8 ±6.05	20	9.09	5	4
III	28.92 ± 0.39	35.28 ± 1.49	24	10.90	7	6
IV	29.76 ± 2.94	29.87 ± 4.88	27	12.27	8	8
V	30.55 ± 3.61	28.28 ± 8.44	36	16.36	21	12

The values are expressed in terms of Standard Error of the Mean.

The instar wise average temperature and its standard deviation of Tasar silkworm *Antheraea mylitta* (Daba TV) rearing of September-October 28.65 ± 1.14 (S.D.), 28.92 ± 0.66 (S.D.), 28.92 ± 0.39 (S.D.), 29.76 ± 2.94 (S.D.) and 30.55 ± 3.61 (S.D.) in I, II, III, IV and V instars, respectively. The instar wise average Relative Humidity and its Standard Deviation of *Antheraea mylitta* (Daba TV) rearing of September-October 35.5 ± 2.25 (S.D.), 48.8 ±6.05 (S.D.), 35.28 ± 1.49 (S.D.), 29.87 ± 4.88 (S.D.) and 28.28 ± 8.44 (S.D.) in the I, II, III, IV and V instars, respectively (Table 3).

The instar-wise average mortality of *Antheraea mylitta* (Daba TV) in September-October were 16, 20, 24, 27, 36 in the I, II, III, IV and V instars, respectively. The instar-wise % of loss of Tasar silkworm, *Antheraea mylitta* (Daba TV) in September-October were **7**.27%, 9.09%, 10.90%, 12.27%, 16.36% in the I, II, III, IV and V instars respectively. The instar-wise larval life span of Tasar silkworm, *Antheraea mylitta* (Daba TV) in September-October were 6, 5, 7, 8, 21 in the I, II, III, IV and V instars, respectively (Table 3).

Table 4: The cocoon parameters of parental combination in breeding of Tasar silkworm, *A. mylitta* D during in various seasons (July-August).

Year	Ecorace	Cocoon weight (gm)	Pupal weight (gm)	Shell weight (gm)	Emergence date	Date of coupling	Hatching of the Eggs (date)	No. of Eggs laid/% of hatching
I year	Andhra local (Male)	4.54	4.04	1.39	2-9-2012	2-9-2012	10-9-2012	150/46
	Daba TV (Female)	11.01	8.10	1.52	2-9-2012			
II year	Andhra local (Male)	4.66	4.19	1.40	19-10-2013	19-10-2013	28-10-2013	220/90
	Daba TV (Female)	9.53	8.05	1.59	18-10-2013			

The values are expressed in terms of Standard Error of the Mean.

Table 5: Larval and Cocoon Parameters of hybrid and normal tasar silkworm, *Antheraea Mylitta* D. during in various seasons (September-October).

Larval /cocoon parameter	Larval length cms		Larval Weight cms		Larval duration days	Cocoon weight gms	Pupal weight gms	Shell weight gms
Instar	IV	V	IV	V				
Hybrid	5.55 ± 0.65	7.97 ± 0.911	2.78 ± 1.14	12.01 ± 2.71	45-47	5.67	4.69	0.98
Control	5.17 ± 0.78	9.78 ± 1.64	**9.348** ± 0.97	**26.31** ± 6.57	40-45	12.11	9.56	1. 65

The values are expressed in terms of Standard Error of the Mean.

The Cocoon weight, Pupal weight, Shell weight of Andhra local (male) undergone breeding during July-August were 4.54 (g), 4.04 (g), and 1.39 (g), respectively, while that of Daba (female) were 11.01 (g), 8.10 (g), and 1.52 (g), respectively. The Cocoon weight Pupal weight, Shell weight of Andhra local (male) undergone breeding during September-October were 4.66 (g), 4.19 (g), 1.40 (g) respectively, while that of Daba (female) were 9.53 (g), 8.05 (g), 1.59 (g), respectively (Table 4).

