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Genetic Variability Within and Among Three Ecoraces of the Tasar Silkworm *Antheraea mylitta* Drury, as Revealed by ISSR and RAPD Markers

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Genetic diversity within and between populations of *Antheraea mylitta* Drury was studied using thirty individuals from three ecoraces using 12 ISSR and 10 RAPD primers. Raily, Daba and Modal ecoraces were collected from Chattisgarh, Jharkhand and Orissa states of India respectively. The ISSR and RAPD primers generated 94.7% and 95.6% polymorphism among the 30 individuals. The cluster analysis grouped these individuals according to their ecorace. The intra-ecoracial heterozygosity estimated with ISSR markers were 0.123 ± 0.18, 0.169 ± 0.17 and 0.214 ± 0.17 respectively for Modal, Raily and Daba ecoraces. Like wise, with RAPD markers the intra-ecoracial heterozygosity was 0.17 ± 0.22 in Modal, 0.229 ± 0.17 in Raily and 0.23 ± 0.19 in Daba ecoraces. However, the significantly low genetic differentiation (G_{ST}) (0.182 for ISSR and 0.161 for RAPD) and the high gene flow (Nm) (2.249 for ISSR and 2.60 for RAPD markers) among the ecoraces revealed that the amount of genetic diversity present among the ecoraces is not significant enough to make drastic genetic drifts among these ecoraces in the near future.

Key words: Silkworm, Daba, Raily, Modal, *Antheraea mylitta*, ISSR, Genetic diversity

Introduction

The tasar silkworm *Antheraea mylitta* Drury belongs to the family Saturniidae, under the super family Bombycoidea. It produces a variety of silk popularly called tasar silk and has high economic value. The tasar silkworm is distributed throughout the humid tropical region of India between 60 – 88°E longitude and 16 – 24°N latitude, covering the states of Karnataka, Andhra Pradesh, Maharashtra, Orissa, Madhya Pradesh, Chhattisgarh, Jharkhand, West Bengal and Bihar. Srivastava *et al.* (2000, 2002) reported 44 ecoraces of *A. mylitta* in India. Out of these ecoraces Raily, Daba, Modal, Sukinda, Bhandara, Andhra local, Sarihan and Laria are commercially important for sericulture industry. Most of these ecoraces were recognized chiefly on the basis of voltinism, host plant preference, fecundity, larval span, larval weight, shell weight and cocoon weight. However, most of these phenotypic variations are highly influenced by the environmental factors such as temperature, relative humidity and rainfall in different seasons (Srivastava *et al.*, 2000). Photoperiod, which has a latitudinal basis, is also seen to influence the pattern of voltinism of these populations (Kar *et al.*, 2000). Altitude is another factor, which changes voltinism in wild silkmoths as *A. mylitta* behaves as trivoltine, bivoltine and univoltine in low, medium and high altitudes respectively (Nayak and Dash, 1991). These observations raise questions on the genetic status of the ecoraces as whether the phenotypic variations exhibited by these ecoraces are the result of variations at genome level or merely due to environmental effects. Further, conservation of these precious wild silkworm genetic resources becomes an absolute necessity under the present circumstances where deforestation coupled with overexploitation threatens the very existence of this silkworm species. Hence, it was felt essential to understand the genetic basis of variability found among different ecoraces of *A. mylitta*, which would be of much use to the biologists, conservationists, geneticists and breeders to formulate appropriate strategies for proper conservation and utilization.

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Attempts were made earlier to characterize different gene pools of these ecoraces with both biochemical and quantitative traits, (Jolly *et al.*, 1974; Thangavelu and Sinha, 1993; Kar *et al.*, 2000; Srivastava *et al.*, 2000). But these studies could not yield substantial information on the genetic identity and diversity of these ecoraces, as these characters were found to be influenced by the environmental factors. Hence, Chaterjee *et al.* (2004) used ISSR markers to estimate the genetic diversity among seven populations of the Raily ecorace and found significantly very low genetic diversity among the populations. However, Mohandas *et al.* (2004) found significant among of genetic diversity among individuals of a single populations of Daba ecorace. These contrasting results together with the need of understanding the genetic diversity among the ecoraces at the molecular level for its proper conservation and utilization prompted us to undertake the present study with three sericulturally important ecoraces namely Daba, Modal and Raily

Materials and Methods

Study materials and sampling

Three ecoraces of *Anthereae mylitta* Drury were selected for this study based on their economic importance, urgent need for conservation. Cocoons of these three ecoraces viz Daba, Modal and Raily, were collected from the forest regions where natural populations are available.

