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Genetic variability and genetic structure of wild and semi-domestic populations of tasar silkworm (*Antheraea mylitta*) ecorace Daba as revealed through ISSR markers

P.K. Kar[1], K. Vijayan[2], T.P. Mohandas[2], C.V. Nair[2], B. Saratchandra[2] & K. Thangavelu[1]
[1]*Central Sericultural Germplasm Resources Centre, Central Silk Board, Thally Road,, P.B. 44635109, Hosur, Tamil Nadu, India (E-mail: prasantakar@rediffmail.com);* [2]*SeriBiotech Research Laboratory, Central Silk Board, Carmelram Post, 560035, Bangalore, Karnataka, India*

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Abstract

The genetic diversity in the wild and semi-domestic populations of Daba ecorace of *Antheraea mylitta* was studied to ascertain the distribution of variability within and among populations of semi-domestic bivoltine (DB), trivoltine (DT) and nature grown wild populations (DN) with inter-simple sequence repeat (ISSR) markers. A total of 138 markers were produced among 56 individuals of the three populations, of which 98% were polymorphic. For the individual populations, the percentage polymorphism was 58.69, 52.9 and 77.54 for DB, DT and DN, respectively. Average number of observed (1.791 ± 0.408) and effective alleles (1.389 ± 0.348) was also high in the wild populations in comparison to the bivoltine and trivoltine semi-domestic populations. Genetic diversity (H_t) in DB, DT and DN was 0.180 ± 0.033, 0.153 ± 0.032 and 0.235 ± 0.033, respectively and within-population genetic diversity (H_s) ranged from 0.166 to 0.259 with a mean of 0.189. Mean gene differentiation (G_{ST}) was found to be 0.25. Shanon's diversity index was 0.278, 0.237 and 0.361 for DB, DT and DN and overall it was 0.391. Gene flow (N_m) among the populations was 1.509. The dendrogram produced by UPGMA with Dice's genetic distance matrices resulted in the formation of three major clusters separating the three populations. Considerable intra- and inter-population variability is found in all three populations. The population structure analysis further suggests that the semi-domestic populations of Daba ecorace are at the threshold of differentiating themselves. The high genetic variability present within wild Daba population of *A. mylitta* is of much importance for conservation as well as utilization in systematic breeding program.

Introduction

The tropical tasar silkworm *Antheraea mylitta* Drury belongs to the family Saturniidae of Lepidoptera and provides a substantial contribution to the raw silk production in India. This species is polyphagous; primarily feeds on *Shorea robusta*, *Terminalia arjuna* and *T. tomentosa* and secondarily lives on at least a dozen other host plants (Jolly et al., 1979). This silkworm has a wide distribution in India over a distinct belt of humid and dense tropical forests sprawling over the central and southern plateau between 80–88° E longitude and 16–24° N latitude covering the states of Karnataka, Andhra Pradesh, Maharashtra, Orissa, Madhya Pradesh, Chhattisgarh, West Bengal, Jharkhand and Bihar (Thangavelu & Sinha, 1993). The majority of tasar silk comes from the cocoons collected from forests. Central Silk Board of India and state government agencies produce tasar silkworm eggs and supply to rearers for raising commercial crops. Presently, eight ecoraces

namely Daba, Sukinda, Sarihan, Raily, Laria, Andhra local, Bhandara local and Modal are commercially exploited in different states. Ecoraces like Modal and Raily are wild in nature and exclusively found in the natural forests, their rearing is practically impossible as they are least amenable to human handling. On the other hand ecoraces Daba and Sukinda are amenable to human handling and therefore called semi-domestic. Daba is one of the races, which has both wild (nature-grown) as well as semi-domestic populations. The wild population behaves as both bi- and tri-voltine in nature while semi-domestic counterparts are exclusively either bivoltine or trivoltine. Usually, after the pupal diapause when the monsoon sets in, the silk moth emerges out from the pupa by piercing through the cocoon and starts the life cycle. The trivoltines complete three cycles in a calendar year, hence the emergence starts early, around the first week of June. On the other hand, the bivoltines complete only two life cycles in a calendar year, and moth emergence takes place sometimes around the 3rd to 4th week of June, thus delay in the diapause break. Similarly, the bivoltine populations enter into diapause much earlier than the trivoltines (Jolly, Sen & Ahsan, 1974). The cocoon characters of these different populations show distinct variability (Srivastava et al., 2002). Since these populations are believed to have originated from a single population in the past and undergone different selection pressure, it was essential to study the genetic basis of this variability using a reliable marker system with molecular markers. Thus, the present study was undertaken with the objective of investigating the inter- and intra-population variability among three closely related populations of *A. mylitta*, for utilization in formulating strategies for conservation as well as breeding programs.