In the F1 hydrid Tasar silkworm, *Antheraea mylitta* D., the larval length in fourth and fifth instars, larval weight, cocoon weight, pupal weight and shell weight were 5.55 ± 0.65 (S.D.), 7.97 ± 0.911 (S.D.), 2.78 ± 1.14 (S.D.), 12.01 ± 2.71 (S.D.) respectively, while those maintained in control were5.17 ± 0.78 (S.D.), 9.78 ± 1.64 (S.D..), 9.348 ± 0.97 (S.D..), 26.31 ± 6.57 (S.D.), respectively. The larval duration was 45-47 and 40-45 days in hybrid and control tasar silkworms (Table 5).

DISCUSSION

During breeding, selection of the parent is the basic tool for improving the genetic structure and improving the productivity status of animal stock in desired direction. The simplest form of selection is choosing parents based on phenotypic traits. From the present studies (Chapter I) it becomes evident that there is a tremendous phenotypic as well as genetic variability among the ecoraces of tropical tasar silkworm, *Antheraea mylitta*. The present studies on trials on hybridization made in breeding season, during the three years, for Tasar silkworm, *Antheraea mylitta* D., Andhra Local ecorace with six other ecoraces (Raily, Modal, Bhandara, Sukinda and Daba BV) has revealed that hybridization was possible only between Andhra Local and Daba TV ecoraces. This was mainly due to variation in moth emergence pattern, synchronization and choice of mating. During hybridization, Daba TV (female) was crossed with Andhra local (male) to produce F1 hybrids, in which Daba TV (female) was the donor parent whereas male counterpart of Andhra local acted as recipient parent.

The studies on hybridization needs to be made through single, double and three-way crosses involving many population lines with respect to fecundity, larval weight, shell weight, silk ratio and other commercial traits [18]. Also, different types of parental mating can balance desired traits leading to high egg recovery, cocoon and silk yield [19]. The present study is a preliminary report on breeding of tasar ecoraces aimed at the improvement of Andhra local ecorace to be bestowed with superior characters *i.e.,* survivability and fecundity of Daba TV and also one of the probable strategies for enhancing the productivity and quality of tasar raw silk by breeding method.

Secondly, the role of environment on genotype is apparent in the productive potential of the progeny needs to be attained with matching seasons and specifically for the trait of commercial importance [20]. In the present studies, the hybridization was carried out during winter (September-October, i.e., second crop season) to exploit the potential of heterosis in commercial season, for two consecutive years of the study, during which the temperature and relative humidity were 23-32°C and 64-78 % R.H., respectively (during some days an R.H. of 29-35% was also noted). While allowing the breeding, the moths were allowed to mate in all the three crops but the compatibility of mating was observed only for second crop owing to optimum environmental conditions for rearing. It is in corroboration with earlier studies- that in commercial crop season (Sep-Nov), selection of silk yield and filament length, the traits of quantitative nature are stressed because of congenial weather and quality of feed in addition to longer larval feeding period [21]. in contrast to fluctuating weather in seed crop season (Jul-Aug). Moreover, environmental conditions have a great influence in effectiveness of parental selection; hence selections take an advantage of season's different characteristics [22-24].

The average fecundity obtained in the present studies in the two years of hybridization was 150-220 eggs with a hatching percent of 60-75%. The oviposition lasted for 4-5 days for F1 hybrids, which is 6-7 days in normal layings. The fecundity of hybrid variety thus obtained seems to be more than that of normal layings (170) of Andhra local and less than that of normal layings (250) of Daba TV. [25]. Thus it can be inferred that by hybridization between Andhra local and Daba TV, the fecundity of the former can be enhanced. This process needs to be standardised by repeating the breeding procedure in successive years as the percentage of fecundity and hatching was found to be increased from the first year to the second year of the study. The parental selection with specific traits lead to explicit individuals in their progeny [26] and when the silkworm strain of low fecundity is crossed with better strain, it regains fecundity levels [27].