Thirteen individual cocoons of Daba ecorace were collected randomly from primary host plant of *Terminalia tomentosa* present in the forest region of south Singhbhum district of Jharkhand between 22.60°N latitude, 85.82°E longitude, and at an average altitude of 209 m from the mean sea level (MSL) covering an area of approximately 120 square kilometers (Fig. 1). These cocoons showed significant variability as the weight of single cocoon varied from 8.3 g to 10.4 g with an average of 9.3 g. The single shell weight also showed variation from 1.5 g to 2.1 g with an average of 1.75 8 g.

Fourteen cocoons of Raily ecorace were collected from the primary food plant of *Shorea robusta* distributed in the Bastar forest division of the state of Chhattisgarh (Fig. 1), which ranges from the north to south within 17°45' to 20°34' N latitude and 81°00' to 82°01' E longitude with altitude ranging from 150 to 1200 m from the mean sea level (MSL). These cocoons also showed considerable variability in most of the characters as the single cocoon weight varied from 13.8 g to 14.4 g, the shell weight varied from 2.8 g to 3.1 g.

Three cocoons of the Modal ecorace could be collected from the forest ranges of Kaptipada in Mayurbhanj district

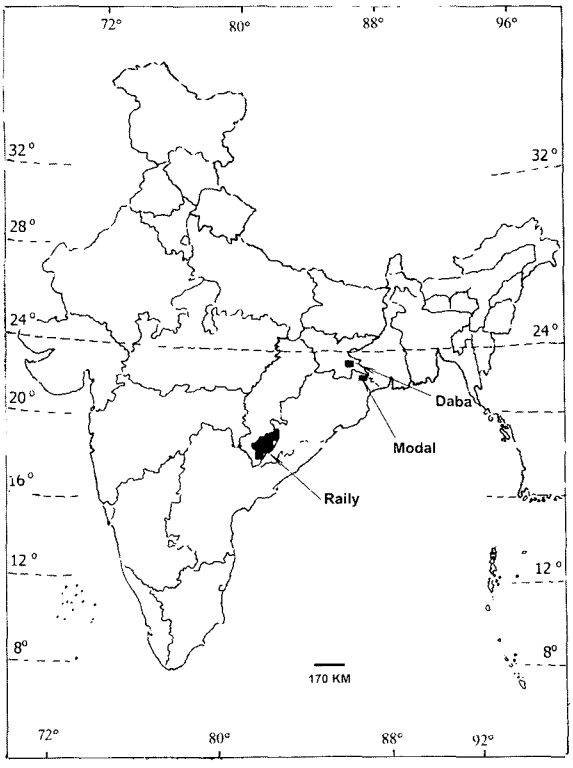

Fig. 1. Geographical distribution of the three ecoraces of *A. mylitta,* namely Daba, Raily and Modal, used in this study.

of Orissa state. This area is at an altitude of 3110 m MSL and is in between 86°25'E longitude and 21°35'N latitude. These cocoons were collected from the host plant of *Shorea robusta*. The mean weight of single cocoon was 10.65 g and single shell weight as 2.11 g.

DNA extraction and PCR amplification

Individual pupa was dissected out from the cocoon and 500 mg of the pupal portion was crushed in liquid nitrogen and made into fine powder. The powder was then transferred to a 25 ml Oakridge tube and 5 ml of lysis buffer (pH 7.5) containing 200 mM Tris-HCl, 25 mM EDTA, 300 mM NaCl and 2% of SDS was added to it before being incubated at 37°C for 16 hrs in the presence of proteinase K. DNA was extracted through the standard phenol chloroform Isoamyl alcahol extraction protocol and precipitated with ethanol in the presence of 3 M sodium acetate. The precipitated DNA was washed with 70% alcohol and dissolved in Tris-EDTA buffer (pH 8.0) for treatment with DNase free RNase at 37°C for 2 hrs. The DNA was again extracted by following the phenol-chloroform extraction steps. The extracted DNA dissolved in TE buffer and diluted to 10 ng/µl working solution through quantification on 0.8% agarose gel using uncut λ-DNA (10 ng/µl) as standard.

