

## GENETIC DIVERSITY OF *PHYLLANTHUS EMBLICA* IN TROPICAL FORESTS OF SOUTH INDIA: IMPACT OF ANTHROPOGENIC PRESSURES

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**PADMINI, S., NAGESWARA RAO, M., GANESHAIYAH, K. N. & UMA SHAANKER, R. 2001.** Genetic diversity of *Phyllanthus emblica* in tropical forests of south India: impact of anthropogenic pressures. In the Indian subcontinent, extraction of non-timber forest products (NTFPs) is a major occupation of the forest dwelling and forest fringe communities. A substantial portion of their livelihood is derived from the extraction of NTFPs. Despite the widespread dependence, little is known of the possible impacts that such extraction has on the regeneration and genetic diversity of the species. We examined the impact of anthropogenic pressures on the regeneration and genetic diversity of *Phyllanthus emblica*, an important NTFP species, across increasing gradients of pressures (disturbance) at two sites in the deciduous forests of south India. Our studies showed that even under moderate levels of disturbance, there was a significant decrease in the regeneration of the species. Populations close to human settlements (likely to be impacted more), had a relatively less proportion of small size class individuals than those farther away (likely to be impacted less). Several seed and seedling features were significantly affected by disturbance. Isozyme analysis indicated a decrease in percentage heterozygosity of populations with disturbance at one of the forest sites. There was a genetic structuring of the populations due to disturbance. Populations close to settlements (and hence impacted) tended to cluster in a group and were separated from those farther away (not impacted). Thus our studies showed that anthropogenic pressures can adversely affect the regeneration and genetic diversity of NTFP species in tropical forests. Further studies might be required to identify critical levels of disturbance or pressures that a species can tolerate such that protocols for its sustainable extraction can be prescribed.

Key words: *Phyllanthus emblica* - anthropogenic pressures - disturbance - India - forests - NTFP - genetic diversity - regeneration - forest communities

**PADMINI, S., NAGESWARA RAO, M., GANESHAIYAH, K. N. & UMA SHAANKER, R. 2001.** Kepelbagaian genetik *Phyllanthus emblica* di hutan tropika di selatan India: dampak tekanan antropogenik. Di subbenua India, pengekstrakan hasil hutan bukan kayu (NTFP) merupakan pekerjaan utama bagi penduduk di kawasan hutan dan pinggir hutan. Sebahagian besar daripada nafkah mereka diperoleh daripada pengekstrakan NTFP. Meskipun begitu, hanya sedikit yang diketahui mengenai dampak pengekstrakan ini terhadap pemulihan dan kepelbagaian genetik spesies tersebut. Kami mengkaji dampak tekanan antropogenik terhadap pemulihan dan kepelbagaian genetik *Phyllanthus emblica*, spesies NTFP yang penting sepanjang kecerunan tekanan (kerusakan) yang semakin meningkat di dua tapak hutan daun luruh di selatan India. Kajian kami menunjukkan bahawa walaupun berada di paras kerusakan yang sederhana, terdapat pengurangan yang bererti dalam pemulihan spesies.

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Populasi spesies yang berdekatan dengan penempatan (mungkin lebih terimpak) mempunyai individu bersaiz kecil yang kurang secara relatif berbanding dengan populasi yang lebih jauh (mungkin kurang terimpak). Beberapa ciri biji benih dan anak benih dipengaruhi dengan bererti oleh kerosakan. Analisis isozim menunjukkan pengurangan peratus keheterozigotan populasi dengan penambahan kerosakan di salah sebuah tapak hutan. Terdapat penstrukturan genetik bagi populasi akibat kerosakan. Populasi yang hampir dengan penempatan (dan dengan itu terimpak) cenderung untuk berkelompok dalam satu kumpulan dan terpisah daripada populasi yang berada jauh (tidak terimpak). Oleh itu kajian kami menunjukkan bahawa tekanan antropogenik memberikan kesan buruk terhadap pemulihan dan kepelbagaian genetik bagi spesies NTFP di hutan tropika. Kajian lanjut mungkin diperlukan untuk mengenal pasti tahap kritikal kerosakan atau tekanan yang dapat ditanggung oleh sesuatu spesies supaya protokol bagi pengestrakan secara berterusan dapat ditentukan.

## Introduction

A large proportion of the population in the tropics depends directly on forest resources for their subsistence as well as commerce. In India, approximately 50 million people living in and around forests are dependent upon non-timber forest product (NTFP) species for their subsistence living (NCHSE 1987). Over 5000 of the 16 000 species of flowering plants in India are reported to be used as NTFP (CSIR 1986). Obviously concern has been expressed over the sustainability of extraction of these resources from the forests. Though the impacts of the extraction and other anthropogenic pressures such as fire, grazing, etc., on forest dynamics and regeneration are recently being addressed (Kruckeburg & Rabinowitz 1985, Daniels *et al.* 1995, Hegde *et al.* 1996, Murali *et al.* 1996, Shankar *et al.* 1996, Shaanker *et al.* 1997, Ganeshiah & Shaanker 1998), we only have a limited understanding of these impacts on the genetic diversity of the populations of NTFP species (Shaanker *et al.* 1997). This understanding is crucial for developing strategies for the sustainable harvesting of these resources by the forest dwelling communities (Shaanker *et al.* 1997).