The hatching for F1 hybrids took place after 6-7 days, while in normal layings it was for 10-15 days for Andhra local and Daba TV. Though the proportion of pupal weights in parental combination were same in the both the years of hybridizations, there was an increase in hatching and fecundity in the second year. Though maternal size influences fecundity and its associated traits, it may be attributed to low temperature and low humidity recorded during that period. This is in corroboration with the statistical studies on fecundity-maternal relations in breeding lepidopterans, which revealed that fecundity was controlled directly by maternal weight and indirectly by rearing temperature and that cooler temperatures produced heavier and more fecund females [28]. It is also inferred that fecundity and hatching are directly related to photoperiod across season and indirectly related to photoperiod across altitude [29]. The reproductive characters on any commercial rearing animal are mainly influenced by climatic factors of the environment, chance of survival and multiplying of the tasar silkmoth [30].

During the F1 hybrid larval development of Tasar silkworm, *A.mylitta*, certain abnormalities were observed when compared to that of the control ones. The instar wise duration was increasing gradually, more so in the fifth instar. The F1 hybrids were found to be relatively slow in their movements than those of the control ones. They were also found to be moving frequently in search of food. The feeding behavior was found to be slower that the normal ones, which might due to want of suitable nutritional requirements. This can be ascribed to the fact that nutrition has direct influence on the health and growth of larvae and better crop yield as the feed quality has direct correlation with cocoon and shell weights, silk ratio and silk filament [31-32].

Though the control and F1 hybrids were reared in the same conditions, a high mortality was observed in the latter due to higher sensitivity to environmental conditions and diseases. The loss of worms due to viral disease was more rampant. Post-harvest studies have shown that cocoons with less weight were formed. The pupal duration was also lengthened when compared to that of control. A decreased pupal weight has probably arisen due to decreased larval weight. These characteristic features suggest that due to poor intake of the leaves, there was less growth and development which made the worms look pale in contrast to glowing normal and healthy tasar silkworms. A reduced nutrition in F1 hybrids must have created a lack of protein pool in the haemolymph of the larvae, due to which silk gland development was affected, consequently giving rise to feeble cocoons. It is mainly because the synthesis of silk fibre protein takes place with the help of amino acids derived from the leaves of food plant consumed by the silkworms. This silk fibre forms the cocoon fibre and excessive amino acids present in the larval body become converted into cocoon shell, which protects the pupa. Generally abnormal silk glands arise due to genetic mutations or due to degenerated cells of middle and posterior parts of silk glands. A recent work on higher concentration of the total protein and carbohydrates in F1 hybrid was reported that larvae acquired robustness due to inter-ecoracial crossing and feeding on higher quantity and quality leaves, which maintained level of proteins in the hemolymph, midgut and fat body [33]. From the present work, it is clear that a serious drive to improvise the rearing conditions, method of feeding and quality of leaves is necessary in order to improve cocoon weight and yield of the hybrid population and achieve the basic objective of breeding.

The larval development of tasar silkworm, *A. mylitta* D., has revealed that the larval length and weights have shown profound decrease in the F1 hybrids. From these studies it can be noted that though fecundity is more in the hybrid generation, the larval size is less, which indicates that though there exists compatibility between parental combinations, the vigour of the race is not maintained in the new breed within the environment. A study on applying the compatibility of parental combination with seasons indicated that silk productivity can be augmented based on phenotypic traits. The present study of breeding focused on improvement of Andhra local ecorace based on parental compatibility with Daba TV can be extended further by applying suitable phenotypic traits along with selection of crop season.

The study has revealed that flimsy cocoons were formed in F1 hybrid population and the larvae survived only till fifth instar in some of the crops, a few stray or no emergence of moths was observed. The failure or delay in emergency may be due to hormonal imbalance. As basically, in the silkworm pupae, the brain secretes a hormone which activates the prothoracic glands which in turn secretes a hormone inducing the metamorphosis into the moth.

