Kasetsart J. (Nat. Sci.) 47 : 8 - 22 (2013)

Population Genetic Structure of Two Forest Grasses with Contrasting Life Forms, *Arundinella setosa* and *Garnotia tenella* in Thailand

Atchara Teerawatananon [1, 2], Trevor R. Hodkinson [3] and Sarawood Sungkaew [2, 4, 5,*]

ABSTRACT

Plastid microsatellite DNA markers and Nei's (1978) unbiased genetic distance, the unweighted-pair-group method of analysis and principal components analysis were employed to assess the intra- and interpopulation genetic variations of two forest grasses with contrasting breeding systems. Seven haplotypes and 4 groups from 11 populations of *Arundinella setosa*, and 11 haplotypes and 4 groups from 8 populations of *Garnotia tenella*, were defined. The high value of genetic diversity within the populations suggested that they consist of multiple genets and indicates nonclonality in individuals of clonally spreading *A. setosa*. For the annual *G. tenella*, it suggested that the populations are not fixed for a plastid DNA type and that seed-mediated geneflow is occurring. The haplotype distributions reflect patterns of plant dispersal via seed. The low population genetic differentiations among populations (G_{ST}: *Arundinella* = 0.049; *Garnotia* = 0.155) suggested that the most variation in haplotypes is distributed within populations. The high value of genetic diversity but low gene flow estimates of both grasses, found in Northern Thailand, could be affected by topographical barriers and habitat fragmentation but no obvious correlations of biogeographical distribution were found that were consistent with previously defined Thai floristic regions. However, groupings in the analyses provided weak evidence for a general east-west divide.

Keywords: *Arundinella*, *Garnotia*, chloroplast DNA, forest grasses, haplotypes, gene diversity, microsatellites, population genetics

INTRODUCTION

In Thailand, the genetic diversity of forest tree species and the influence of forest clearance and fragmentation on their diversity have been studied (Pakkad *et al*., 2004). However, there has been little research on the population genetics of grasses in Thailand while research has been extensive in other areas of the world. For example, Hodkinson *et al.* (2002) used amplified fragment length polymorphism (AFLP) to study genetic variation in the panicoid grass genus *Miscanthus* Anderss. from Southeast Asia, Gao (2004) studied the population genetic structure of wild rice *Oryza*

1 Natural History Museum, National Science Museum, Technopolis, Pathum Thani 12120, Thailand.

2 Center for Advanced Studies in Tropical Natural Resources, Kasetsart University, Bangkok 10900, Thailand.

3 School of Natural Sciences, Botany Building, Trinity College Dublin, University of Dublin, Dublin 2, Ireland.

4 Department of Forest Biology, Faculty of Forestry, Kasetsart University, Bangkok 10900, Thailand.

5 Center of Excellency for Bamboos, Kasetsart University, Bangkok 10900, Thailand.

* Corresponding author, e-mail: fforsws@ku.ac.th

Received date : 13/02/12 Accepted date : 07/12/12

rufipogon Griff. from microsatellite variation, and McGrath *et al.* (2007) studied genetic diversity in the pooid grass *Lolium perenne* L. in Europe using plastid microsatellite markers.

In order to investigate the biogeographical patterns of population genetic structure on Thai native forest grass, two grass species, *Arundinella setosa* var. *setosa* (hereafter *A. setosa*) and *G. tenella* which have contrasting life forms (*Arundinella setosa* is perennial, while *G. tenella* is annual), were chosen as model species to study the genetic variation at the intrapopulational level, as well as between populations using the plastid microsatellite markers developed and tested for grasses by McGrath *et al.* (2006). More specifically, the aims of the study were to: (1) amplify polymorphic cpDNA microsatellite regions of *A. setosa* and *G. tenella* collected from Thailand and test the diversity of the markers of McGrath *et al.* (2006) on these two genera; (2) investigate the extent of haplotype diversity of *A. setosa* and *G. tenella* in Thailand to determine if the diversity was structured into geographically meaningful patterns and asses if this was affected by forest fragmentation; and (3) compare diversity and differentiation in the grasses in relation to their life forms (annual versus perennial).

MATERIALS AND METHODS

Plant material and DNA extraction

Plant material was obtained from a number of sources listed in Table 1. In total, 55 samples were collected from 11 populations of *A. setosa* and 40 samples from 8 populations of *G. tenella*. Five individuals of each population were sampled from natural habitats distributed across Thailand (see Figures 1 and 3). Plants were randomly sampled, depending on availability, over a geographical range of at least 50 km for

Table 1 Plant material used in the microsatellite study and summary of genetic variation based on three SSR loci within populations of *A. setosa* (A1-A11) and *G. tenella* (G1-G8).