Molecular markers are known to have many advantages over morphological and biochemical markers, as they are more stable and independent of environmental influences (Bernatzky & Tanksley, 1989; Gepts, 1993). With the advent of polymerase chain reaction (PCR), a number of DNA marker systems like simple sequence repeat (SSR), random amplified polymorphic DNA (RAPD), inter-simple sequence repeat polymorphism (ISSR), expressed sequence tag (EST) and amplified fragment length polymorphism (AFLP) have been used to study the population genetics of plants and animals (Williams et al., 1990; Zietkiewicz, Rafalski & Labuda, 1994; Tsumura, Ohba & Strauss, 1996; Dayanandan, Bawa & Kesseli, 1997; Gabrielsen & Brochmann, 1998; Wolfe, Xiang & Kephart, 1998; Knox & Palmer, 1999). Use of SSR and EST requires prior knowledge on the genome of the organism. Since, no information is available on the genome of tasar silkworm; it was decided to use a primer system, which does not warrant such knowledge. Inter-simple sequence repeats (ISSR) marker system has been extensively used recently for genetic analysis of plant and animals alike (Zietkiewicz, Rafalski & Labuda, 1994; Tsumura, Ohba & Strauss, 1996; Ghislain et al., 1999; Prevost & Wilkinson, 1999; Reddy, Nagaraju & Abraham, 1999; Bornet et al., 2002; Vijayan, 2004). ISSR markers are useful in detecting genetic polymorphisms among closely related plants or animals by generating a large number of markers that target multiple microsatellite loci distributed across the genome. Further, they are simpler to use than the SSR technique as prior knowledge of the target sequences flanking the repeat regions is not required (Zietkiewicz, Rafalski & Labuda, 1994, Nagaoka & Ogihara, 1997). Recently, Chatterjee et al. (2004) used ISSR primers to study the genetic diversity present among different populations of Raily ecotypes of *A. mylitta*. Further, utility of ISSR markers for population genetic analysis has already been demonstrated (Deshpande et al., 2001). Hence, in this study ISSR markers were used to unravel the genetic relationship of three distinct populations of Daba ecorace of *A. mylitta*.

Material and methods

Genetic materials

Cocoons of *Antheraea mylitta* Drury, ecorace Daba were collected from the natural habitats of south Singhbhum district of Jharkhand state of India (22.60° N latitude and 85.82° E longitude) and also from semi-domestic populations (bivoltine and trivoltine) reared in forests near to human settlements of Korba district of Chhattisgarh state (24.51° N latitude and 82.40° E longitude). The semi-domestic populations are reared by the forest dwellers (*Adivasis*) by procuring tasar silkworm eggs, commonly known as disease free layings

(dfls) from the state government agencies. At the onset of monsoon in late June and July, the pupal diapause of tasar silkworm breaks and thousands of silk moths emerge out from cocoons and mate randomly. The silkworms come out of the egg would be allowed to enter in trees and shrubs, on which they feed. The mature worms spin cocoons on the branches of the host plants. These cocoons were collected for the study.

For the wild populations 16 cocoons were obtained and used for recording quantitative traits and also to extract DNA. Similarly, twenty cocoons from each of the bivoltine and trivoltine population were used for semi-domestic populations.

DNA extraction

Individual cocoons were cut open and pupae were crushed in pre-chilled mortar and pestle with liquid nitrogen, added lysis buffer (pH 7.5) containing 200 mM Tris–HCl, 25 mM EDTA, 300 mM NaCl and 2% of SDS and incubated at 37°C overnight in the presence of Proteinase-K. DNA was extracted through phenol:chloroform:isoamyl alcohol extraction method 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). The RNA present in the extracts was removed through the treatment with RNAse. The DNA was re-precipitated following second round of phenol:chloroform:isoamyl alcohol extraction steps. The extracted DNA was dried, dissolved in TE buffer and diluted to 10 ng/μl through serial dilution and quantification on 0.8% agarose gel against uncut λ-DNA (10 ng/μl) as standard.