In this paper we examined the impacts of anthropogenic pressures including extraction of fruits on the seedling recruitment, regeneration and genetic diversity of populations of *Phyllanthus emblica*, an important NTFP species, in tropical forests of south India. We discussed the results in the light of the need to identify threshold levels of disturbance that the species can tolerate such that the protocols for its sustained management can be formulated.

## Materials and methods

### *The tree*

*Phyllanthus emblica* (synonym: *Emblca officinalis*; family: Euphorbiaceae) is a wind-pollinated and animal-dispersed tree species (the fruits are eaten by large mammals such as elephant, gaur, deer, monkey, etc.) occurring in the dry deciduous forests of central and southern India. The trees flower in February–March and mature their fruits in November–December. The fruit, a berry with six nutlets, is a rich source of vitamin C and forms an integral component of ayurvedic medicines (Nadkarni 1976, Shaanker & Ganeshiah 1997). Trees begin to bear fruits after 3–4 years when they

attain a dbh of > 5 cm (Shankar *et al.* 1996). The fruits are harvested for pickle and medicinal purposes and are reported to contribute significantly to the household economy of the indigenous people (Hegde *et al.* 1996).

### *Site description*

The study was conducted at two sites, namely Biligiri Ranganswamy Temple Wildlife Sanctuary (BRT) (11° 40'–20° 09' N and 77° 05'–77° 15' E, Karnataka State) and Mudumalai Wildlife Sanctuary (MWS) (11° 32'–11° 43' N and 76° 22'–76° 45' E, Tamil Nadu State) in south India. Forests at these sites have a long history of association with human activities and hence are subjected to anthropogenic pressures such as harvesting, fire, grazing, etc.

At BRT, an indigenous tribe, the Soligas, live within the forest and depend upon a number of non-timber forest product species for their subsistence and cash economy (Ganeshaiyah & Shaanker 1998). At MWS, the indigenous tribes have been evicted from the forests since the last 30 years and are rehabilitated outside the forest. These tribes along with other communities who have migrated from nearby towns and villages, actively extract NTFPs from the forest.

At both sites a number of NTFP species (approximately 20–25) are extracted from the sanctuary for subsistence and commerce. However the predominant ones are *P. emblica*, *Terminalia bellirica*, *T. chebula*, *Acacia sinuata* and *Sapindus emarginata* (Murali *et al.* 1996, Ganeshaiyah & Shaanker 1998). The extraction of the fruits of *Phyllanthus* commences at the onset of winter in mid-November and continues until mid-February. The fruits are either individually picked from the trees or beaten to the ground from where they are then picked. Not infrequently, the fruit bearing branches are lopped and then the fruits extracted.

### *Sampling design*

At each forest site three levels of anthropogenic pressures based on the levels of extraction and proximity to human settlement were identified, namely, least (control), mildly and highly disturbed areas. At BRT, the highly disturbed area is located 1 km from the major BRT settlement. The area is frequently trespassed by the people of BRT and other settlements and thus is evidently highly impacted. The mildly disturbed area is located 3 km from a Soliga settlement, while the least disturbed area is about 6–7 km away from this settlement.

At MWS, the highly disturbed area is located at the site of rehabilitation of the evicted forest dwellers. This area is highly impacted through a number of anthropogenic pressures including extraction of NTFPs, grazing, fire and felling. The mildly disturbed area is situated about 5–6 km from the site of rehabilitation. The least disturbed area was located in an adjoining forest in the Bandipur Tiger Reserve about 6–7 km away from the rehabilitation site. Due to its protected status, extraction of NTFPs is illegal in this area.

The study was conducted across these gradients of disturbance at each of the forest sites. As an index of the extent of disturbance (due to anthropogenic pressures), the proportion of cut and broken stems was estimated at each of the disturbance levels for both sites (Murali *et al.* 1996). Transects (n = 5) of length 1 km and width 10 m

were laid at each of the three disturbance levels at each forest site. The number of cut and broken stems (due to human activities) was recorded for each transect and expressed as proportion of the total number of trees (> 10 cm dbh) in the transect.

#### *Seed and seedling vigour parameters*

Fruits from randomly chosen trees ( $n = 20$  trees) from the transects (total transect area at each disturbance level was 5 ha) of each of the disturbance levels from both sites, were collected and sun dried. Following this seeds were harvested from the dried fruits and weighed. A known number (for sample size refer to caption of Figure 1b) of seeds were sown in Petri dishes lined with moistened filter paper and the per cent germination of seeds was recorded. Seeds that did not germinate were categorised into those that (1) showed signs of predation, indicated by damage of seeds or by the emergence of maggots and (2) showed no signs of predation. From this count we computed the per cent seed predation. The root growth of seedlings was recorded six days after germination and the seedling vigour computed as product of root growth and per cent germination of seeds (Abdul Baki & Anderson 1973).

