RESEARCH ARTICLE

Genetic variation in ecoraces of tropical tasar silkworm, *Antheraea mylitta* using SSR markers

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Abstract

The tropical tasar silkworm, *Antheraea mylitta*, polyphagous sericigenous insect mostly found in the tropical areas of India. It is found in these regions as ecotypes or ecoraces. It feeds primarily on plants, a variety of secondary plants like *Terminalia arjuna* and *T. tomentosa*. Tasar culture is a traditional livelihood for lakhs of tribal populace in the areas of Jharkhand, Chhatisgarh, Orissa, Maharashtra, Andhra Pradesh, West Bengal and Uttar Pradesh. In the present study, the genetic diversity of these ecoraces is identified by DNA markers, namely simple sequence repeats (SSRs), most of which produced polymorphic bands.

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Introduction

Microsatellites or simple sequence repeats (SSRs) are tandemly repeated units of one to six nucleotides, abundant in prokaryotic and eukaryotic genomes (Weber 1990; Field *et al.* 1996). The SSR markers have proven potential in diversity analysis owing to their codominant nature, high level of polymorphism, amenability to high throughput analysis and as informative markers to address population genetic questions in a given species.

The advent of polymerase chain reaction (PCR) and the availability of high-throughput automated sequencers have increased the use, detectability and popularity of microsatellite markers, which have become a highly informative and versatile class of genetic markers (Litt and Luty 1989; Tautz 1989; Weber and May 1989; Schlotterer 2004). On the other hand, more stable SSR markers with lower variability can be used to reconstruct more ancient evolutionary events (Meyer *et al.* 1995).

Microsatellites take advantages of the abundant and ubiquitously distributed sequence repeats (SSR) in the eukaryotic genome (Hamada *et al.* 1982; Weber and May 1989; Dietrich *et al.* 1992). The variation in repeat number can be visualized as difference in the length of the PCR amplified products. Formerly, 13 diverse strains of silkworm, *Bombyx mori*, were analysed using the SSR-anchored PCR or inter-SSR-PCR (ISSR-PCR) (Reddy *et al.* 1999a, b).

Assessment of genetic diversity is essential for efficient management and conservation of any animal genetic resources in gene banks. Since, SSRs are codominant markers and can reveal multiple alleles at a single locus and also have been extensively used in the diversity analysis of animal and plant system, these molecular markers are selected in this study. This work has been undertaken to characterize the ecoraces of *Antheraea mylitta* from different parts of tropical forest zones, as basis for identification and genetic diversity among the tasar populations. Based on these reports, a comprehensive breeding programme could be evolved to conserve the dwindling population of *A. mylitta,* Andhra local ecorace.

Phylogenetic studies on ancestral position of microsatellite sequences in *A. assama* were used to distinguish Saturniidae and Bombycidae members. The nucleotide sequences were aligned manually and used for construction of phylogenetic trees based on maximum parsimony and maximum likelihood methods. A study based on the studies of phylogenetic relationships of different ecoraces and genetic variation revealed that RFLP markers were used to distinguish the closely related ecoraces of tropical tasar silk producing insect *A. mylitta* Drury (Mahendran *et al.* 2006a, b).

The tasar silkworm, *A. mylitta* Drury, an Andhra local ecorace, is found exclusively in the state of Telangana, is well known for its superior commercial qualities. It is one of

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the verge of extinction due to certain weaknesses, like poor egg-laying behaviour, voltinism, erratic emergence, nonuniform silk deposition in cocoons and pupal mortality. The present investigation is an attempt to study the genetic proximity of the other ecoraces of *A. mylitta*, to bring about an idea of breeding of Andhra local ecorace with other ecoraces, without losing its beneficial commercial characters and suggest methods to overcome its limitations. The knowledge of genetic diversity in crop improvement is essential for high yield and gain hybrid vigour (Razdan *et al.* 1994). In the present study, we present the PCR-SSR based phylogenetic analysis of seven tasar silkworm populations. For these studies, programs like POPGENE 1.32 (SSR and ISSR) and STRUCTURE (SSR) and CLUSTALW (EST) were used (see electronic supplementary data at http://www.ias.ac.in/jgenet/).

Other phylogenetic reports include a number of molecular markers like random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), fluorescent dye labelled ISSR PCR reaction (FISSR-PCR) and single-nucleotide polymorphism (SNP) analyses have been developed to distinguish the genetic diversity among silkworm species (Yasukochi 1998; Reddy *et al.* 1999a, b; Tan *et al.* 2001; Nagaraju *et al.* 2002; Cheng *et al.* 2004; Mahendran *et al.* 2005, 2006a, b).

A recent work on the phylogeny of seven ecotypes of *A. assamensis* has been taken up by Seribiotech Research Laboratory, Begaluru (Arunkumar *et al.* 2012). The present work is being envisaged to involve larger populations and representatives encompassing various regions of our country for phylogenetic studies and evolve future breeding programme of Andhra local ecorace as a strategy to conserve the dwindling population which is on the brink of extinction.