PCR amplification of the DNA with ISSR primers

Ten inter-simple sequence repeat (ISSR) primers from 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 (MBI Fermentas), 0.2 mM dNTP, 2.5 mM $MgCl_2$, 0.15 μl M primer and 1.0 U of *Taq* DNA polymerase. The PCR schedule was 94 °C for 2 min followed by 40 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 2 min and a final extension of 10 min at 72 °C.

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 carried out with a constant voltage of 60 V from Amersham Pharmacia power supply units, EPS 400/200 for 3 h. Gels were stained with ethidium bromide (0.5 μg/ml) and photographed with the gel documentation system Syngene, USA. The experiment was repeated thrice and those bands, which appeared consistently in all the three gels, were scored and used for analysis. Weak bands were not

Table 1. List of ISSR primers used and polymorphism generated among the tasar silkworms

Sl. no.	5′–3′ Primer sequence	Number of fragments			% Polymorphism		
		DB	DT	DN	DB	DT	DN
807	AGAGAGAGAGAGAGAGT	13	9	16	100	100	100
809	AGAGAGAGAGAGAGAGG	12	11	12	83.33	90.91	100
811	GAGAGAGAGAGAGAGAC	9	5	11	100	100	100
812	GAGAGAGAGAGAGAGAA	7	6	12	100	100	91.67
825	ACACACACACACACACT	5	7	12	100	100	100
834	AGAGAGAGAGAGAGAGYT	9	8	10	100	100	90
840	GAGAGAGAGAGAGAGAYT	8	8	11	100	100	90.91
855	ACACACACACACACACYT	8	9	10	62.5	77.78	70
881	GGGTGGGGTGGGGTG	6	5	10	100	80	80
884	HBHAGAGAGAGAGAGAG	10	9	15	90	100	80

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

considered for analysis. Binary scoring of the profiles as presence (1) and absence (0) was adopted.

Statistical analyses

The genetic variability in the population was calculated using Nei's (1973) coefficient of gene differentiation (G_{ST}) in POPGENE version 1.32 (Yeh & Boyle, 1997). 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 in the following equation:

$$G_{ST} = D_{ST}/H_t = (H_t - H_s)/H_t$$

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 is defined by the following equation:

$$H_t = 1 - \sum p_{i^2}$$

where p_i is the frequency of ith allele at a locus in a population. Hence, H_s was defined in terms of gene diversities. However, for random mating subpopulations, gene diversities can be defined as expected heterozygosities under Hardy–Weinberg equilibrium averaged among subpopulations (H_s) and of the total population (H_t). The main difference between G_{ST} and F_{ST} (Wright, 1978) is that in G_{ST} the estimation of the heterozygosities relies on allele frequencies (Nei, 1987), whereas in F_{ST} to estimate the H_s the individual genotypes have to be known. The estimate of gene flow from G_{ST} was calculated as

$$\mathrm{Nm} = 0.5(1 - G_{ST})/G_{ST}$$

The F-statistics were estimated using the software package TFPGA (Miller, 1997) where Weir and Cockerham's (1984) methods of calculating Wright's F-statistics are applied to the data. Here theta (θP) value corresponds to Wright's F_{ST}. Jackknifing was done to obtain the variance estimate and bootstrapping was performed (1000 replications) to generate confidence intervals. The exact test (Raymond & Rousset, 1995) for population differentiation was also performed.

The genetic diversity within the population was also estimated by pair-wise 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 & Sokal, 1973). A dendrogram was generated from the above matrix using unweighted pair group method with arithmetical averages (UPGMA; Sneath & Sokal, 1973) on PHYLIP 3.5c software program (Felsenstein, 1993). The robustness of the dendrogram was tested by estimating cophenetic correlation for the dendrogram and comparing it with the original genetic dissimilarity matrix, using Mantel's matrix correspondence test (Mantel, 1967; Liedloff, 1999).

Results

Variability in quantitative traits

The quantitative characters of cocoons influence the silk production and these showed significant variations among the three varieties of Daba ecorace of *A. mylitta*. Average single cocoon weight for Daba trivoltine (DT), Daba bivoltine (DB) and Daba wild (DN) were 8.3 ± 0.58, 10.63 ± 0.35 and 12.57 ± 0.88 g, respectively. Similarly average single shell weight was 1.45 ± 0.18, 1.83 ± 0.23 and 2.18 ± 0.56 g in the above populations, respectively. It is well documented that the tasar silkworm *Antheraea mylitta* shows considerable variation within and between populations in different quantitative parameters and voltinism (Thangavelu & Sinha, 1993; Kar, Srivastava &Naqvi, 2000; Srivastava et al., 2002). The variability in the cocoon characters observed in the present study agrees with these findings. The nature grown wild cocoons were superior with a robust built whereas this was lowest in DT among all.