#### *Isozyme analysis*

Fruits ( $n = 20-25$ ) from 25–30 randomly chosen trees from the transects were collected from each of the three disturbance levels at each site. The fruits were sun dried and the seeds separated and stored under ambient conditions. A random sample of seeds from each of the disturbance level were sown in Petri dishes lined with moistened filter paper and allowed to germinate at 30 °C and 75% relative humidity. Seedlings (5–7-day-old) were extracted in modified Chase buffer containing 0.01 M EDTA, 0.001 M KCl, 0.002 M MgCl<sub>2</sub>, 4% PVP-40, 1% PEG, 1% PVPP, 0.027 mM NADP, 0.003 M NAD, 0.3 M DTT, and 10% glycerol in 0.2 M phosphate buffer, pH 7.5 (Murawaski & Bawa 1994) and centrifuged. The supernatant was absorbed on Whatman No. 3 filter paper wicks and stored at -70 °C. The wicks were loaded onto 10% starch gels (starch hydrolysed from Sigma Inc., USA) and electrophoresed at 4 °C at 150–200 volts and 50 mA current for about 7 hours. The gel buffer was 0.05 M histidine-HCl containing 1.4 mM EDTA (pH 7) and the tray buffer contained 0.125 M Tris buffer brought to pH 7 with 1 M citric acid (Cheliak & Pitel 1984). After electrophoresis, the gels were horizontally sliced and stained using agar-overlay method (Wickneswari & Norwati 1992). Twenty-four enzyme systems were stained, of which only seven enzymes, namely isocitrate dehydrogenase (IDH), menadiione reductase (MR), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGDH), phosphogluco isomerase (PGI), phosphogluco mutase (PGM) and triose phosphate isomerase (TPI) yielded good stainability. However, of these only four (6-PGDH, PGM, PGI and IDH) yielded consistent and reliable staining patterns and hence chosen for the diversity analysis.

#### *Data analysis*

The isozyme data was analysed by (1) phenotypically scoring the isozyme bands, and (2) genetically interpreting the bands.

## Phenotypic scoring and analysis

For this analysis we treated every band as an electromorph; presence of a band was scored as 1 and its absence as 0. Nineteen unique electromorphs were recovered from the four enzyme systems analysed. The electromorph data was subjected to principal component analysis (Ludwig & Reynolds 1986, Shaanker & Ganeshiah 1997) to examine the dispersion of individuals belonging to the different disturbance levels.

## Genotypic scoring and analysis

Only three (6-PGDH, PGI and IDH) of the four enzymes could be unambiguously interpreted genetically. The genetic analysis was therefore restricted to these enzymes only. The data was subjected to population genetic analysis following POPGENE (Version 1.2 Microsoft for Windows). The measurements of genetic diversity included allele frequency, effective number of alleles, per cent polymorphic loci, and per cent observed and expected heterozygosity. We also computed Nei's genetic distance among the populations of various disturbance levels. A dendrogram representing the genetic distance between the populations was computed using the UPGMA method (Nei 1972). Finally an estimate of gene flow among the populations ( $N_m$ ) was computed from the multilocus value of  $F_{st}$  following POPGENE. We also assessed the sensitivity of alleles to disturbance by computing the difference in allele frequency between the control and disturbed populations (Shaanker *et al.* 1997).

## Results

**Disturbance gradients:** Within each site, the disturbance index (DI) increased with the proximity of populations sampled to human settlements (Figure 1a) indicating that the regimes of disturbance chosen for the study reflected increasing levels of anthropogenic pressures. The average levels of disturbance were, however, higher at MWS than at BRT hills.

**Seed and seedling parameters:** Seedling vigour measured as a product of seed germination and root growth, decreased with increase in disturbance at both sites (Figure 1d) suggesting the possible occurrence of inbreeding depression at the highly disturbed or harvested areas at both the forest sites. There was a significant increase in the infestation of seeds with disturbance (Figure 1c). At BRT the average seed weight decreased from control to disturbed areas, while there was no such pattern at MWS (Figure 1b).

**Regeneration:** At both sites, the proportion of individuals < 10 cm dbh was significantly reduced with increase in disturbance (Figure 2). At MWS, for instance, seedlings and saplings constituted nearly 80 per cent of the population in the control or least disturbed area in contrast to only 6 per cent in the highly disturbed area.

**Phenotypic analysis:** Principal component analyses based on the electromorph data indicated a clear separation of individuals corresponding to the three disturbance levels at both sites (Figures 3a & b). This suggests a structuring of the populations across the gradients of disturbance. The first three axes accounted for 45 and 47.5 per cent of the variability at BRT and MWS respectively. The major contribution to