In the present studies, though the hybrid generation was reared on healthy and nutrient leaves, the fecundity and hatching percentage were enhanced in F1, the larval growth was found to be less and so is the quality of cocoons. This may be due to decreased intake of quality leaves which ultimately leads to low silk yield. Some reports have shown that there was a dramatic increase in the formation of flimsy cocoons in winter season due to physioclimatic stress [34]. The food consumption has direct relation with cocoon quality and Tasar feed will be also be helpful in minimizing bad impact of unfavourable conditions. This can be corroborated by a report that semi-synthetic diet fed larvae showed higher cocoon weight, shell weight and shell ratio than complete outdoor fed larvae whereas minimum when larvae were fed fresh leaf in indoor conditions up to II stage [35]. It clearly indicates that the F1 hybrids in the present study which were reared in indoor conditions, in the laboratory, and later shifted to the outdoor conditions up to spinning stage, required more proteins in their diet. This must have led to a low protein concentration in the haemolymph of the larvae, leading to a depletion of proteins required for cocoon formation. The low pupal weights in F1 hybrids can be attributed to less larval size. The fact that the pupae of F1 generation could not emerge may be due to physiological inability caused due to the reduced availability of proteins in fifth instar haemolymph. This is in accordance with the studies that changes in fifth instar proteins are related to biosynthesis of silk proteins and metamorphosis preparation from larva to pupa [36]. The physiological potential of the life performance of the insect is always challenged by abundance of food and its quality, various abiotic factors, presence of predators, parasites and disease [37-39]. The quality and quantity of food intake, its digestibility and their utilization influences growth, development, weight, survival, silk synthesis and reproduction [40].

Temperature affects nearly all biological processes, including the structure of proteins, biological membranes and rates of biochemical and physiological reactions [41-44]. All organisms are affected by their surrounding environment and the environmental factors play an important role in shaping ecology and evolution of biological systems. Environmental stress is especially important at many levels of biological organization [45-46]. In this context, environmental stress is regarded as an "environmental factor causing a change in a biological system, which is potentially injurious" [47]. and which has some fitness consequences [48]. It is evident that F1 hybrid variety in the present studies was physiologically not fit owing to non-acclimatization to temperature and other environment related conditions, poor feeding, leading to less larval weight, cocoon weight and inability in emergence of adult moth.

From the present studies it is observed that during F1 hybrid larval development a low temperature and low percent of relative humidity were recorded. The F1 hybrid mortality was seen, mostly due to viral disease. The percentage mortality has increased from first to fifth instar. The low mortality in early instars may be due to the fact that the hybrid stocks were maintained under indoor conditions in first and second instars, while late age larvae were reared in the field of *Terminalia* plantation in outdoor conditions. This is in corroboration with the recent studies that adopting indoor rearing method show significant reduction of crop loss due to diseases, pests and predators [49]. Also, medium temperature and relative humidity regimes coincide with highest larval mortality, which is similar to observations by [50] in *Bombyx mori*. Medium temperature and relative humidity apparently stimulate the disease causing pathogens [51]. In the present investigation, the minimal survivability of F1 hybrids is attributed to non-acclimatisation of new genotype to the environmental conditions.

Andhra local Ecorace of Tasar silkworm, *Antheraea mylitta* Drury, which is an exclusive race of the state of Andhra Pradesh, is well known for its superior commercial qualities. It is on the verge of extinction due to certain weaknesses like poor egg laying behavior, voltinism, erratic emergence, non-uniform silk deposition in cocoons and pupal mortality. The present investigation is an attempt to study the genetic proximity of Andhra local ecorace with other ecoraces of *Antheraea mylitta,* and bring about an idea of evolving a new breed without losing its beneficial commercial characters and suggest methods to overcome its weaknesses.