ISSR profiles

The band profiles generated by ISSR primers showed considerable polymorphism among the populations and example of the same with UBC-840 (Figure 1) clearly exhibit variability among the

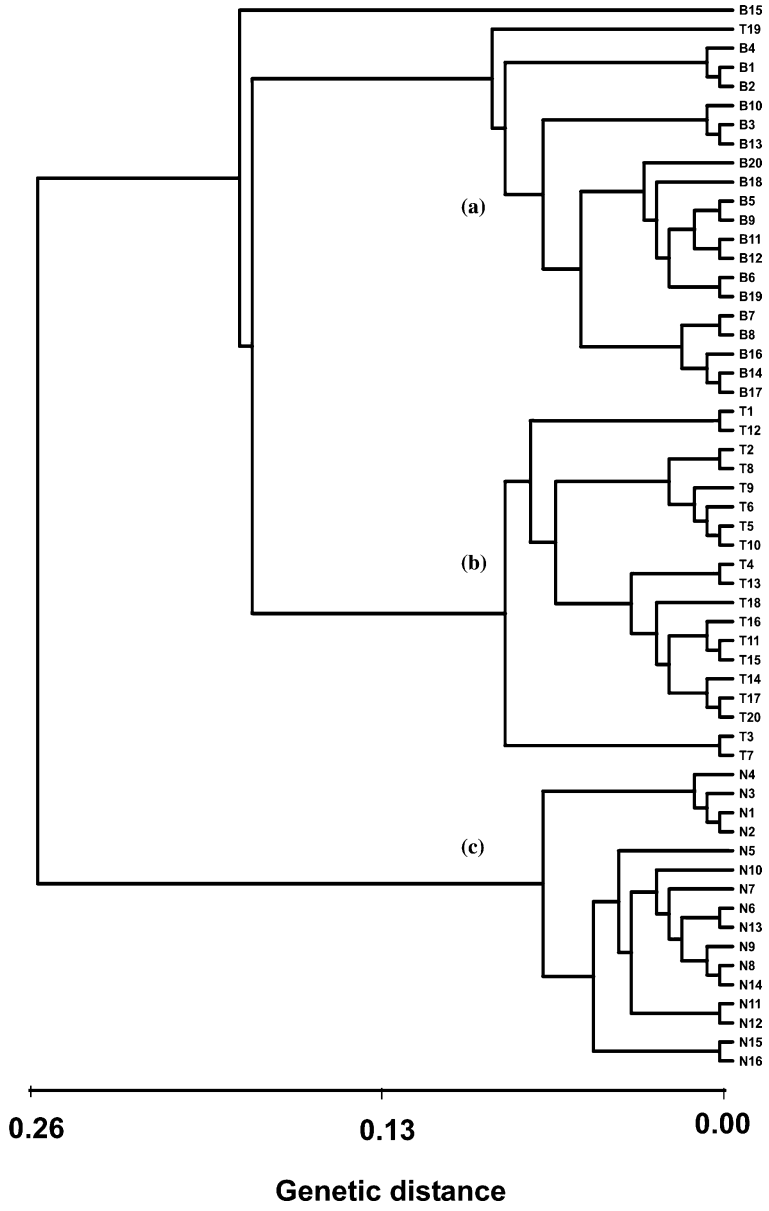

Figure 1. Dendrogram based on UPGMA (Dice's coefficient), showing phenetic relationship among 56 individuals, a, b and c denote semi-domestic Daba bivoltine, trivoltine and natural wild populations. B, T and N denote the individuals of bivoltine, trivoltine and nature grown Daba populations, respectively.

semi-domestic (DB, DT) and wild populations (DN). The 10 ISSR primers generated a total of 138 bands, of which 136 were polymorphic thus showing 98.55% polymorphism among the tasar silkworms. The bands were in the range of ~250–3000 bp. The number of bands generated by individual primers varied from 5 (UBC811, in DT) to 16 (UBC 807 in DN) (Table 1). The extent of polymorphism within a population also varied. DN population had considerably more polymorphism, ranging from 90.91 to 100%. The range of polymorphism in DB and DT was 62.5–100% and 77.78–100%, respectively (Table 1). Percentage polymorphic loci (no or 99% criterion) were found to be 58.696, 52.899 and 77.536 for DB, DT and DN populations whereas overall polymorphism

was 98.551. For 95% confidence limit criterion, the percentage polymorphic loci were calculated to be 57.971, 47.826 and 69.565 for these populations and 78.986 for all loci taken together. Maximum polymorphism was recorded for the primer UBC807 in all cases.