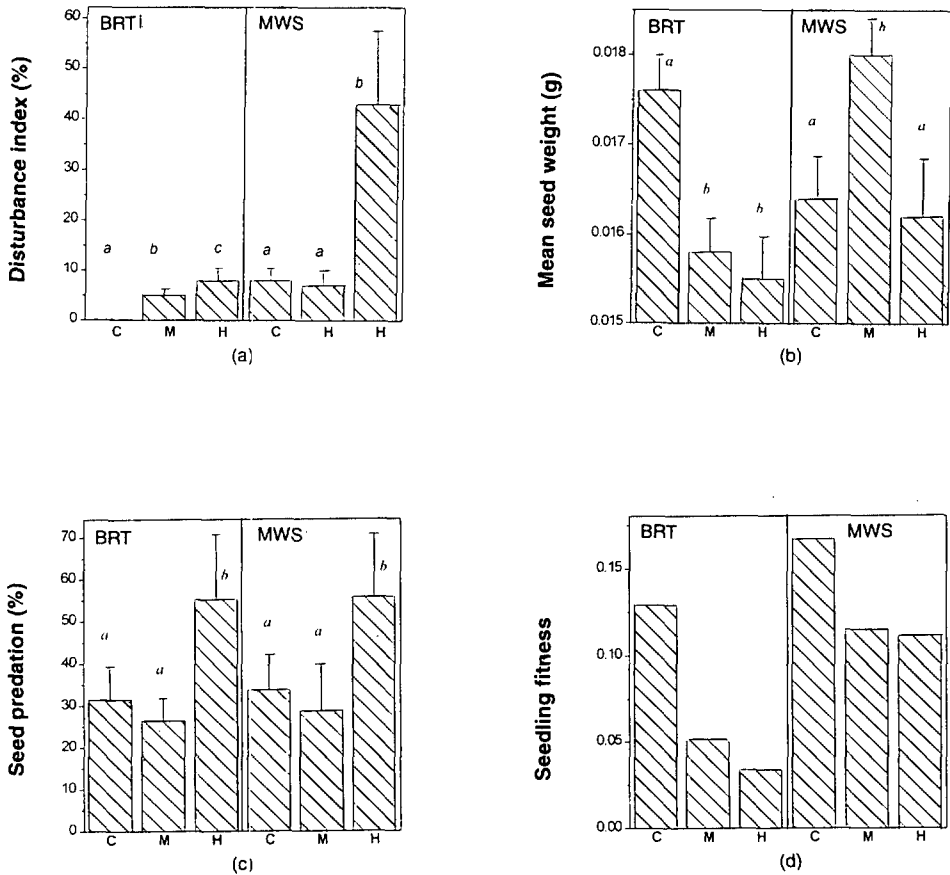
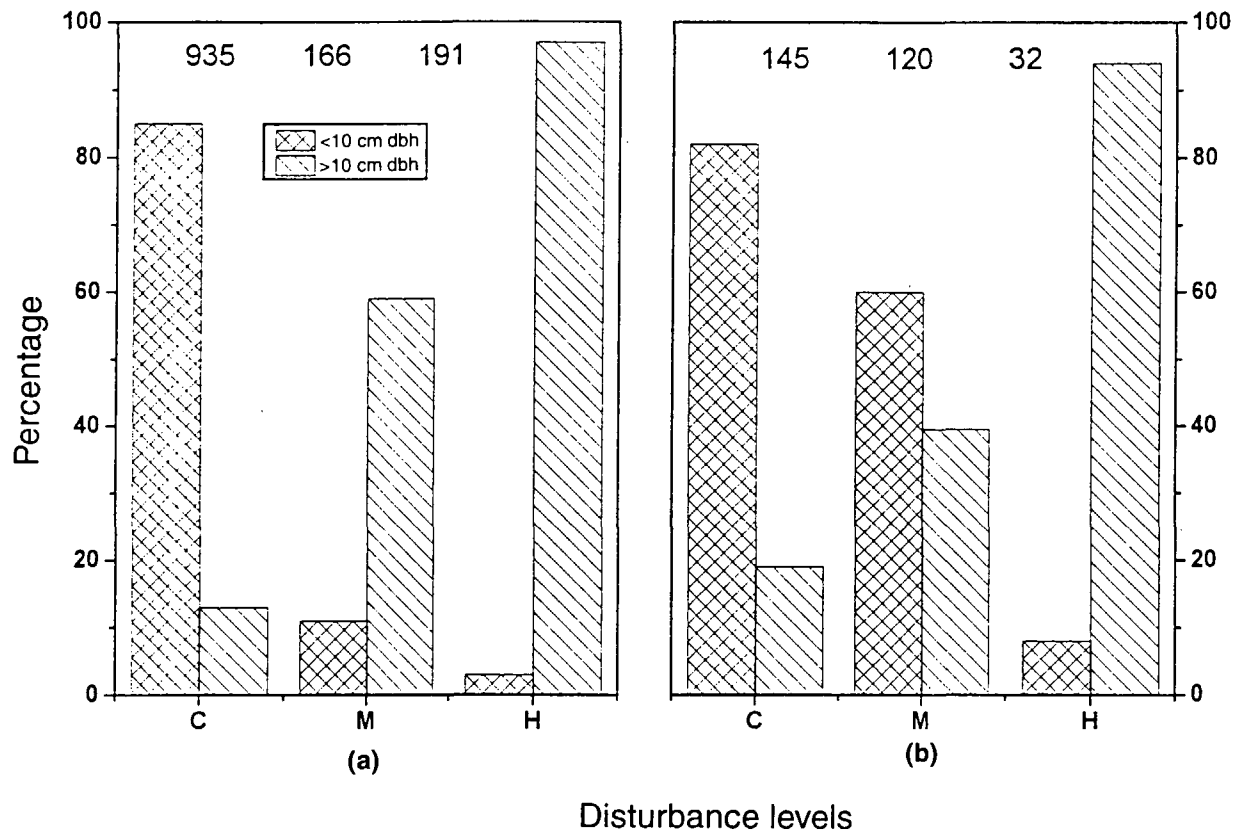
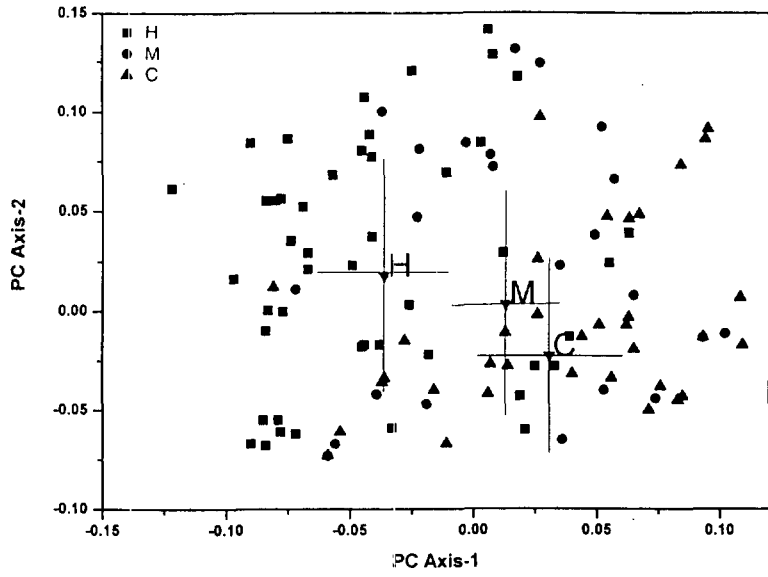