PCR amplification with ISSR primers

Eleven ISSR primers from the University of British Columbia (ISSR Kit # 9) were used for the study (Table 1). PCR amplification was done in an MJ Research Thermal-Cycler, PTC200, using 20 µl reaction mixture containing 30 ng DNA, 2.0 µl of 10 × PCR Buffer of Genei, Bangalore, India, 0.2 mM dNTP, 2.5 mM $MgCl_2$, 0.15 µM individual ISSR Primer and 1 U of Taq DNA polymerase. The PCR schedule was 94°C for 2 min followed by 40 cycles of 94°C for 30 sec, 50°C for 30 sec, 72°C for 2 min and a final extension of 10 min at 72°C.

PCR amplification with RAPD primers

Initially twenty-three RAPD primers (Operon Technologies, Inc., USA) were screened for PCR amplification with four DNA samples from Daba ecorace. On the basis of the formation of robust and polymorphic band profiles, 10 RAPD primers (Table 2) were selected for the study. PCR amplifications of genomic DNA were conducted according to Williams *et al.* (1990) using 20 µl of reaction mixture containing 1× PCR Buffer (MBI, Fermantas) 0.2 mM each of dGTP, dATP, dCTP, and dTTP; 2.0 mM $MgCl_2$; 100 pM Primer; 10 ng genomic DNA and 1 U Taq polymerase. The PCR schedule followed was 93°C for 2 min followed by 35 cycles of 93°C for 1 min, 36°C for 1 min, 72°C for 2 min and a final incubation at 72°C for 15 min.

The PCR products were resolved on 1.5% agarose (GIBCO, Bethesda Research Laboratory, Paisley, Scotland) gel in Tris Boric Acid/EDTA buffer (1 × TBE) using 0.6 cm thick gel of 20 cm (w) × 25 cm (L) and electrophoresis was done at a constant voltage of 60 V using power supply units EPS 400/200 (Amersham Pharmacia) for three hrs. Gels were stained with ethidium bromide (0.5 µg/ml) and photographed with a gel documentation system (Syngene corporation, Frederick, MD, USA).

Table 1. List of ISSR primers used and polymorphism generated

Sl. no.	5'-3' Primer sequence	No. of fragments	Polymorphic bands	% DNA polymorphism
807	AGAGAGAGAGAGAGAGT	10	8	80
808	AGAGAGAGAGAGAGAGC	10	9	90
810	GAGAGAGAGAGAGAGAT	9	9	100
811	GAGAGAGAGAGAGAGAC	10	10	100
834	AGAGAGAGAGAGAGAGYT	14	13	92.9
835	AGAGAGAGAGAGAGAGYC	7	6	85.7
840	GAGAGAGAGAGAGAGAYT	11	10	90.9
855	ACACACACACACACACYT	10	8	80
864	ATGATGATGATGATGATG	11	9	81.8
881	GGGTGGGGTGGGGTG	11	8	72.7
884	HBHAGAGAGAGAGAGAG	11	8	72.7
Total		114	98	Avg-85.9

Note: Y = (A,G,C,T); B = (C,G,T); H = (A,C,T)

Table 2. List of RAPD primers used and polymorphism generated

Sl. no.	5'-3' Primer sequence	No. of fragments	Polymorphic bands	% DNA polymorphism
OPW-4	CAGAAGCGGA	13	11	84.6
OPW-5	GGCGGATAAG	11	9	81.8
OPW-6	AGGCCCGATG	13	9	69.2
OPW-9	GTGACCGAGT	9	6	66.7
OPW12-	TGGGCAGAAG	8	7	87.5
OPW-13	CACAGCGACA	14	13	92.9
OPW-16	CAGCCTACCA	7	5	71.4
OPW-17	GTCCTGGGTT	8	7	87.5
OPW-18	TTCAGGGCAC	8	8	100
OPW-20	TGTGGCAGCA	8	7	90.6
Total		99	84	Avg:84.8

Binary scoring of the profiles was done on the basis of presence (1) or absence (0) of bands.