Genetic variability revealed through ISSR markers

A summary of genetic variation statistics for all loci is depicted in Table 2. Average number of alleles observed were 1.547 ± 0.114, 1.493 ± 0.115 and 1.791 ± 0.108 for DB, DT and DN, respectively, while it was 1.987 ± 0.016 when all populations were taken together. Similarly, in DB, DT and DN the average number of effective alleles were 1.292 ± 0.076, 1.248 ± 0.074 and 1.389 ± 0.089 while average for the total populations was 1.367 ± 0.043. When all populations were taken together, the observed number of alleles ranged from 1.385 (UBC825, DB and UBC811, DT) to 1.923 (UBC825, DN) while effective number of

Table 2. Observed and effective number of alleles in three populations for all ISSR primers

Primers	Alleles in Daba (BV)		Alleles in Daba (TV)		Alleles in Daba (wild)	
	Observed	Effective	Observed	Effective	Observed	Effective
UBC807	1.619 ± 0.108	1.270 ± 0.056	1.429 ± 0.112	1.189 ± 0.053	1.762 ± 0.94	1.382 ± 0.073
UBC809	1.667 ± 0.102	1.458 ± 0.075	1.667 ± 0.102	1.418 ± 0.080	1.800 ± 0.083	1.460 ± 0.083
UBC811	1.692 ± 0.098	1.232 ± 0.039	1.385 ± 0.109	1.161 ± 0.047	1.846 ± 0.067	1.373 ± 0.073
UBC812	1.583 ± 0.111	1.280 ± 0.064	1.500 ± 0.115	1.195 ± 0.058	1.833 ± 0.072	1.355 ± 0.073
UBC825	1.385 ± 0.109	1.268 ± 0.079	1.538 ± 0.114	1.283 ± 0.072	1.923 ± 0.037	1.385 ± 0.063
UBC834	1.818 ± 0.068	1.374 ± 0.065	1.727 ± 0.091	1.323 ± 0.070	1.818 ± 0.077	1.433 ± 0.078
UBC840	1.615 ± 0.109	1.250 ± 0.064	1.615 ± 0.109	1.211 ± 0.045	1.769 ± 0.092	1.332 ± 0.071
UBC855	1.385 ± 0.109	1.255 ± 0.072	1.538 ± 0.114	1.283 ± 0.062	1.538 ± 0.128	1.330 ± 0.091
UBC881	1.600 ± 0.110	1.313 ± 0.064	1.400 ± 0.110	1.140 ± 0.044	1.800 ± 0.083	1.529 ± 0.093
UBC884	1.529 ± 0.114	1.237 ± 0.057	1.529 ± 0.114	1.261 ± 0.060	1.706 ± 0.107	1.351 ± 0.085

Table 3. Population genetics parameters for the three populations of Daba ecorace of *Antheraea mylitta*

Genetic parameters	Daba bivoltine	Daba trivoltine	Daba wild	Overall
Percentage polymorphic loci (no or 99% criterion)	58.696	52.899	77.536	98.551
Percentage polymorphic loci (95% criterion)	57.971	47.826	69.565	78.986
Number of observed alleles: n_a	1.547 ± 0.114	1.493 ± 0.115	1.791 ± 0.108	1.987 ± 0.016
Number of effective alleles: n_e	1.292 ± 0.076	1.248 ± 0.074	1.389 ± 0.089	1.367 ± 0.043
Nei's gene diversity: H	0.180 ± 0.042	0.153 ± 0.041	0.235 ± 0.047	0.246 ± 0.021
Shanon's information index: I	0.278 ± 0.060	0.237 ± 0.060	0.361 ± 0.065	0.391 ± 0.027
Total genetic diversity (H_t) or expected heterozygosity	0.180 ± 0.033	0.153 ± 0.032	0.235 ± 0.033	0.253 ± 0.024
Sample genetic diversity (H_s) or average heterozygosity (direct count)	0.180 ± 0.033	0.153 ± 0.032	0.235 ± 0.033	0.232 ± 0.016
Estimated gene flow (Nm)				1.509
Gene differentiation (G_{ST})				0.251
Theta value (θP)(F_{ST})				0.308
95% confidence limit from bootstrapping over loci (1000 replications)	θP Upper			0.378
	θP Lower			0.240
Jacknifing over loci				0.308 ± 0.036
Exact test for population differentiation			χ^2	1491.81
			df	276
			P	0.0000