Selective use of parental races or parents for heterosis, a method of backcrossing to exploit the traits of commercial importance and applying the advantage of Genotype × Environment (G×E) interactions are indispensable. In spite of current knowledge on sophisticated transgenic silkworm, appropriate application of on-hand parental resource material and methodologies can expedite tasar silk productivity improvement in addition to up-keep the agro based cottage industry's cost-effectiveness and biodiversity conservation [52]. The selection of breeding method is to develop a breed with stability and productivity in terms of quantity and quality of silk and for such aspire the apt method is repeated backcrossing [53]. Several scientists have also suggested that superior silkworm varieties can be evolved using repeated backcrossing with choice of donor and recipient parents [54, 55, 10]. Silkworm hybrids have shown improved reeling performances over pure races [56] and the silk filament length is positively correlated with cocoon weight and negatively with silk filament denier [57]. The present study is a preliminary report on the probable strategies for enhancing the productivity and quality of tasar raw silk by breeding method.

The quantity and quality are fundamentally important even for economics of commercial seed production and use of right parents and combinations can optimize productivity improvement [10]. In spite of better female component, the optimal reproductive success can be attained with availability of appropriate male counterpart. The fertility, vital character of egg hatching, depends on potency of male in transferring sperms along with secretions of accessory glands [58-59].

The breeding program conducted here is aimed at introgression of survivability traits of Daba TV into Andhra local ecorace, purely based on time of emergence and mating compatibility. Several studies have stated that breeding programme can be facilitated through molecular marker technique to produce varieties with desirable characters.

Hence, there is an urgent need to identify the genes responsible for beneficial traits like survivability and devise new parental combinations. The parental combination of high pupal female and high shell male combination has show enhanced silk yield and fecundity. In the present study high pupal and high shell female Daba TV owing to its high survivability with that of male Andhra local, aiming at more fecundity in the F1 generation as maternal weight directly influences fecundity. As the F1 hybrids have shown low rearing performance and survivability, this study needs further exploration by standardizing the procedure by initial identification of gene by molecular markers, selection of parents and repeated back-crossing to ensure the introgression of desired trait. A few scientists have also reported that double cross hybrids mainifested maximum heterosis than the single and three way cross hybrids. [60], while [61] and [62] indicated the fact that dominant gene effects determine the commercial characters in *Antheraea mylitta* D.

CONCLUSION

In the present study, which primarily focused on Tasar silkworm, Andhra local ecorace, it is found that it has low denier, high reliability and shell ratio, which are important commercial qualities of silk production, opens an avenue to take up measures to overcome its weaknesses like low hatchability, irregular emergence, disease incidence, etc. and pave way for its conservation, by effective breeding measures and genetic studies of other ecoraces.

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REFERENCES

1. Jolly MS, Sen SK, Ashan, MM. *Tasar culture*. *Ambika publishers*, Bombay. 1992-1993, 1974: 134-147.

2. Jolly, M. S., Sen, S. K. and Das, M. G. Tasar Culture, *Ambika Publishers,* Bombay. 1992-93, 1975: 134-147.

3. Thangavelu K, Recent studies in Indian tasar and other wild silkmoths. *Wild Silkmoth* 1991: 20-29.

4. Thangavelu K, Population ecology of *A. mylitta* Drury (Lepidoptera Saturnidae). *Wild Silkmoths.* 1992, 92, 99-104.

5. Srivastava AK, Sinha AK, Sinha BRRP. Descriptor of topical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). 2003: CTR&TI, Ranchi, India.

6. Kavane RP, Sathe TV. On a new variety (*kolhapurensis*) of *Antheraea mylitta* from India. *Biological forum An international Journal*. 2009: 1(2): 5-47.

7. Kumaresan P, Sinha RK, Mohan B, Thangavelu K. Conservation of mutivoltine silkworm (*Bombyx mori* L.) Germplasm in India an overview. *International Journal of Industrial Entomology.* 2004: 9(1): 1-13.

8. Mirhoseini SZ, Seydavi AR, Ghanipoor M, Etebari K. General and special combining ability estimation and heterosis in new varieties of silkworm *Bombyx mori*. *Journal of Biological Science.* 2004: 4, 725-730.