Statistical analyses

The genetic diversity among the 30 silkworms was estimated by pairwise comparison of the silkworm using Dice's coefficient (D) = $1 - (2N_{ab}/(2N_{ab} + N_a + N_b))$, where N_{ab} is the number of bands that are shared by the genotypes 'a' and 'b' and N_a is the number of bands present in 'a' and N_b is the number of bands present in 'b' (Sneath and Sokal, 1973). A dendrogram was generated from the above matrix using unweighted pair group method with arithmetical averages (UPGMA; Sneath and Sokal, 1973) on PHYLIP 3.5c software program (Felsenstein, 1993). In order to test the ecorace against the marker systems it generated, the discriminant function analysis (DFA) was carried out with SPSS/PC + 10.0 program (M.J. Norusis, SPSS Inc., Chicago) using the option of within group covariance and Mahalanobis D^2 statistics (Mahalanobis, 1936). The ecoraces were coded numerically as 1 to 3. Squared Mahalanobis distance was used for testing the group centroid of each individual of the ecoraces. Mahalanobis distance is the distance between a case and the centroid (mean of all the cases of the group) for each group (of the dependent) in attributed space (n-dimensional space defined by n variables). A case will have one Mahalanobis distance for each group, and it would be classified as belonging to the group for which its Mahalanobis distance is smallest. Thus, smaller the Mahalanobis distance, the closer is the case to the group centroid and more likely it is to be classed as belonging to that group (Mahalanobis, 1936).

The genetic variability present within and between ecoraces, in terms of percentage of polymorphism (P) and heterozygosity (H), Shannon's information index (I) were estimated based on band profiles. Nei's (Nei, 1973) measures of heterozygosity between ecoraces (Ht) within ecoraces and the coefficient of population differentiation (G_{ST}) were estimated using POPGENE version 1.3 (Yeh, 1998). In POPGENE the genetic divergence among different populations is calculated using a multiallelic analogue of F_{ST} among a finite number of populations, which is otherwise called the coefficient of gene differentiation (Nei, 1973). This is stated as $G_{ST} = D_{ST}/H_t = (H_t - H_s)/H_t$, where D_{ST} is the average gene diversity between subpopulations, including the comparisons of subpopulations with themselves, with $D_{ST} = (H_t - H_s)$. G_{ST} is an extension of Nei's (1972) genetic distance between a pair of populations to the case of hierarchical structure of populations (Nei, 1973). $H_t = (1 - \sum p_i^2)$, where p_i is the frequency of i^{th} allele at a locus in a population and $\sum$ is the summation of all alleles. Hence, the H_s in the equation were defined in terms of gene diversities. However, for random mating subpopulations, gene diversities can be defined as expected heterozygosity under Hardy-Weinberg equilibrium averaged among subpopulations (H_s) and of the total population (H_t). The estimate of gene flow from G_{ST} was also calculated as $(N_m) = 0.5\ (1 - G_{ST})/G_{ST}$.

Results

DNA polymorphism as revealed by ISSR and RAPD primers

A high amount of polymorphism was observed at the DNA level with both primer systems (Fig. 2, 3). The 11

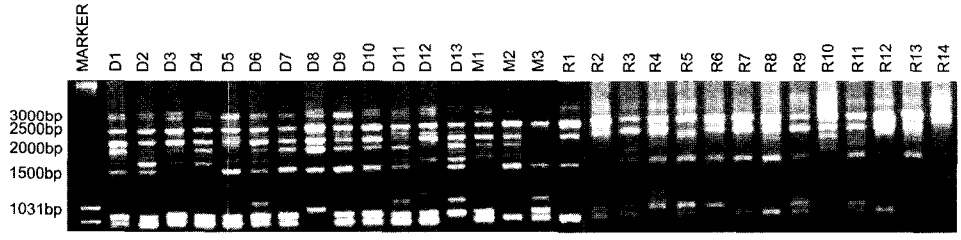

Fig. 2. ISSR marker profiles generated with UBC-864 and resolved on 1.5% agarose gel(D-Daba; M-Model and R-Raily).

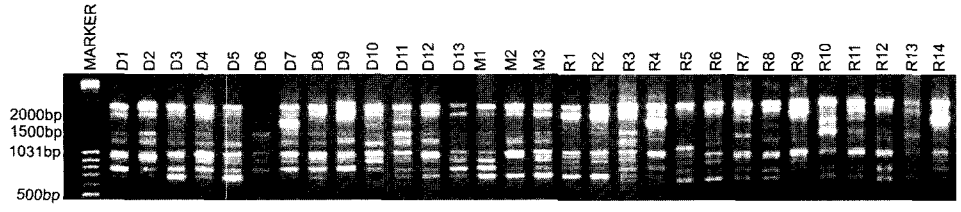

Fig. 3. RAPD marker profiles generated with OPW-6 and resolved on 1.5% agarose gel.