alleles ranged from 1.14 (UBC881, DT) to 1.529 (UBC881, DN) (Table 3).

Gene diversity in the sub-divided populations

The genetic diversity in the sub-divided populations is presented in Tables 3 and 4. The total genetic diversity (H_t) varied between 0.199 and 0.337 with a mean of 0.253. Within-population genetic diversity (H_s) ranged from 0.166 to 0.259 with a mean of 0.189. Gene differentiation (G_{ST}) ranged from 0.099 (UBC825) to 0.339 (UBC884) with a mean of 0.251. Nei's diversity ranged from 0.192 (UBC811) to 0.336 (UBC809) with a mean of 0.246 ± 0.021. When individual populations were considered, total genetic diversity in DB, DT and DN was 0.180 ± 0.033, 0.153 ± 0.032 and 0.235 ± 0.033, respectively. The sample genetic diversity was calculated to be the same as the total genetic diversity when the populations were taken separately. An alternative approach for calculating the within population variation is Shanon's diversity index (Bussel, 1999) which does not assume Hardy-Weinberg equilibrium. Average Shanon's diversity index was 0.278, 0.237 and 0.361 For DB, DT and DN and overall it was 0.391 (Table 3). Primer-wise minimum value was 0.326 (UBC811) and maximum was 0.503 (UBC809) (Table 4). Gene flow estimate (N_m) was estimated to be 1.509. Genetic analysis through TFPGA software revealed the theta value (θP) for overall population to be 0.308 which is equivalent to Wright's F_{ST}. Under 95% confidence limit from bootstrapping over all loci (1000 replications), θP Upper was 0.378 and θP Lower was 0.24. The jacknifing over loci also generated high value, 0.308 ± 0.036. The χ^2 value, calculated through exact test for population differentiation was found to be very high (1491.81; $P < 0.0000$).

Phylogenetic analysis

The average genetic distance based on Dice's coefficient is presented in Table 5. The genetic distance within DB, DT and DN was 0.382 ± 0.068, 0.381 ± 0.069 and 0.361 ± 0.051 while it was 0.504 ± 0.116 among the three populations. Genetic similarity and distance values

Table 4. Nei's F-statistics and Shanon's information index in the subdivided populations for all ISSR primers studied

Primer	Overall gene diversity (H_t)	Within sample gene diversity (H_s)	Gene differentiation (G_{ST})	Gene diversity (h)	Shanon's information index (I)
UBC807	0.202	0.176	0.116	0.196	0.334
UBC809	0.337	0.259	0.204	0.336	0.503
UBC811	0.199	0.166	0.124	0.192	0.326
UBC812	0.225	0.171	0.162	0.218	0.355
UBC825	0.214	0.187	0.099	0.208	0.330
UBC834	0.299	0.230	0.170	0.293	0.451
UBC840	0.208	0.171	0.152	0.205	0.352
UBC855	0.280	0.174	0.254	0.272	0.405
UBC881	0.256	0.193	0.197	0.244	0.391
UBC884	0.312	0.175	0.339	0.300	0.463

Table 5. Genetic distance (Dice's) within and among three populations of Daba ecorace of *Antheraea mylitta*

Genetic distance	Within Daba bivoltine	Within Daba trivoltine	Within Daba natural populations	Among all populations taken together
Average	0.382	0.381	0.361	0.504
SD	0.068	0.069	0.051	0.116
Maximum	0.618 DB6 & DB15	0.567 DT19 & DT13	0.524 DN8 & DN14	0.812 DT8 & DN5
Minimum	0.210 DB11 & DB12	0.206 DT11 & DT15	0.213 DN11 & DN12	0.206 DT1 & DT15

Table 6. Nei's original (1972) and unbiased (1978) measures of genetic identity and genetic distance among three populations of Daba ecorace of *Antheraea mylitta*

Populations	Daba bivoltine	Daba trivoltine	Daba natural populations
Daba bivoltine	–	0.9684 (0.9634)	0.8503 (0.8437)
Daba trivoltine	0.0321 (0.0371)	–	0.8459 (0.8397)
Daba natural populations	0.1622 (0.1699)	0.1674 (0.1747)	–

Genetic identity (above diagonal) and genetic distance (below diagonal); figures in parentheses indicate the unbiased measures values.