9. Reddy RM, Suryanarayana N, Prakash NBV. Heterosis potential in selective parental F1 hybrids of divergent geographic ecoraces of tropical tasar silkworm, *Antheraea mylitta* D. (Lepidoptera: Saturniidae). *Acad. J. Entomol*. 2008: 1, 32-35.

10. Reddy RM, Sinha MK, Hansda G, Prakash NBV. Application of parents by selection for basic and commercial seed efficiency in tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). *Academic Journal of Entomology. 2009:* 2 (2): 56-61.

11. Reddy RM, Sinha MK, Tarakanadha B, Prakash, NBV. Appropriate handling of tasar silk moth for seed productivity and quality. *Indian Silk.* 2010b: 48: 16-18.

12. Shamitha G. Comparative studies on tasar silkworm (*Antheraea mylitta* Drury). Andhra local ecorace under outdoor and total Indoor conditions. *Ph.D. thesis*, Department of Zoology, Kakatiya University, Warangal. 1998.

13. Petkov N, Nacheva I, Tsenov P, Grekov D, Golovko,V, Braslavskii M. Hybridization schemes for developing new silkworm (*Bombyx mori* L.) races by using populations from different eco-geographical zones. *Acta Agric. Serbica.* 2000: 5, 3-9.

14. Verma AK, Chattopadhyay GK, Sengupta M, Sengupta AK, Das SK, Urs SR. Expression of heterotic genetic interaction among multivoltine recurrent backcross/congenic lines for higher shell weight of silkworm *Bombyx mori* L. *Int. J. Ind. Entomol.* 2003: 7, 21-27.

15. Reddy RM, Sinha MK, Prasad B.C. Application of parental selection for productivity improvement in tropical tasar silkworm *Antheraea mylitta* Drury: A review. *J. Entomol*. 2010a: 7, 129-140.

16. Jolly, MS, Bardaiyar VN, Narasimhanna MN, Razdan JL. Diallel cross analysis of qualitative characters off our races of Indian tasar silkworm *Antheraea mylitta* D. *Indian J. Seric.* 1969: 8(1): 23-25.

17. Kuang-Ming CC, Ta-Yuan C. The tasar silkworm. *Journal of Silkworm*. 1958: XI, 111-125.

18. Bardaiyar VN, Jolly MS, Benchamin KV, Sinha BRRP. Heterosis in relation to single, three and double crosses in *Antheraea mylitta* D. *Ind. Jr. Seric*. 1976: 15, 3-8.

19. Yamaguchi A. Future directions of bivoltine silkworm breeding in India. *Indian Silk.* 2001: 39, 26-28.

20. Reddy RM, Sinha AK, Kumar R, Prasad BC. Parental combination and rearing season compatibility for silk yield in tropical tasar silkworm, *Antheraea mylitta* Drury. *World Applied Sciences Journal*. 2010: 9(8),855-859.

21. Jong DG, Bijma P. Selection and phenotypic plasticity in evolutionary biology and animal breeding. *Livest. Prod. Sci*. 2002: 78, 195-214.

22. Kumar NS, Kumar CMK, Basavaraja HK, Malreddy N, Rameshbabu M, Datta RK. Comparative performance of robust and productive bivoltine hybrids of silkworm (*Bombyx mori* L.) under high temperature conditions. *Sericologia*. 1999: 39, 567-571.

23. Munoz MFC, Tonhati H, Costa CN, Sarmiento DR, Echeverri, DM.Factors that cause genotype by environment interaction and use of a multiple-trait herd-cluster model for milk yield of Holstein cattle from Brazil and Colombia. *J. Dairy Sci*. 2004: 87, 2687-2692.

24. Mulder HA, Bijma P. Effects of genotype × environment interaction on genetic gain in breeding programs. *J. Anim. Sci*. 2005: 83, 49-61.