ISSR primers generated a total of 114 bands, of which 108 were polymorphic thus showing 94.7% polymorphism among the silkworms of the three ecoraces. The bands were between ~450 bp to ~3000 bp. The number of bands generated by individual primers varied from 7 (835) to 14 (UBC 884) (Table 1). Similarly, the ten RAPD primers generated a total of 99 bands, out of which 95 were polymorphic (Table 2); thereby it revealed 95.6% polymorphism among the silkworms. The bands were between ~250 bp to ~3500 bp.

Genetic distance and grouping of silkworms

The genetic distance estimated on the basis of ISSR markers among the silkworms, irrespective of the ecorace to which they belong varied from 0.173 (R2 vs. R1) to 0.622 (R13 vs. D4). Further, 28 pairing showed genetic distance above 0.500. This higher genetic distance was mainly observed between individuals of Raily and Daba ecoraces. The intra-population variability was 0.337 in Daba, 0.308 in Modal and 0.385 in Raily. The inter population variability between Daba and Raily ecoraces was 0.447, the same between Daba and Modal was 0.347. The inter-population distance between Modal and Raily was 0.426.

Similarly, the genetic distance based on RAPD markers were in the range of 0.131 (D1 vs D2) to 0.593 (R4 vs. M1). In this case also, eight pairs showed a genetic distance above 0.500. Here, an important point observed is that D6 was distant from the individuals of Raily (R5, R7, R8 and R13). The intra-population genetic diversity in the ecorace Daba was 0.272. In Modal ecorace the intrapopulation diversity was 0.341 and the same in Raily ecorace was 0.379. The inter population distance measured by averaging the distance of all pairs between Modal and Daba silkworm individuals was 0.344, and the same between Modal and Raily was 0.391. In case of Raily and Daba the inter ecoracial distance was 0.382.

Cluster analysis

The cluster analysis with ISSR markers grouped the thirty silkworms into seven clusters (Fig. 4). The grouping was, to a great extent, in accordance with their ecoracial affiliations, though there are one or two exceptions. In cluster A and B all the silkworms were exclusively from the Daba ecorace where as in group C out of the eight members two were from Daba, two were from the Modal ecorace and the rest four were from the Raily ecoraces. Similarly, the group D contained two silkworms one each from Daba and Modal ecoraces. In the group E only two silkworms were present one from Daba and the other from Raily. In group F and G all the silkworms were from the Raily ecorace. The dendrogram realized from the RAPD markers also depicted almost a similar clustering pattern where individual silkworms of Daba, baring a few excep-

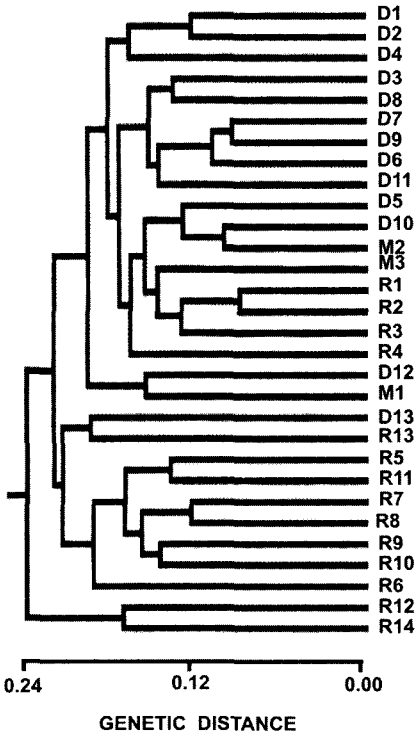

Fig. 4. Dendrogram based on ISSR markers showing genetic relationships among the individuals of the three ecoraces.