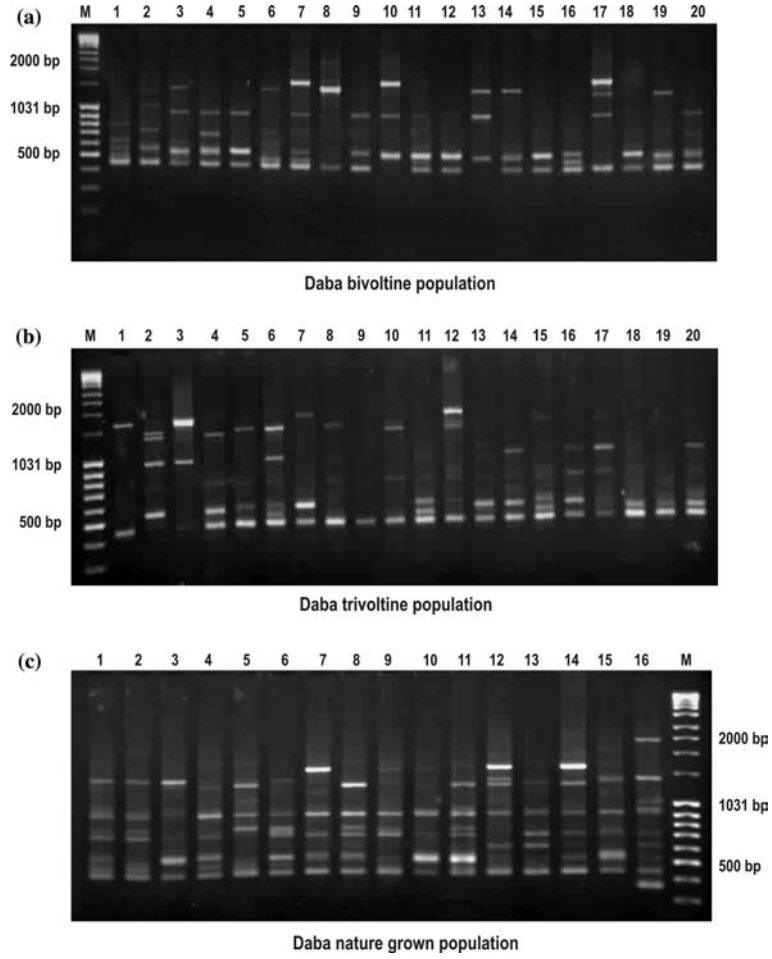

Figure 2. ISSR band profiles (UBC840) in semi-domestic Daba bivoltine and trivoltine (20 individuals each), and natural wild (16 individuals) populations, M denotes molecular size markers.

based on Nei's original (1972) and unbiased (1978) measures are presented in Table 6. DB and DT are found to have genetic distance of 0.1622 and 0.1674 with DN, while they themselves are at a distance of 0.0321. The dendrogram produced by UPGMA of the Dice's genetic distance matrices for all the populations (56 samples) is presented in Figure 2. Three major clusters were identified from the dendrogram where DB, DT and DN populations separated from each other (A, B, and C clusters). Only one individual (DB15) remained as a separate isolate. It may be noted that one tri-

voltine individual (DT19) remained clustered with Daba bivoltine populations. Considerable intra-population variability is found in all three populations. All the three populations have four intra-population clusters each. A high co-phenetic correlation coefficient of these clusters ($r = 0.89$; $p < 0.001$) obtained through the Mantel test revealed the robustness of the cluster analysis.