Figure 1. Disturbance levels

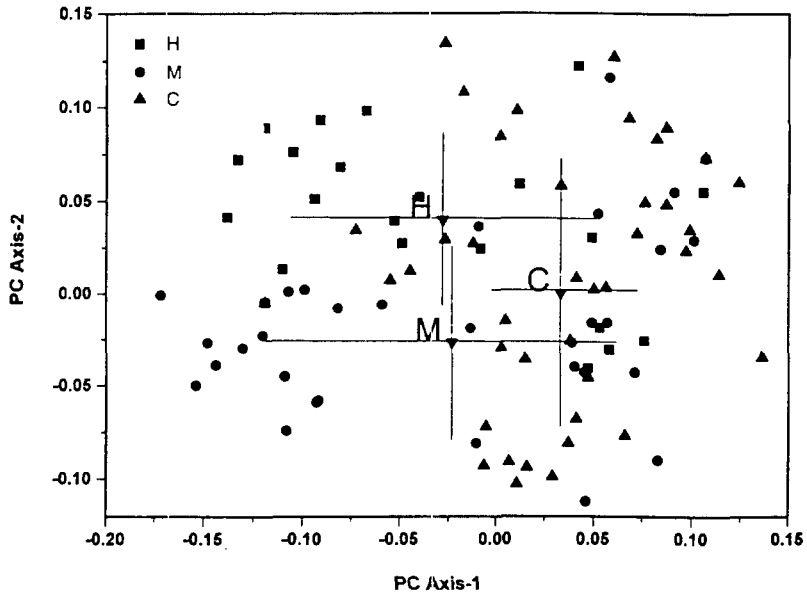
- Disturbance index (DI) of the areas selected for the study at BRT and MWS. C = control, M = mildly disturbed and H = highly disturbed. Means followed by dissimilar letters are significantly different ( $t$ -test,  $p < 0.05$ ).
- Mean weight of seeds of *Phyllanthus emblica* in the control (C), mildly disturbed (M) and highly disturbed (H) areas at BRT and MWS. Means followed by dissimilar letters are significantly different ( $t$ -test,  $p < 0.05$ ). Sample numbers of seeds sown at BRT: C = 70, M = 80, H = 56 and at MWS: C = 80, M = 80, H = 70.
- Per cent predation of seeds in control (C), mildly disturbed (M) and highly disturbed (H) areas at BRT and MWS. Means followed by dissimilar letters are significantly different ( $t$ -test,  $p < 0.05$ ). The sample numbers are as given in Figure 1b.
- Seedling vigour of *Phyllanthus emblica* in the control (C), mildly disturbed (M) and highly disturbed (H) areas at BRT and MWS. The sample numbers are as given in Figure 1b. (Seedling vigour was computed based on the mean germination percentage and the mean radicle length for each disturbance regime.)



**Figure 2.** Percentage seedlings and saplings (< 10 cm dbh) and adults (> 10 cm dbh) at the various disturbance levels at (a) BRT and (b) MWS. C = control, M = mildly disturbed and H = highly disturbed. Numbers refer to total number of individuals censused in five transects of 1 km × 10 m.



(a)



(b)

**Figure 3.** Principal component analysis (PCA) of populations of *Phyllanthus emblica* from the three disturbance regimes at (a) BRT and (b) MWS. The standard deviations from the mean for both axes for each of the populations are depicted: C = control, M = mildly disturbed and H = highly disturbed. The PCA is based on the electromorph frequencies.



the principal component loading at BRT was from the electromorphs 6-PGDH1, 6-PGDH3 and PGI3, while at MWS it was from 6-PGDH1, PGI5, PGI1 and MRI.

**Genetic analysis:** The genetic analysis involved three loci showing diallelic (6-PGDH and PGI) and triallelic (IDH) variations (Table 1). All the three loci were polymorphic for all the populations studied. There was no clear pattern of decrease in the genetic variability parameters with disturbance at BRT. However, at MWS several parameters such as effective allele number ( $n_e$ ) as well as observed and expected heterozygosity decreased with increase in disturbance (Tables 2a & b). The percentage observed heterozygosity decreased from 0.619 in the least disturbed area to 0.425 in the highly disturbed area ( $t$ -test,  $p < 0.05$ ). At MWS, all populations had more heterozygotes than expected, but this was true only for the mildly disturbed population at BRT.