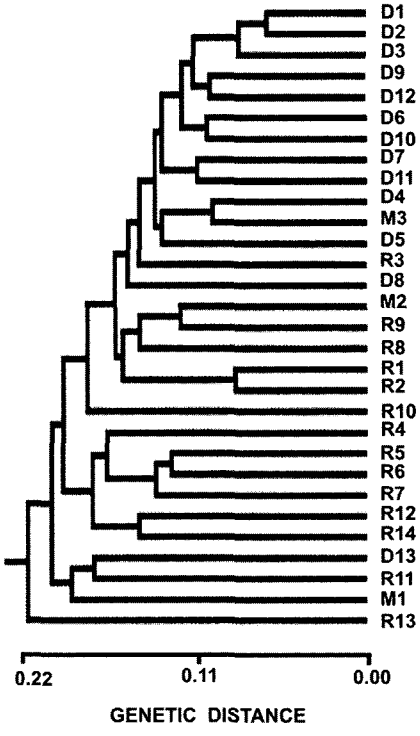

Fig. 5. Dendrogram based on RAPD markers showing genetic relationships among the individuals of the three ecoraces.

tion grouped at one end of the cluster while the same from Raily grouped in the other end of the dendrogram and the three silkworms of the Modal ecorace dispersed among the Daba and Raily ecoraces (Fig. 5). Thus, it is clear from the dendrogram that considerable amount of genetic mixing is present among these three ecoraces.

Discriminant function analysis with ISSR and RAPD markers

Discriminant Function Analysis led to the identification of 'two' functions which together showed 100% of variability among the ecoraces. The Wilk's lambda estimates for these functions were significant ($p < 0.000$). The chi-square value for the first function was 180.39. The distribution of the 18 individuals showed highly distinct grouping without any overlapping (Fig. 6). The Daba ecoraces were distributed in an area, which covered from 9 to 16 in the X-axis (Function-1) and -5 to -1.7 in Y-axis (Function-2). Similarly, silkworms of Modal ecoraces were distributed in an area which covered from -25 to 30 in the X-axis (Function-1) and 7 to 9 in Y-axis (Function-2) and silkworms of Raily ecorace were distributed between 20 – 30 in X-axis (Function-1) and -1 to 4 in Y-axis (Function-2). From the canonical distribution it is clear that all the three ecoraces differed one other significantly.

The discriminant function analysis with RAPD markers extracted two functions for the ecoraces. The first function alone accounted 98% variability and the canonical correlations for these functions were 0.999 to 0.970. The Wilk's lamda for these functions were highly significant ($p < 0.000$). The distribution of the 18 individuals against these two functions showed no overlapping among the

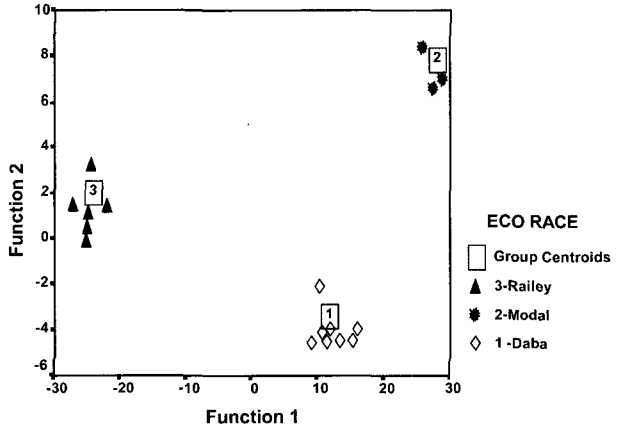

Fig. 6. Scatter plot showing distribution of 30 tasar silkworms, grouped based on their ecoracial affiliation, against the first two canonical discriminant functions according to the genetic variability observed using the ISSR fingerprinting.

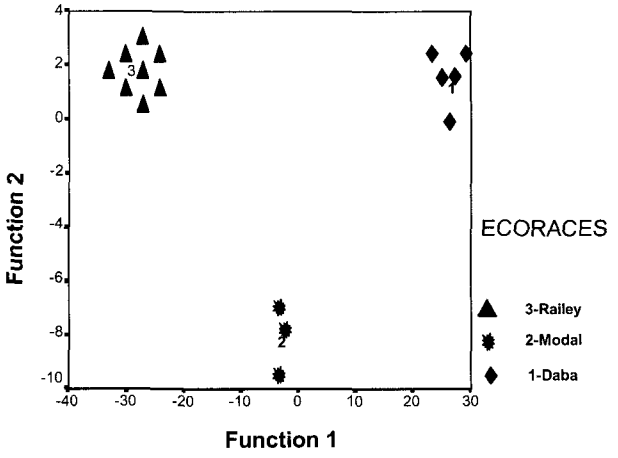

Fig. 7. Scatter plot showing distribution of 30 tasar silkworms, grouped based on their ecoracial affiliation, against the first two canonical discriminant functions according to the genetic variability observed using the RAPD fingerprinting.