Discussion

The study clearly demonstrates that higher polymorphism is present in the natural populations of Daba ecorace compared to semi-domestic populations, which could be due to the greater chance of inbreeding in semi-domestic populations. The natural population, on the other hand, exploits the chance of random mating in the natural habitat, where the moths have the chance of mating even with other ecoraces as the male moths of this species can fly miles in search of females. This higher polymorphism is further evident from the average number of observed (1.791) and effective alleles (1.389) in the wild population in comparison to the other two. This higher genetic variability present in the three populations of Daba supports the higher amount of phenotypic variability reported in the quantitative traits (Thangavelu & Sinha, 1993) which is, thus, not purely due to the environmental factors, as considered earlier, but is due to the variability accumulated at the genetic level over years. Since, the natural populations of outbreeding species are expected to hold higher genetic diversity than the inbreeding ones, the genetic polymorphism unraveled by the ISSR markers in these silkworms of Daba ecorace is thus, in the expected line. This observation further corroborates the findings of Chatterjee et al. (2004) in the Raily, another wild ecorace of *A. mylitta*, which feeds on *Shorea robusta* and is endemic to Bastar region of Chhattisgarh state in India.

The cluster analysis also revealed clear genetic difference among the populations. The dendrogram clearly separates the individuals of the three populations into separate clusters barring one or two individuals. The high co-phenetic correlation coefficient of these clusters ($r = 0.89$; $p < 0.001$) obtained through the Mantel test reveals that the clustering truly depicts the genetic variability present among the individual silkworms. The depth of the nodes clearly indicated that the intra-population distances are always less than the inter-population distances. The dendrogram further revealed the higher genetic distance of the wild population from the other two semi-domestic populations. This higher genetic distance shown by the wild population points to the possibility of further improvement in the quantitative traits of this ecorace through heterosis breeding between the wild and semi-domestic populations. The higher genetic variability present in the wild population could be regarded as an adaptive strategy for increasing the population fitness in a spatio-temporally heterogeneous and uncertain environment; and that the broad-niche (habitat generalist) species are characterized by high heterozygosity as compared with narrow-niche (habitat specialist) species. Several studies support this hypothesis (Hedrick et al, 1976; Hedrick, 1986). The high diversity found in Daba wild might have been maintained from generation to generation over years. The maintenance of genetic polymorphism in natural populations can also reflect the process of adaptation to environmental heterogeneity (Hedrick et al., 1976; Hedrick, 1986).

Genetic structure analysis is essential to understand the evolutionary processes such as gene flow, natural selection, and genetic drift taking place in a population. The moderate mean coefficient of genetic differentiation ($G_{ST} = 0.251$) observed among the populations indicated that the populations are in the verge of genetic differentiation as they have accumulated about 25% genetic variability among themselves. The θP value ranged from 0.24 to 0.378 under 95% confidence limit from bootstrapping over loci (1000 replications) further points to the moderate genetic differentiation among the populations. The gene flow estimate of 1.509 shows that gene flow among the populations is not very high. The exchange of genes between populations is known to homogenize allele frequencies between populations and determines the relative effect of selection and genetic drift. High gene flow between populations precludes local adaptation and will also impede the process of speciation (Barton & Hewitt, 1985), but an N_m value greater than 1.0 is considered necessary to prevent divergence resulting from genetic drift (Wright, 1951). The value of gene flow (N_m) < 1.0 (fewer than one migrant per genera-

tion into a population) or equivalently, a value of gene differentiation (G_{ST}) > 0.25 is generally regarded as the threshold quantities beyond which significant population differentiation occurs (Slatkin, 1987). The moderately low gene flow and high G_{ST} values suggest that the Daba (bivoltine) and Daba (trivoltine) populations are at the threshold of differentiation. Further genetic drift in these populations may result in the formation of sub-ecoraces genetically more different from original Daba natural population. Semi-domestication over long period of time and the selection pressure under man made rearing conditions (generally the farmers select the big and healthy cocoons for breeding though inbreeding generally reduces the cocoon size) might have resulted this genetic diversification of the semi-domestic populations.

The study revealed that the genetic diversity in semi-domestic populations is considerably reduced as compared to that of the wild population. Selection and inbreeding may have caused this departure of the wild populations as reported in brown sea mustard (Huh & Huh, 2002). Hence, the genetic variability present in the natural population of this precious ecorace is to be preserved and also to be used for breeding with the semi-domestic populations to maintain the genetic diversity and also to harness the hybrid vigor for economically important traits. Thus, the information generated in this study could be used for strategic planning to conserve this precious genetic material for its effective utilization in breeding program, as adopted in different crop plants (Birmeta, Nyborn & Bekele, 2004) to enhance the livelihood of the forest folks.

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