Materials and methods

Randomly selected male and female moths from populations representing seven selected areas of our country: Andhra local ecorace from Warangal district (Telangana) and Daba TV and Daba BV ecoraces from Mahadevpur, Karimnagar districts of Telangana, Bhandara ecorace from Maharasthra (Bhandara), Sukinda from Orissa (Sukindergarh), Raily from Chhatisgarh (Bastar) and Modal from Orissa (Keonjhar) were collected for the present study. The present investigation is an analysis of polymorphism unraveled by 10 SSR primers for 16 populations of *A. mylitta* belonging to seven ecotypes (figure 1, a&b).

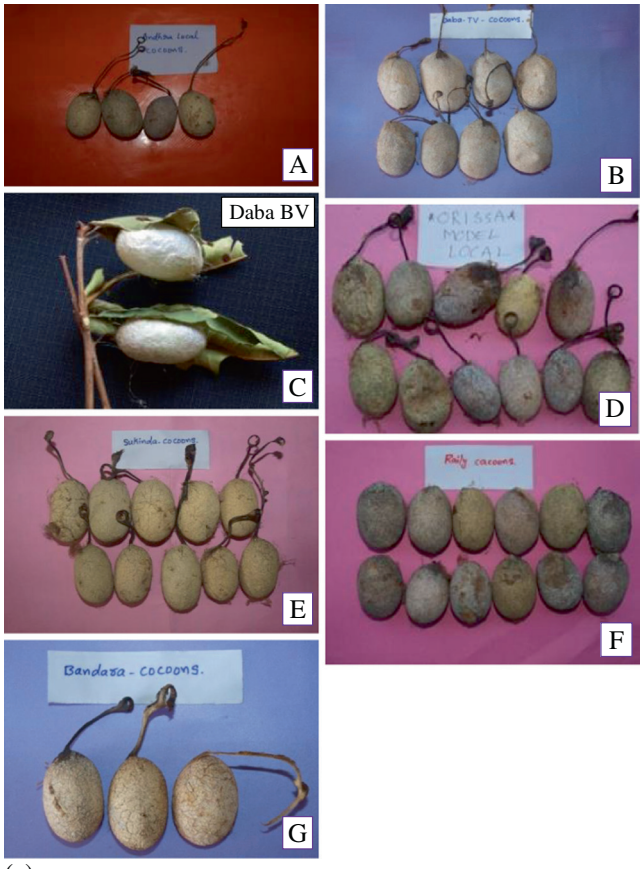
Genomic DNA isolation

Genomic DNA was extracted from 16 randomly selected individual moths from each generation of each line by using phenol–chloroform method (Suzuki *et al.* 1972), later it was modified as suggested by Nagaraju and Nagaraju (1995). DNA was dissolved in TE buffer (Tris-EDTA, pH 8.0) and quantification was done on 0.8% agarose gel and a uniform concentration of 100 ng/μL was obtained after serial dilution with the TE buffer (pH 8.0) or using Nanodrop.

SSR-based molecular analysis of polymorphic study of different ecoraces of A. mylitta

Microsatellite markers: Microsatellites or simple sequence repeats (SSRs) are small repeats of one or few tandemly arranged nucleotides spread throughout eukaryotic genomes. They are single locus markers and are codominant. Of the 12 primer combinations tried (a generous gift from Dr Nagaraju (deceased), CDFD, 2012), 10 combinations have shown to have desired size fragments and five: 001, Amysat023, Amysat025, 40 and 41 (of allelic size 211, 166, 247, 209 and 169, respectively) showed polymorphism (table 1).

PCR amplification of DNA with SSR primers: PCR is a technique used to amplify small target DNA molecules in many folds using two oligonucleotide primers that hybridize to opposite strands. The location of the primers in the template determines the length of the amplicon. The PCR was carried out in 25 μL reaction. Of the 25 μL, 1 μL of template (50 ng/μL) was mixed with 2.5 μL of 10 $\times$ PCR buffer, 0.2 μL of 25 mM dNTP's (individual nucleotide sets



(a)

Figure 1. *Continued.*

(b)

Figure 1. (a) Cocoons of various tasar ecoraces- (A–G) Andhra local, Daba TV, Daba BV, Modal, Sukinda, Raily and Bhandara. (b) Moths of various tasar ecoraces: Andhra local, Daba TV, Daba BV, Modal, Sukinda, Raily and Bhandara.

Table 1. List of SSR primers*.