25. Suryanarayana N, Srivastava AK. *Monograph of Tropical Tasar Silkworm*. CTR & TI, Ranchi, India, 2005: pp. 1-87.

26. Darlington CD, Mather K. *The Elements of Genetics*. The Macmillan Co., New York, London. 1952.

27. Aruga H. *Principles of Sericulture*. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, Bombay, Calcutta, 1994: pp. 99-111.

28. Miller WE. Extrinsic effects on fecundity-maternal weight relations in capital-breeding Lepidoptera. *Journal of the Lepidopterists' Society.* 2005: 59(3), 143160.

29. Benchamin KV, Gapuz JV, Jayaramaraju P. Influence of photoperiod on emergence, fecundity and fertility in multivoltine breeds of silkworm *Bombyx mori* L. *Indian J. Seric*. 1990: 29(1), 110-118.

30. Dey DG, Nayak BK, Mohanty N, Guru BC. Reproductive characteristics of modal ecorace of wild tasar silkmoth, *Antheraea paphia* Linn. in different altitudes of Similipal Biosphere Reserve, Orissa, India. *The BioScan.* 2010: 5(1), 41-45.

31. Dash A.K., Nayak, B.K. and Dash, M.C. The effect of different food plants on cocoon crop performance in the Indian tasar silkworm *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). *J. Research on the Lepidoptera*. 1992: 31(1-2), 127-

131.

32. Yadav GS, Mahobiam GP. Effect of different food leaves on rearing performance in Indian tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae), *UP J. Zoo*. 2010: 30(2), 145-152.

33. Lokesh G, Tirkey SR, Srivastava AK, Kar PK, Sinha MK. Biochemical Performance and Quantitative Assessment of F1 Hybrid of Two Ecoraces of Tropical Tasar Silkworm *Antheraea Mylitta* Drury (Lepidoptera: Saturniidae). *Int. J. Indust. Entomol*. 2013: 26(2), 67-73.

34. Dash AK, Nayak BK. Seasonal variation of occurrence of deformed cocoons of the tasar silkmoths *Antheraea mylitta* (Drury) in India. *J. Lepid. Soc*. 1990: 44, 34-36.

35. Kumar GS, Shamitha G. Studies on the Proteins and Proteolytic activity of indoor reared tasar silkworm, *Antheraea mylitta* D. (Daba TV). *International Research Journal of Biological Sciences.* 2013: 2(5), 24-29.

36. Li XH, Wu XF, Yue WF, Liu J, Li G, MiaoY. Proteomic analysis of the silkworm (Bombyx *mori* L.) hemolymph during developmental stage. *J. Proteome Res*. 2006: 5, 2809-2814.

37. Slansky FJr, Scriber JM. Food consumption and utilization: comprehensive insect physiology, biochemistry and pharmacology. *In*: Kerkut, G.A. and Gilbert, L.I. (eds.), *Regulation, Digestion, Nutrition, Excretion*. Peragamon Press, Oxford. 1985: 4, 87-163.

38. Rath SS, Sinha BRRP, Thangavelu K. Nutritional efficiency in *Antheraea mylitta* D. during food deprivation. *Intl. J. Industrial Entomolo*. 2004a: 9(1): 111-115.

39. Rath SS, Narain R, Prasad BC. Impacts of physiological condition of fifth instar larvae of *Antheraea mylitta* on rate of feeding and assimilation and its nutritional requirements. *Proceeding of National Academy of Science India*. 2004b: 74B(III) & (IV), 237-243.

40. Rath S. Food utilization efficiency in *Antheraea mylitta* fed on *Terminalia arjuna* leaves. *Academic journal of Entomology.* 2010: 3(1), 23-28.

41. Hazel JR. Thermal adaptation in biological membranes: Is homeo viscous adaptation the explanation? *Annu. Rev. Physiol.* 1995: 57, 19-42.