The mean Nei's genetic distance among the populations at BRT was 0.0435 and that at MWS was 0.0363 (Tables 3a & b). At both sites, the genetic distance was least between the mildly and highly disturbed populations compared to that between the disturbed and control populations. The relatively smaller genetic distance among the populations from MWS was also reflected in the higher estimate of gene flow ( $N_m$ ) among these populations (6.29) compared to that among populations from BRT (4.35). UPGMA cluster analysis based on Nei's genetic distance indicated that at both sites, populations from the mildly and highly disturbed areas clustered together and were separated from the control populations (Figure 4a). Intriguingly, a pooled cluster analysis including the populations from both sites showed that populations from the respective disturbance regimes clustered together (Figure 4b). In other words, it appeared that the populations of *Phyllanthus* are affected nearly similarly by the anthropogenic pressures at both sites.

We examined the pattern of change in the frequency of alleles due to disturbance by computing the differences in allele frequency between the control and the disturbed populations (Figure 5). A few of the alleles tend to be adversely affected by disturbance with a lowered frequency in the disturbed populations. On the other hand, a few other alleles actually showed increased frequency. In general, the direction and pattern of change in the frequency of alleles seemed to be common for the two sites.

## Discussion

Our studies showed that anthropogenic pressures, including harvesting of the fruits of *P. emblica* have significantly reduced the recruitment and regeneration of the species. At both BRT and MWS the seedling vigour decreased from the least disturbed to the highly disturbed populations. In particular, populations close to human settlements (highly disturbed) seemed to suffer a high rate of seed predation compared to populations farther away. The increased predation in areas close to settlements could probably be due to the pestilence load from the agricultural crops in and around the settlements. The populations in the disturbed areas seemed to have poor recruitment of seedlings due to increased predation and low seedling vigour. The populations close to human settlement, and hence more disturbed, had a relatively less proportion of small-sized class individuals compared to those farther away (and hence less disturbed). These results suggest that harvesting, along with other anthropogenic pressures, can severely limit the ecological sustainability of the

**Table 1.** Allele frequencies of populations of *Phyllanthus emblica* from various disturbance levels in BRT and MWS

Locus	Allele	BRT				MWS			
		Control	Mildly disturbed	Highly disturbed	Mean	Control	Mildly disturbed	Highly disturbed	Mean
6-PGDH	a	0.34	0.4727	0.54	0.4559	0.5143	0.5652	0.5	0.5230
	b	0.66	0.5273	0.45	0.5441	0.4857	0.4348	0.5	0.4770
PGI	a	0.62	0.8455	0.9091	0.8039	0.5286	0.7174	0.8621	0.6897
	b	0.38	0.1545	0.0909	0.1961	0.4714	0.2826	0.1379	0.3103
IDH	a	0.04	0.0364	0	0.0294	0.0857	0	0.0345	0.0460
	b	0.84	0.7273	0.9545	0.8039	0.8857	0.8261	0.8276	0.8506
	c	0.12	0.2364	0.0455	0.1667	0.0286	0.1739	0.1379	0.1034

**Table 2a.** Genetic diversity parameters of populations of *Phyllanthus emblica* from different disturbance levels at BRT

Disturbance levels	$n_a$	$n_e$	I	P loci (%)	$H_o$ (%)	$H_e$ (%)
Control	2.33 ± 0.577 <sup>a</sup>	1.698 ± 0.2723 <sup>a</sup>	0.6116 ± 0.0719 <sup>a</sup>	100	0.3200 ± 0.2800 <sup>a</sup>	0.4076 ± 0.1076 <sup>a</sup>
Mildly disturbed	2.00 ± 0 <sup>b</sup>	1.4255 ± 0.4860 <sup>b</sup>	0.3929 ± 0.2634 <sup>b</sup>	100	0.3636 ± 0.4810 <sup>a</sup>	0.2551 ± 0.222 <sup>b</sup>
Highly disturbed	2.33 ± 0.577 <sup>a</sup>	1.6847 ± 0.3207 <sup>a</sup>	0.6051 ± 0.1512 <sup>a</sup>	100	0.4182 ± 0.4821 <sup>a</sup>	0.6052 ± 0.1213 <sup>c</sup>

$n_a$  = mean number of alleles,  $n_e$  = effective number of alleles, I = Shannon index, P loci = polymorphic loci,  $H_o$  = observed heterozygosity,  $H_e$  = expected heterozygosity

Means followed by dissimilar superscripts (column wise) are significant at  $p < 0.05$  (t-test).

**Table 2b.** Genetic diversity parameters of populations of *Phyllanthus emblica* from different disturbance levels at MWS

Disturbance levels	$n_a$	$n_e$	I	P loci (%)	$H_o$ (%)	$H_e$ (%)
Control	2.33 ± 0.577 <sup>a</sup>	1.7510 ± 0.4240 <sup>a</sup>	0.6013 ± 0.1573 <sup>a</sup>	100	0.6190 ± 0.5378 <sup>a</sup>	0.4076 ± 0.1708 <sup>a</sup>
Mildly disturbed	2.00 ± 0 <sup>b</sup>	1.6839 ± 0.2817 <sup>ac</sup>	0.5807 ± 0.1120 <sup>a</sup>	100	0.4493 ± 0.4355 <sup>ac</sup>	0.4035 ± 0.1048 <sup>ac</sup>
Highly disturbed	2.33 ± 0.577 <sup>a</sup>	1.5762 ± 0.3704 <sup>bc</sup>	0.5468 ± 0.1460 <sup>a</sup>	100	0.4253 ± 0.5165 <sup>bc</sup>	0.3503 ± 0.1403 <sup>bc</sup>