Locus	Annealing temperature (°C)	$MgCl_2$ (mM)	Allele size (bp)	Comment
Amysat001	57	1.5	211	Worked
Amysat013	55	1.5	194	
Amysat015	57	1.5	196	
Amysat019	55	1.5	248	
Amysat021	55	1.5	190	
Amysat023	48	1.5	166	
Amysat025	50	2.0	247	
Amysat032	48	1.5	166	Not worked
Amysat037	54	1.5	122	
Amysat038	53	2.0	153	Worked
Amysat040	53	2.0	209	
Amysat041	53	2.0	169	

*Donated by Late Dr J. Nagaraju in 2012, CDFD, Hyderabad.

from Fermentas), 1.5–2.0 μL of 25 mM $MgCl_2$, 0.5 U of polymerase enzyme (*Taq*, Fermentas), 1 μL of 10 pmol forward and reverse primers. PCR reaction was performed in Eppendorf Thermal cycler and cycling conditions varied for every fragment. The amplified product was analysed by gel electrophoresis. PCR cycling conditions were standardized for each fragment to be amplified based on the melting temperature (T_m) of primers by using Research Master Cycler PTC 200, Eppendorf. The PCR schedule was 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 53–57 for 30 s, 72°C for 45 s and a final extension of 10 min at 72°C (Reddy *et al.* 1999b).

Separation and detection of SSR-PCR products: After the PCR amplification, the reaction mixture was loaded on a 3% agarose gel (3:1 ratio of high resolution agarose: agarose) Tris-boric acid-EDTA (TBE) buffer and resolved at 50 V in an electrophoresis system. The gel was illuminated with UV and the SSR profile were photographed using a gel documentation system (model no12 200069, 230 V capacity).

Table 2a. Phylogenetic analysis using SSR primers. Nei's original measures of genetic identity and genetic distance (Nei 1972).

Pop ID	1	2	3	4	5	6	7
1	****	0.7581	0.6808	0.7519	0.8317	0.6939	0.8214
2	0.2770	****	0.8506	0.8860	0.8648	0.8228	0.8243
3	0.3845	0.1618	****	0.7791	0.7610	0.7683	0.7746
4	0.2851	0.1211	0.2496	****	0.8488	0.9267	0.8012
5	0.1842	0.1452	0.2731	0.1639	****	0.8196	0.8611
6	0.3654	0.1951	0.2636	0.0761	0.1990	****	0.7677
7	0.1967	0.1932	0.2554	0.2217	0.1495	0.2644	****

The data analysis was done using popgene ver. 1.32 (32-bit, designed for Windows 95, 98, 2000, ME and NT) and the following observations were made. AL, DT, DB, Modal, Sukinda, Raily were found to have a genetic distance of 0.1967, 0.1932, 0.2554, 0.2217, 0.1495 and 0.2644, respectively, with that of Bhandara. The dendrogram produced by UPGMA of Nei's genetic distance for all populations ($7 \times 16 = 112$) is presented in figure 3.

Table 2b. Calculation of mean genetic distance from the table 2(a).

	Ecoraces	Mean values
1	Andhra local	0.1822
2	Daba TV	0.2646
3	Daba BV	0.18625
4	Modal	0.1858
5	Sukinda	0.2081
6	Raily	0.21348

Genetic data analysis: The phylogenetic relationship among tasar ecoraces were analysed by generating the phylogenetic tree by Nei (1972) genetic distance using UPGMA analysis through POPGENE software ver. 1.32 (Yeh *et al.* 1999). The PCR amplified bands were scored visually by different ecoraces of *A. mylitta* on the basis of their presence (1) or absence (0). The scores obtained were then pooled for constructing a single data matrix, which was used for estimating the proportion of polymorphic loci, Nei (1973) gene diversity (h), gene flow (N_m), coefficient of gene differentiation (GST), Nei (1978) unbiased genetic distance (*D*). Significant test and construction of a unweighted pair group method of arithmetic means (UPGMA) dendrogram among populations were carried out by using POPGENE ver. 1.32 (Yeh *et al.* 1999). Band sharing based intrapopulation similarity indices (S1) were calculated for all possible comparisons according to the following formula: similarity index (S1) = 2NAB/(NA+NB).

Genetic distance (D): Genetic distances are designed to express the genetic differences between two populations as a single number. If there are no differences, the distance could be set to zero, whereas if the populations have no allele in common at any locus the distance may be set equal to its maximum value, 1. The genetic distance (*D*) was calculated by POPGENE software (Yeh *et al.* 1999) using Nei (1972) standard genetic distance equation.

Scoring for codominant markers (SSR): With codominant markers, such as allozymes, RFLP and SSR, each recognizable allele at a given locus is ordinarily associated with a single band at a unique position in the gel. Thus, in the case of diploid organisms for a given locus, a homozygote will have one band and a heterozygote will have two. Null alleles (no band) are rarely seen. Also, if there are multiple alleles per locus, as expected for SSRs, which are highly variable, the total number of bands expressed by all the individuals in a sample will likely to be much greater than the number of loci involved.

In the profile of dendrograms for SSR using POPGENE 1.32, the level of polymorphism is expressed as the percentage of all loci that are polymorphic. It also gives detail about number of alleles, gene flow, genetic distance, gene diversity etc. (table 2, a&b).