42. Somero GN. Proteins and temperature. *Annu. Rev. Physiol*. 1995: 57, 43-68.

43. Logue JA, deVries AL, Fodor E, Cossins AR. Lipid compositional correlates of temperature-adaptive interspecific differences in membrane physical structure. *J. Exp. Biol.* 2000: 203, 2105-2115.

44. Willmer P, Stone G, Johnston I. *Environmental physiology of animals* (Oxford: Blackwell Science). 2004.

45. Hoffmann AA, Parsons PA. *Extreme environmental change and evolution.* Cambridge University Press, Cambridge. *1997.*

46. Hoffmann AA, Hercus MJ. Environmental stress as an evolutionary force. *Bioscience*. 2000: 50, 217-226.

47. Hoffmann AA, Parsons PA. *Evolutionary genetics and environmental stress.* Oxford University Press, New York

48. Kumar D, Pandey JP, Sinha AK, Salaj S, Mishra PK, Prasad BC. Evaluation of Novel Tasar Silkworm Feed for *Antheraea mylitta*: It's impact on rearing, Cocoon Trait and Biomolecular profile. *American Journal of Biochemistry and Molecular Biology* 2013: 3(1), 167-174.

49. Dasgupta MR. Monographs on cottage industries, No. 1, Disease of Silkworms Govt. Indian press, Calcutta. 1950: 202 pp.

50. Mishra CSK. Larval mortality of Indian tasar silkworm *(Antheraea mylitta)* (Saturniidae) due to pebrine infection. *Journal of the Lepidopterists' Society.* 1992: 46(2), 106-109.

51. Reddy RM. Parental selection for productivity improvement in Indian tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). *Nature Proceedings.* 2011: doi:10.1038/npre.2011.6331.1, Posted 4 Sep 2011.

52. Tazima Y. Silkworm Moths. *In*: Mason, I.L. (ed.), *Evolution of Domesticated Animals*.Longman, New York, London. 1984. pp. 416-424.

53. Raju PJ, Krishnamurthy NB. Breeding of two bivoltines, MG511 and MG512 of silkworm, *Bombyx mori* L. for higher viability and productivity. *Sericologia.* 1993: 33, 577-587.

54. Das SK. Techniques of breeding for evolving improved breeds of bivoltine mulberry silkworm *Bombyx mori* L. *Pers. Cytol. Genet*. 2001: 10, 129-134.

55. Takabayashi C, Hariraj G, Tsuboi H, Tsughe M. Comparative studies between exploitation reeling tension and reeling performance of Indian pure and hybrid races. *Sericologia.* 1994: 34, 287-295.

56. Sekharappa BM, Radhakrishna PG, Keshavareddy KS, Dandin SB. Breeding of bivoltine silkworm races with better survival and high shell content for tropics-Karnataka. *Sericologia.* 1999: 39, 205-213.

57. Ravikumar G, Rajeshwary H, Ojha NG, Thangavelu K. Effect of multiple mating on fecundity and fertility in the tropical tasar silkworm, *Antheraea mylitta* D. (Lepidoptera: Saturniidae). *Entomon*. 1995: 20, 15-17.

58. Krishnaprasad NK, Sannappa B, Varalakshmi R. Effect of multiple mating on grainage performance of newly evolved bivoltine breeds of *Bombyx mori* L. *Bull. Ind. Acad. Seric*. 2002: 6, 50-55.

59. Bardaiyar VN, Jolly MS, Benchamin KV, Sinha BRRP. Heterosis in relation to single three way and double cross in *Antheraea mylitta* D. *Indian J. Seri*. 1976: 15, 3-8.

60. Siddiqui AA, Sengupta AK, Kumar A, Sengupta K. Genotypic and two phenotypic variability of some characters in *Antheraea mylitta* D. *Sericologia.* 1988b: 28, 187-192.

61. Sinha SS, Sinha AK. Conservation strategies for important wild silk moth populations of *Antheraea mylitta* D. *Int. J. Wild Silkmoth and Silk.* 1994: 1(2), 159-162.