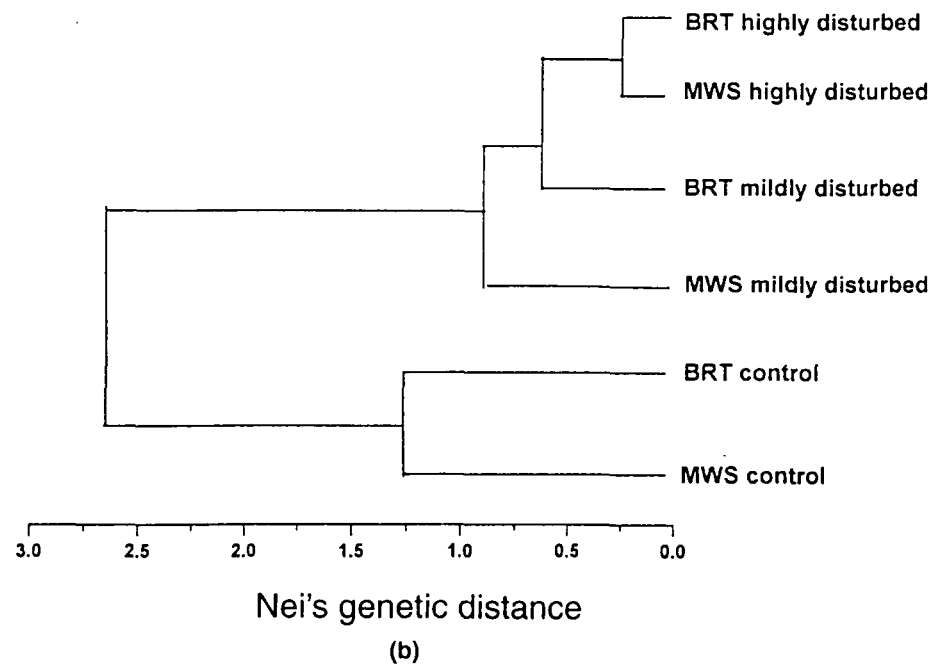
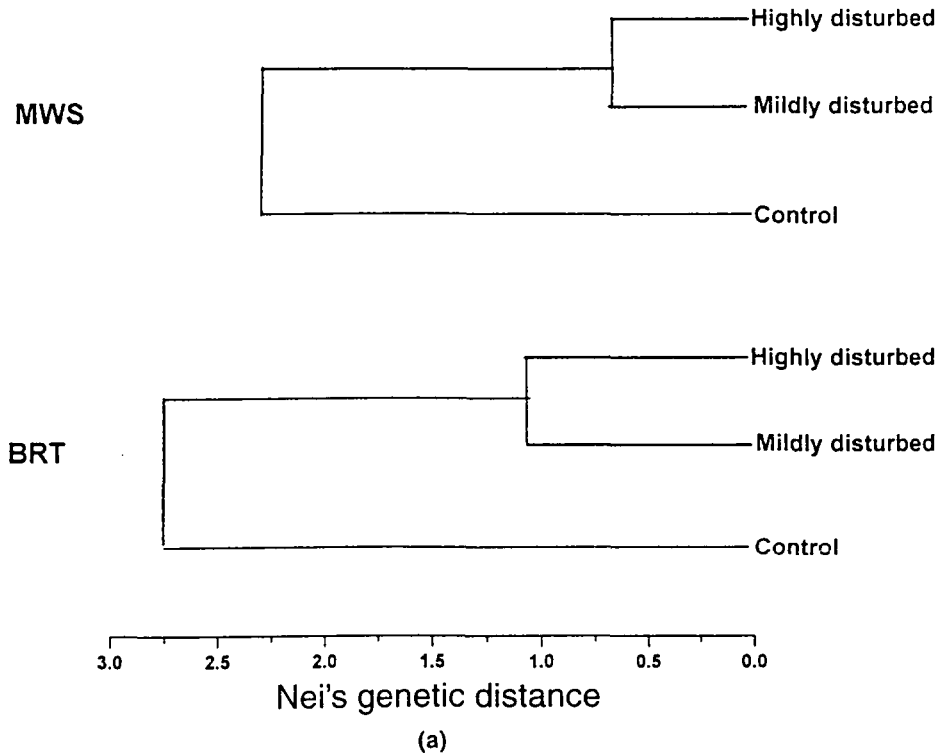
Abbreviations are as presented in the footnote of Table 2a