Results and discussion

Eukaryotic genomes are densely interspersed with tandem repeats termed 'microsatellites' or SSR. These are short DNA sequence motifs (2–5 bp long) that occur at multiple sites and reveal a high degree of allelic diversity which can be typed through PCR. Analysis of SSRs provides a codominant, highly reproducible and genetically informative marker system. This method is labour-intensive, as complete sequence information is necessary to design primers for the single copy sequences flanking the microsatellites being scored (Reddy *et al.* 1999a, b).

Genetic characterization of seven genotypes with SSR primers was done. Of the 12 primer combinations tried (a generous gift from Dr Nagaraju (deceased), CDFD, 2012), 10 primer combinations have shown to have desired size fragments and seven primers Amysat001, Amysat013, Amysat023, Amysat025, Amysat38, Amysat40 and Amysat 41 (of allelic size 211, 194, 166, 247, 153, 209 and 169, respectively) have shown polymorphism. All the 16 populations of seven ecoraces screened with these seven primers have shown polymorphism. The SSR amplification of seven silkworm strains/ecoraces (16 individuals in each, with seven primers, which generated polymorphism) yielded a total of 887 bands, of which 420 were (47.3%) polymorphic. Most of

the bands were observed within the range 130–500 bp, which is in accordance with the allelic size of the primers taken for studies.

Among the 29 alleles, in 19 alleles, there was polymorphism showing a minimum of two and a maximum of four bands in each lane. The seven markers were polymorphic within the strain. The amplification products originated from individuals of the same strain clearly displayed similar patterns.

Most of the amplification products were centered around 200 bp region in the Amysat001, 013, 015, 021, 025, 040 and 041 primers; in Amysat019 and Amysat038, they were found centered around 250 and 150 bp regions, while in Amysat025, the bands were seen in 250–300 bp region. As could be seen from the PCR amplification of DNA with Amysat023, almost all the seven strains have revealed specific products at 400 bp (Andhra local, Daba TV); 120 bp (Daba BV, Modal); 200 bp (Sukinda, Raily); 180 bp (Bhandara). Most of the markers were present in almost all the individuals. As we have examined 16 individuals in each strain and we have tested only 10 primers, it is possible that we have not scored all the markers that are specific to a particular strain. Similarly, in Amysat038, some strain specificity was observed at 300–400 bp while in Amysat041 primer, some strain specificity was observed in Andhra local and Sukinda at 200 bp. The rest of the five primers, namely 015, 019 and 021 (allelic size, 196, 248 and 190, respectively) yielded monomorphic bands with the genomic DNA of these ecoraces. During scoring, all the bands present in both polymorphic and monomorphic profiles were selected. From the findings, it is abundantly clear that SSR Amysat023 (figure 2) primer generated highest polymorphism among the ecoraces followed by Amysat025, 041, 038, 001, 040 and 13, and these primers can be used effectively in genetic and phylogenetic studies.

In the present investigation, screening of genomic DNA from 16 individuals of seven populations using 10 SSR primers yielded several reproducible amplicons. The average