ecoraces (Fig. 7). The Daba ecoraces were distributed in an area, which covered from 22 to 30 in the X-axis (Function-1) and -1 to 3 in Y-axis (Function-2). Modal were distributed in an area which covered from -1 to -3 in the X-axis (Function-1) and -10 to -5 in Y-axis (Function-2) and Raily ecoraces were distributed in an area which covered from -20 to -40 in X-axis (Function-1) and 0 to 3 in Y-axis (Function-2).

Genetic diversity and Heterozygosity

The genetic analysis of the population and subpopulation of the three ecoraces revealed that the average number of observed alleles (na) per population varied from 1.30 ± 0.39 in Daba to 1.69 ± 0.46 for Raily for ISSR markers and from 1.40 ± 0.49 in Modal to 1.89 ± 0.31 in Raily in RAPD primers (Table 3). The average number of effective number of alleles (ne) varied from 1.21 ± 0.34 in Modal to 1.35 ± 0.32 in Daba in ISSR markers and from 1.31±0.41 in Modal to 1.39 ± 0.38 in Daba in RAPD markers. The Nei's genetic heterozygosity (h) was highest in Raily (0.17 ± 0.17) and least in Modal (0.123 ± 0.18) with ISSR primers. The same with RAPD was highest in Daba (0.23 ± 0.19) and least in Modal (0.17 ± 0.22). The Shannon's information index (I) was 0.337 ± 0.23 in Daba, 0.268 ± 0.24 in Raily and 0.183 ± 0.27 in Modal with ISSR markers and 0.364 ± 0.23 in Raily, 0.349 ± 0.27 in Daba and 0.242 ± 0.30 in Modal with the RAPD markers. The percentage of polymorphism present within each ecorace varied from 32.48% in Modal to 90.34% in Daba for the ISSR markers and from 40.0% in Modal to 89.0% in Raily for the RAPD markers.

The mean coefficients of genetic differentiation value G_{ST}, across the loci were 0.182 and 0.161 respectively for

Table 3. Gene diversity present in three ecoraces of the tasar silkworm *Antheraea mylitta*

Ecorace	Number of observed alleles (na)	Number of effective alleles (ne)	Heterozygosity (h)	Shannon's information index (I)	Polymorphism (%)
			ISSR markers		
Daba	1.303 ± 0.399	1.345 ± 0.324	0.214 ± 0.173	0.337 ± 0.239	80.34
Modal	1.324 ± 0.470	1.211 ± 0.339	0.123 ± 0.187	0.183 ± 0.271	32.48
Raily	1.692 ± 0.463	1.271 ± 0.326	0.169 ± 0.176	0.268 ± 0.243	69.23
			RAPD markers		
Daba	1.730 ± 0.942	1.393 ± 0.375	0.230 ± 0.196	0.349 ± 0.271	73.00
Modal	1.400 ± 0.492	1.307 ± 0.411	0.168 ± 0.215	0.242 ± 0.303	40.00
Raily	1.890 ± 0.314	1.368 ± 0.331	0.229 ± 0.168	0.364 ± 0.225	89.00

Table 4. Genetic heterozygosity within and between ecoraces along with genetic differentiation and gene flow

Population	H_t	H_s	G_{ST}	N_m
		ISSR markers		
Daba vs Modal	0.216 ± 0.027	0.192 ± 0.025	0.115	3.842
Daba vs Raily	0.191 ± 0.026	0.169 ± 0.021	0.114	3.869
Raily vs Modal	0.184 ± 0.030	0.147 ± 0.021	0.205	1.936
Among ecoraces	0.207 ± 0.025	0.169 ± 0.017	0.182	2.249
		RAPD markers		
Daba vs Modal	0.227 ± 0.039	0.199 ± 0.123	0.123	3.552
Daba vs Raily	0.262 ± 0.027	0.229 ± 0.021	0.121	3.636
Raily vs Modal	0.230 ± 0.031	0.199 ± 0.024	0.134	3.232
Among ecoraces	0.249 ± 0.029	0.209 ± 0.022	0.161	2.604