**Table 3a.** Genetic distance among the populations from the three disturbance levels at BRT

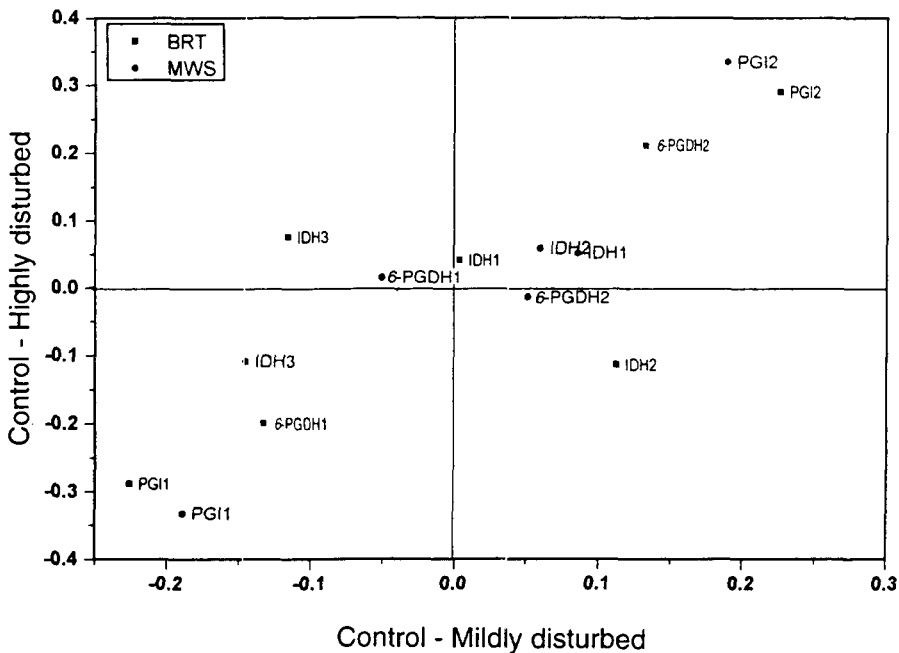
Disturbance regimes	Control	Mildly disturbed
Mildly disturbed	0.0632	-
Highly disturbed	0.0460	0.0214

**Table 3b.** Genetic distance among the populations from the three disturbance levels at MWS

Disturbance regimes	Control	Mildly disturbed
Mildly disturbed	0.0305	-
Highly disturbed	0.0651	0.0133



**Figure 4.** Cluster analysis of (a) the populations from the three disturbance levels at BRT and MWS and (b) pooled over all populations from BRT and MWS. Nei's (1972) genetic distance coefficients and UPGMA was used. Genetic distance was computed based on allele frequencies.



**Figure 5.** Relation between differences in allele frequencies between control and mildly disturbed as well as control and highly disturbed populations of *Phyllanthus emblica* from BRT and MWS

populations. Further, the low recruitment and regeneration may also lead to a spatial structuring of the populations and eventually perhaps in their genetic structuring as well.

We examined the genetic diversity parameters of the populations along the disturbance gradients at the two forest sites. At BRT, we found no clear pattern of change in the population genetic diversity parameters following increase in disturbance. However, at MWS there was a significant decrease in the percentage of observed and expected heterozygosity from the least disturbed to the highly disturbed populations. The effective number of alleles required to maintain the current levels of heterozygosity also decreased with disturbance. Thus it appeared, though only from the MWS site, that increased anthropogenic pressures could lead to a loss of genetic variability of the population. However, these results are only suggestive and are constrained by the analysis being limited to only three gene loci. Several workers have recently addressed the impacts of disturbance or logging on the percentage heterozygosity of populations of tree species (Burchert *et al.* 1997, Kowit *et al.* 1997, Wickneswari *et al.* 1997). For instance Kowit *et al.* (1997) reported that the percentage of expected heterozygosity increased with disturbance levels for pioneer species, and decreased or remained unchanged for non-pioneer species. *Phyllanthus emblica* is wind-pollinated and animal-dispersed and could be regarded as a mid-successional species. Though disturbance in the habitat lead to a relative opening of the canopy (Shaanker *et al.* 1997) and thereby facilitating pollen flow, patchiness of the population with a severe reduction in the population size as revealed by the present study, could have restricted the gene flow in this species.

At both forest sites, there appeared to be genetic differentiation of the populations along the gradients of disturbance suggesting that individuals from different

disturbance regimes may have assorted themselves with specific allelic configurations. Such structuring can result directly from the impact of various anthropogenic pressures on the populations and/or from the geographic separation of the populations. However, the geographic differentiation of the populations is less likely because of the following. First, geographical distances separating the various disturbance levels are nearly equal (about 5–7 km) and the populations are embedded in the forest matrix without significant fragmentation. These features could have fairly facilitated the flow of genes through pollen (by wind) or seeds (through animal dispersers such as monkeys and deers). Second, genetic distance and cluster analysis of populations at both sites showed that populations from the mildly and highly disturbed area tend to group together in a cluster while that from the control form a separate cluster. Indeed a pooled cluster analysis involving all the populations from both sites showed that there was a preferential clustering of populations from the respective disturbance regimes. Thus it appeared that the observed genetic differentiation of populations of *P. emblica* may be influenced more by anthropogenic pressures rather than by geographic separation.

Our study underscores the necessity to maintain harvesting intensities and anthropogenic pressures that would sustain the population of the species such that it provides for sufficient genetic variability to be maintained. Perhaps this study provides the first of the attempts to understand the patterns of genetic diversity within and among populations of tropical forest trees as influenced by anthropogenic pressures in peninsular India. Such study needs to be extended to address similar questions on a range of disturbance levels and species, such that the threshold levels of disturbance for different species can be estimated and protocols for the sustainable management of the non-timber forest product species be proposed. However, since the complexity of the ecosystems and the genetic systems studied are very high it is imperative that future studies include a large number of populations with an adequate sampling of individuals within populations.

### Acknowledgements

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