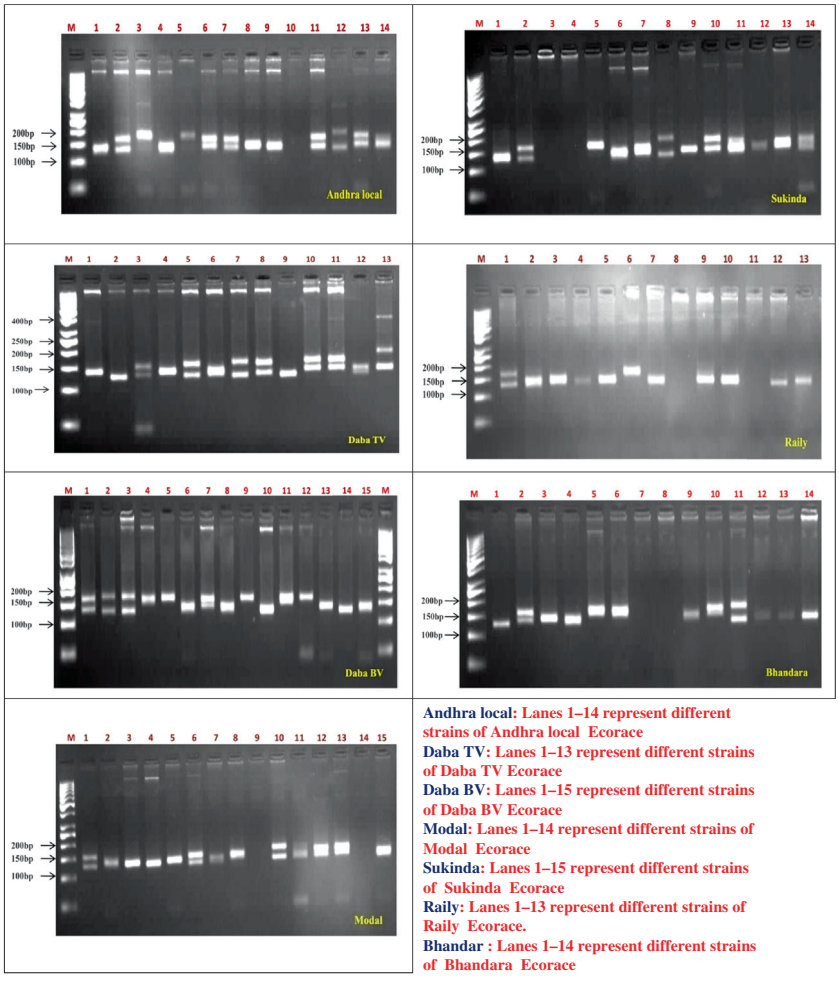


Figure 2. SSR profiles generated from genomic DNA of 16 strains from different individuals of (A.L, D. TV, D. BV, Modal, Sukinda, Raily, Bhandara) ecoraces of tasar silk worm, *Antheraea mylitta* using the primer Amysat023.

number of amplicons produced per DNA sample was 2–6 per primers, with sizes ranging from 130 to 500 bp. The percentage of polymorphism was 70.24 and 80.60 in primers Amtsat023 and Amtsat025, respectively (table 4).

In the present study, among 10 polymorphic microsatellites used on seven populations, six loci showed within population polymorphism in all populations. The most diverse locus (Amysat023) has six alleles and the others (Amysat001, 025, 0140 and 041) have two alleles. The advent of molecular biological techniques clearly showed the advantages of molecular markers over morphobiochemical markers to analyse population diversity, as they are stable and environment independent. Hence these are preferred to phenotypic traits to genetic variation in populations. A number of DNA marker systems such as SSR, ISSR, EST and AFLP have been used to study the population genetics of different organisms including insects. Genetic diversity and differentiation among different populations of the wild silkworm *A. mylitta* was examined using ISSR markers. Use of SSR and EST primers require prior knowledge on the genome of the organism whereas development of ISSR primers does not require prior information of the genome. Of late, ISSR marker system is used to analyse the genetic variability among *Samia cynthia ricini* populations (Vijayan *et al.* 2006).

A century ago, *Antheraea* sp. were widely distributed throughout the tropical and subtropical belts of India (Siddique *et al.* 1992). In spite of the massive deforestation, they are still found in well-demarcated ecozones (Jolly *et al.* 1974) and mostly differentiated based on phenotypic traits (cocoon weight, colour etc.). Hence proper genetic characterization is an important step for appropriate conservation and utilization of wild-genetic materials in breeding programme (Chatterjee *et al.* 2004).

These molecular markers have been extensively used to study genetic diversity of population dynamics in many species of plants and animals (Zietkiewicz *et al.* 1994; Tsumura *et al.* 1996; Dayanandan *et al.* 1997; Gabierslsen and Brochmann 1998; Wolfe *et al.* 1998; Knox and Palmer 1999). The RFLP and PCR-based techniques have been used to study the genetic relationship among silkworm strains (Xia *et al.* 1998; Reddy *et al.* 1999a, b; Nagaraju *et al.* 2001; Lu *et al.* 2002; Li *et al.* 2005; Velu *et al.* 2008).

The advent of PCR and availability of high-throughput automated sequencers have increased the use and popularity of microsatellites or SSRs as highly informative molecular markers (Litt and Luty 1989; Tautz 1989; Weber and May 1989; Schlotterer 2004). Information about distribution and variability of microsatellite sequences in the genome of a species can elucidate its genetic evolution, e.g. in human beings and *Drosophila* (Slatkin 1995; Schlötterer *et al.* 1997). The microsatellite isolation in Lepidoptera has found to be of great use in population studies and evolutionary dynamics in these insects and other organisms (Meglecz *et al*. 2004; Zhang 2004; Prasad *et al*. 2005).

Genetic relations of various genotypes by scoring the PCR-SSR profiles were done. The polymorphic loci generated by SSR marker systems were scored using POPGENE 1.32. The level of polymorphism was expressed as the percentage of all loci that are polymorphic in the profile of dendrograms for SSR.

In the present study, the germplasm collected from various zones of India displayed variable genetic polymorphism and was found to be highest in Bhandara (72.41%), followed by the samples of Daba TV, Daba BV and Modal from Karimnagar, Warangal of Telangana and Keonjhar have displayed (68.98%) and Raily from Bastar of Maharashtra (65.52%). The samples Sukinda and Andhra local from Karimnagar and Sukindergarh were least diverse and displayed only 55.17% polymorphism (table 3).

AL, DT, DB, Modal, Sukinda and Raily were found to have a genetic distance of 0.1967, 0.1932, 0.2554, 0.2217, 0.1495 and 0.2644, respectively with that of Bhandara (table 2a). The dendrogram produced by UPGMA of Nei's genetic distance for all populations ($7 \times 16 = 112$) is presented in (figure 3).