ISSR and RAPD markers (Table 4). When pair-wise comparison of these ecoraces showed that highest genetic differentiation was between Raily and Modal (0.205 and 0.134 respectively for ISSR and RAPD markers). The gene diversity within an ecorace (H_s) was highest in Daba and least in Modal. The genetic diversity between populations was highest in Daba vs. Modal for ISSR markers and Daba vs Raily for RAPD markers. The gene flow (N_m) among the species was 2.249 for ISSR markers and 2.60 for RAPD markers. Pair-wise evaluation revealed that considerably higher amount of gene flow occurs between Daba and Modal for ISSR markers and between Daba and Raily for RAPD markers.

Discussion

The present study demonstrated high DNA polymorphism among the three, Daba, Modal and Raily ecoraces of *A. mylitta*. Chatterjee *et al.* (2004), while assessing the genetic diversity among the populations of Raily, included single individuals from four other ecoraces of *A. mylitta* and found higher amount of DNA polymorphism among these individuals. However, it is needless to state that results from single individuals of a highly heterozygoius species need to be confirmed with detailed studies. The present study was an attempt in this direction were large populations were used from the ecoraces. The results of the present study confirm the earlier findings that there could be significant amount of genetic diversity among the ecoraces, hence the morphological variation shown by these ecoraces are not mere result of environmental effects. However, it is to be admitted that the sample size of Modal ecorace is too small to derive a conclusive opinion on the overall genetic variability present in that ecorace. However, in the light of the DNA polymorphism observed in the other two ecorace it can be presumed that the DNA polymorphism obtained in Modal ecorace can be treated as a representation of the total genetic variability available in the ecorace. Further, it could be seen that RAPD and ISSR analysis has provided valid markers for the demonstration of population genetic parameters.

The genetic distance analysis followed by cluster analysis revealed that considerable amount of genetic differentiation has occurred among the ecoraces of this single species. Hence, the phenotypic differences exhibited by them are not merely due to environmental influences but have resulted from the diversity at the genetic level. This

genetic diversity present among the ecoraces might have occurred from geographic isolation, due to fragmentation of the forest, coupled with selection, as these ecoraces are believed to have developed from a single contiguous population of this species existed in the pre-Christian era (Ghosh, 1949). Studies on biological, physiological, genetical and behavioral characters of some of these ecoraces point to the fact that the genetic variability exhibited by these ecoraces is the result of adaptation changes necessitated from the different eco-geographical conditions and the availability of food plants (Thangavelu *et al.*, 2000).

The results of discriminant function analysis (DFA), further, support the contention that sufficient diversity has been accumulated among the ecoraces at the genomic level that the phenotypic variability exhibited by these ecoraces are not merely due to environmental effect. The discriminant function analysis clearly indicated that the grouping on the basis of their geographical origin (as Ecorace) was correct and the three ecoraces were genetically distant enough to put them in areas quite distant from each other. In the canonical figure the position of the ecoraces clearly revealed that Daba (Jharkhand) is genetically at a further distant from Raily (Chattisgarh) than the Modal (Orissa) ecorace. The geographic distance of the respective habitats of these ecoraces also supports this finding.

The population structure analyses, however, revealed significantly higher genetic mixing among these ecoraces, which is evident from the high gene flow (> 3). This higher gene migration among the ecoraces could be due to the random mating among these ecoraces, due to human interference like transporting cocoons from one region to the other, migration of male moths at the time of mating as the male moths can travel many miles to find a female moth. In population genetics, a gene flow (N_m) less than one migrant per generation into a population or equivalent thereof, the gene differentiation (G_{ST}) greater then 0.25 is regarded as the threshold quantities beyond which significant population differentiation can occur among the populations (Slatkin, 1987). According to Slatkin (1987) gene flow near to four among populations is sufficient to prevent genetic drift causing local adaptations within the two populations. Thus, the high gene flow observed between two ecoraces of *A. mylitta* might prevent speciation resulting from drastic genetic drift among the ecoraces within the near future. However, to preserve the identity and specific characteristics, genetic resources of these ecoraces need to be conserved in the core ecopockets of respective habitats. Further, the results clear indicate that the genetic relationship among these ecoraces is still complex and remains obscure, hence, need to be investigated in detail using more populations from each ecorace.

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