Phenogram based on UPGMA algorithm from SSR data of ecorace genetic diversity analysis of *A. mylitta* does not show region-wise clustering. Two distinct clusters, a and b, have been obtained. (i) Cluster 4: consists of samples collected from Karimnagar (Telangana) and Bhandara (Maharashtra), in which Andhra local-Bhandara formed a cluster, which are found to be genetically close. (ii) Cluster 3: consists of samples collected from Karimnagar (Telangana) and Sukindergarh (Orissa) (cluster 2) Keonjhar (Orissa) and Bastar (Chhatisgarh) (cluster 1). (iii) Cluster 2: among these the

Table 3. Genetic diversity analysis in the ecoraces of *A. mylitta*.

Ecorace	Place of collection	Number of polymorphic loci	Percentage of polymorphic loci
Andhra local	Karimnagar, Telangana	16	55.17
Daba TV	Karimnagar, Telangana	20	68.97
Daba BV	Warangal, Telangana	20	68.97
Modal	Keonjhar, Orissa	20	68.97
Sukinda	Sukindergarh, Orissa	16	55.17
Raily	Bastar, Chhatisgarh	19	65.52
Bhandara	Maharashtra	21	72.41

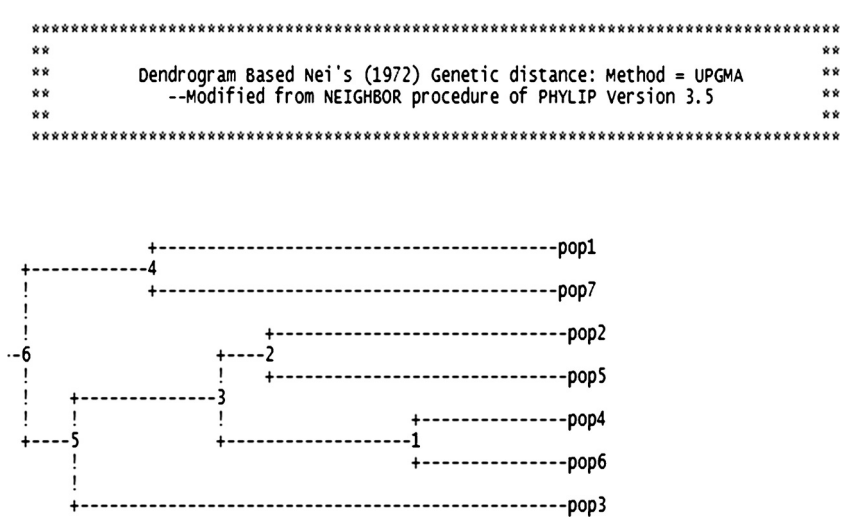


Figure 3. UPGMA dendrogram depicting ecorace genetic diversity of *Antheraea mylitta* D genotypes, obtained by PCR-SSR marker data.

Table 4. List of primers used for SSR analysis and the resulting polymorphic DNA bands.

	Primer	Annealing temperature (°C)	Total number of bands amplified	Number of polymorphic bands	Polymorphism (%)
1	Amysat001	57	124	38	30.64
2	Amysat013	55	99	4	4.04
3	Amysat015	57	99	7	7.07
4	Amysat019	55	95	–	–
5	Amysat021	55	103	2	1.94
6	Amysat023	48	121	85	70.24
7	Amysat025	50	165	133	80.60
8	Amysat038	53	140	74	52.85
9	Amysat040	53	112	54	48.21
10	Amysat041	53	118	70	59.32

populations of Daba TV, Sukinda formed cluster 2, which are found to be genetically close. (iv) Cluster 1: Modal and Raily ecoraces formed an individual cluster 1, which are found to be genetically close. While Daba BV seems to have diverse genotype and a rare one which was not included in any cluster, and found to be closer to cluster 1 than cluster 2 (figure 3).

It can be inferred from the dendrograms (figure 3) obtained using SSR primers Andhra local – Bhandara; Daba TV – Sukinda, Modal – Raily are found to be genetically close, while Daba BV seem to have diverse genotype.

From table 2, a&b on genetic distance, it can be seen that Daba TV (mean value = 0.2646) shows higher genetic distance among the other six populations (i.e., Andhra local, Daba BV, Modal, Sukinda and Raily), which implies that Daba TV is genetically distant from other ecoraces. It can also be observed that the lowest genetic distance was found in ecoraces Andhra local, Daba BV, Modal (same genetic distance of 0.18) have shown more genetic closeness. Lower the genetic distance, genetically closer are the ecoraces (table 4).

The order of genetic closeness may be summarized as: Daba TV < Raily < Sukinda < Daba BV < Modal < Andhra local. Raily is genetically less close than Daba TV. Sukinda is genetically less close than Raily. Daba BV is genetically less close than Sukinda. Modal is genetically less close than Daba BV. Andhra local ecorace is genetically less close than Modal. Andhra local and Bhandara are found to be close within the populations according to genetic distance and phylogenetic tree.

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References

Arunkumar K. P., Sahu A. K., Mohanty A. R., Awasthi A. K., Pradeep A. R., Urs S. R. and Nagaraju J. 2012 Genetic diversity and population structure of Indian Golden Silkmoth (*Antheraea assama*). *PLoS One* **7**, e43716.

Chatterjee S. N., Vijayan K., Roy G. C. and Nair C. V. 2004 ISSR profiling of genetic variability in the ecotypes of *Antheraea mylitta* Drury, the tropical tasar silkworm. *Genetika* **40**, 152–159.

Cheng T. C., Xia Q. Y., Qian J. F., Liu C., Lin Y., Zha X. F. and Xiang Z. H. 2004 Mining single nucleotide polymorphisms from EST data of silkworm, *Bombyx mori*, inbred strain Dazao. *Insect Biochem. Mol. Biol.* **34**, 523–530.

Dayanandan S., Bawa K. S. and Kesseli R. 1997 Conservation of microsatellite among tropical trees (leguminosae). *Am. J. Bot.* **84**, 1658–1663.

Dietrich W., Katz M., Lincoln S. E. and Shin H. S. 1992 A genetic map of the mouse suitable for typing intraspecific crosses. *Genetics* **131**, 423–447.

Field D. and Wills C. 1996 Long, polymorphic microsatellites in simple organisms. *Proc. R. Soc. B.* **263**, 209–215.

Gabierslsen T. M. and Brochmann C. 1998 Sex after all: high levels of diversity detected in theatric clonally plant saxifrage cernua using RAPD markers. *Mol. Ecol.* **10**, 1701–1708.

Hamada H., Petrino M. and Kakunaza T. 1982 A novel repeated element with Z-DNA forming potential in widely found in evolutionaly diverse eukaryotic genomes. *Proc. Natl. Acad. Sci. USA* **79**, 6465–6469.

Jolly M. S., Sen S. K. and Ashan M. M. 1974 *Tasar Culture*, pp. 1–166. Ambika Publishers, Mumbai, India.

Knox E. B. and Palmer J. D. 1999 The chloroplast genome arrangement of *Lobelia thuliniana* (Lobiliaceae): expression of inverted repeat in an ancestor of the companulales. *Plant Syst. Evol.* **214**, 49–64.

Li M., Shen L., Xu A., Miao X., Hou C., Sun P. *et al.* 2005 Genetic diversity among silkworm (*Bombyx mori* L., Lep., Bombycidae) germplasms revealed by microsatellites. *Genome* **48**, 802–810.

Litt M. and Luty J. A. 1989 A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet.* **44**, 397–401.

Lu C., Yu H. S. and Xiang Z. H. 2002 Molecular systematic studies on Chinese mandarina silkworm (*Bombyx mandarina* M.) and domestic silkworm (*Bombyx mori* L.). *Sci. Agric. Sin.* **35**, 94–101.

Mahendran B., Padhi B. K., Ghosh S. K. and Kundu S. C. 2005 Genetic variation in ecoraces of tropical tasar silkworm, *Antheraea mylitta* D. using RFLP technique. *Curr. Sci.* **90**, 100–103.

Mahendran B., Ghosh S. K. and Kundu S. C. 2006a Molecular phylogeny of silk producing insects based on internal transcribed spacer DNA1. *J. Biochem. Mol. Biol.* **39**, 522–529.

Mahendran B., Padhi B. K., Ghosh S. K. and Kundu S. C. 2006b Genetic variation in ecoraces of tropical tasar silkworm, *Antheraea mylitta* D. using RFLF technique. *Curr. Sci.* **90**, 100–103.

Meglecz E., Petenian F., Danchin E., D'Acier A. C. and Rasplus J. Y. 2004 High similarity between flanking regions of different microsatellites detected within each of two species of Lepidoptera: *Parnassius apollo* and *Euphydryas aurinia*. *Mol. Ecol.* **13**, 1693–1700.

Meyer E., Wiegand P., Rand S. P., Kuhlmann D., Brack M. and Brinkmann B. 1995 Microsatellite polymorphisms reveal phylogenetic relationships in primates. *J. Mol. Evol.* **41**, 10–14.

Nagaraju G. M. and Nagaraju J. 1995 Genome fingerprinting of the silkworm *Bombyx mori* using random arbitrary primers. *Electrophoresis* **16**, 1633–1638.

Nagaraju J., Reddy K. D., Nagaraja G. M. and Sethuraman B. N. 2001 Comparison of multilocus RFLPs and PCR-based marker systems for genetic analysis of the silkworm. *Bombyx mori. Heredity* **86**, 588–597.

Nagaraju J., Kathirvel M., Muthulakshmi M., Subbiah E. V. and Kumar L. D. 2002 FISSR-PCR: a simple and sensitive assay for high throughput genotyping and genetic mapping. *Mol. Cell. Probes.* **16**, 67–72.

Nei M. 1972 Genetic distance between populations. *Am. Nat.* **106**, 283–292.

Nei M. 1973 Analysis of gene diversity in subdivisional populations. *Proc. Natl. Acad. Sci. USA* **70**, 3321–3323.

Nei M. 1978 Estimation of average heterozygosity and genetic distance form a small number of individuals. *Genetics* **89**, 583–590.

Prasad M. D., Muthulakshmi M., Madhu M., Archak S., Mita K. and Nagaraju J. 2005 Survey and analysis of microsatellites in the silkworm, *Bombyx mori*: frequency, distribution, mutations, marker potential and their conservation in heterologous species. *Genetics* **169**, 197–214.

Razdan J. L., Siddiqui A. A., Bahal R. K. and Mukherjee P. 1994 Identification of region specific silkworm (*Bombyx mori* L.) hybrids. *Sericologia* **34**, 641–648.

Reddy K. D., Abraham E. G. and Nagaraju J. 1999a Microsatellites of the silkworm, *Bombyx mori*: abundance, polymorphism and strain characterization. *Genome* **42**, 1057–1065.

Reddy K. D., Nagaraju J. and Abraham E. G. 1999b Genetic charecterization of the silkworm Bombyx mori by simple sequence repeat (SSR)-anchored PCR. *Heredity* **83**, 681–687.

Schlotterer C. 2004 The evolution of molecular markers–just a matter of fashion. *Nat. Rev. Genet.* **5**, 63–69.

Schlötterer C., Vogl C. and Tautz D. 1997 Polymorphism and locus-specific effects on polymorphism at microsatellite loci in natural *Drosophila melanogaster* populations. *Genetics* **146**, 309–320.

Siddique A. A., Chatterjee S. N., Goel A. K. and Sengupta A. K. 1992 Genetic divergence in tasar silkworm *A. mylitta* D. *Seircologia* **32**, 425–431.

Slatkin M. 1995 Hitch hiking and associative overdominance at a microsatellite locus. *Mol. Biol. Evol.* **12**, 473–480.

Suzuki Y., Gage L. and Brown D. D. 1972 The genes for silk fibroin in *Bombyx mori*. *J. Mol. Biol.* **70**, 637–649.

Tan Y. D., Wan C., Zhu Y., Lu C., Xiang Z. and Deng H. W. 2001 An amplified fragment length polymorphism map of the silkworm. *Genetics* **157**, 1277–1284.

Tautz D. 1989 Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.* **17**, 6463–6471.

Tsumura Y. K., Ohba K. and Strauss S. H. 1996 Diversity and inheritance of inter-simple repeat polymorphism in Douglas fir (*Pseudootsuga menziesii*) and Sugi (*Crypromeria japonica*). *Theor. Appl. Genet.* **92**, 40–45.

Velu D., Ponnuvel K. M., Muthulakshmi M., Sinha R. K. and Qadri S. M. 2008 Analysis of genetic relationship in mutant silkworm strains of *Bombyx mori* using inter simple sequence repeat (ISSR) markers. *J. Genet. Genomics* **35**, 291–297.

Vijayan K., Anuradha H. J., Nair C. V., Pradeep A. R., Awasthi A. K., Saratchandra B. *et al.* 2006 Genetic diversity and differentiation among populations of the Indian eri silkworm, *Samia cynthia-ricini*, revealed by ISSR markers. *J. Insect Sci.* **6**, 1–11.

Weber J. L. 1990 Informativeness of human (dC-dA)*n*. (dG-dT)*n* polymorphisms. *Genomics* **7**, 524–530.

Weber J. L. and May P. E. 1989 Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* **44**, 388–396.

Wolfe G. R., Hendris D. L. and Salvuchi M. E. 1998 Thermo protective role for sorbitol in the silver leaf white fly *Bermista argentifolii*. *J. Insect. Physiol.* **44**, 597–603.

Xia Q. Y., Zhou Z. Y., Lu C. and Xiang Z. H. 1998 Molecular phylogenetic study on the racial differentiation of *Bombyx mori* by random amplified polymorphic DNA (RAPD) markers. *Acta Entomol. Sin.* **41**, 32–40.

Yasukochi Y. 1998 A dense genetic map of the silkworm, *Bombyx mori*, covering all chromosomes based on 1018 molecular markers. *Genetics* **150**, 1513–1525.

Yeh F. C., Yang R. C. and Boyle T. 1999 *POPGENE version 1.31. Microsoft Window-based Freeware for Population Genetic Analysis. Department of Renewable Resources*. University of Alberta, Edmonton, AB Canada.

Zhang D. 2004 Lepidopteran microsatellite DNA: redundant but promising. *Trends Ecol. Evol.* **19**, 507–509.

Zietkiewicz E., Rafalski A. and Labuda D. 1994 Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* **20**, 176–183.

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