Population	Voucher specimens	Location (Thailand)	*N*	*h*	*P*
Arundinella					
A1	*AT & SS* 293 to 297	Suan Phung, Ratchaburi, South-Western	4	0.160	33.3
A2	*AT & SS* 419 to 423	Khao Sung, Prachuap Khiri Khan, South-Western	5	0.187	33.3
A3	*AT & SS* 435 to 439	Khao Kiew, Chon Buri, South-Eastern	4	0.160	33.3
A4	*AT & SS* 465 to 470	Nam Nao, Phetchabun, North-Eastern	5	0.213	66. 7
A5	*AT & SS* 474 to 478	Phu Phan, Sakol Nakhon, North-Eastern	5	0.267	66. 7
A6	*AT & SS* 522/1 to 522/5	Sai Yok Noi, Kanchanaburi, South-Western	4	0.107	33.3
A7	*AT & SS* 535 to 537 & 539 to 540	Wat Chan, Chiang Mai, Northern	4	0.160	33.3
A8	*AT & SS* 547 to 551	Ang Khang, Chiang Mai, Northern	3	0	0
A9	*AT & SS* 555 to 558 & 560	Mae Rim, Chiang Mai, Northern	3	0	0
A10	*AT & SS* 589 to 593	Ban Tak, Tak, Northern	4	0.107	33.3
A11	*AT & SS* 595 to 599	Huai Kha Khaeng, Uthai Thani, South-Western	5	0.213	66.7
		Mean	4.2	0.143	24.22
Garnotia					
G1	*AT & SS* 162/0 to 4 & 162/6	Khao Yai, Nakhon Ratchasima, Eastern	5	0.320	66.67
G2	*AT & SS* 316/1 to 4 & 316/6 to 7	Khao Kra Jome, Ratchaburi, South-Western	5	0.267	66.67
G3	*AT & SS* 700 to 704	Thong Pha Phum, Kanchanaburi, South-Western	5	0.213	66.67
G4	*AT & SS* 700 to 704	Um Phang, Tak, Northern	5	0.213	33.33
G5	*AT & SS* 741 to 746	Doi Suthep, Chiang Mai, Northern	5	0.267	66.67
G6	*AT & SS* 804 to 806 & 808 to 809	Phu Hin Rong Kla, Pithsanulok, Northern	4	0.107	33.33
G7	*AT & SS* 822 to 826 & 827	Phu Kradueng, Loei, North-Eastern	6	0.373	100.00
G8	*AT & SS* 872 to 876	Na Yong, Trang, Peninsular	5	0.320	66.67
		Mean	5	0.26	62.5

A = *Arundinella setosa*; G = *Garnotia tenella*; AT = A. Teerawatananon; SS = S. Sungkaew; *N* = Total number of alleles detected for the three loci; *h* = Gene diversity; *P* = Percentage of polymorphic loci.

each population. Fresh leaves were dried with silica gel in order to rapidly desiccate the material and reduce DNA degradation (Chase and Hills, 1991). Total genomic DNA was extracted using a modified hot CTAB method of Doyle and Doyle (1987) as outlined in Hodkinson *et al.* (2007).

Microsatellite amplification and data analysis

Five chloroplast simple sequence repeat primers (*TeaSSR1*, *TeaSSR2*, *TeaSSR3*, *TeaSSR4*, and *TeaSSR5*) as described in McGrath *et al.* (2006) were used. Polymerase chain reaction (PCR) studies were carried out using the Applied Biosystems GeneAmp® PCR System 9700 (Applied Biosystems; Foster City, CA, USA) (Life Technologies; Carlsbad, CA, USA). Initial denaturation was for 5 min at 95 ºC, followed by 35 cycles of 1 min at 95 ºC, 1 min at 60 ºC, 1 min at 72 ºC and 10 min final extension at 72 ºC. The PCR products were sized using an ABI PrismTM 310 automated DNA sequencer (Applied Biosystems; Foster City, CA, USA) and alleles were assigned using Genescan® Analysis Software version 3.1 and Genotyper® Software Version 3.7 (Applied Biosystems; Foster City, CA, USA). Primers were labeled with FAM, JOE or NED dyes (fluroscein derivatives that have specially resolvable fluorescent spectra) and an internal standard, GeneScan 500 Rox, was included for sizing the alleles.

Haplotypes were constructed by combining the allele data for each locus. Analysis into the extent of diversity within and among the populations from each location was carried out using a statistical software program for population genetics (Popgene 32, Version 1.3.1; Yeh *et al.*, 1999), including genetic diversity statistics and estimates of population genetic differentiation and gene flow for haploid data (Nei, 1973, 1987). Haplotypes were grouped using Nei's (1978) genetic distances and unweighted-pair-group method of analysis (UPGMA) using Popgene 32 Version 1.3.1, and PCA for population genetic distances (using GENALEX6; Peakall and Smouse, 2006). The geographic patterns of haplotype frequencies of each population were also represented by 'donut charts' (modified pie charts) on a map of Thailand to show their geographical sources. Analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992; in GENALEX 6) was also carried out on the data set to test the variation within and among the populations of regions, detected using UPGMA.

RESULTS

Five regions of simple sequence repeat were analyzed to reveal microsatellite variation in 55 individuals from 11 populations of *A. setosa* and 40 individuals from 8 populations of *G. tenella*. Three out of the five marker loci (*TeaSSR3*, *TeaSSR4* and *TeaSSR5*) were genotyped, while the other two (*TeaSSR1* and *TeaSSR2*) could not be reliably amplified for all samples, but their allele size range was 227–230 and 196–202 bp, respectively (see also Table 2). *TeaSSR3* and *TeaSSR4* are mononucleotide repeat markers (poly A markers) and it is likely that the ranges in allele size recorded for these markers were due to losses or gains of an 'A' nucleotide at this site. For *TeaSSR5*, the repeat type reported by McGrath *et al.* (2006) was 'CTT'. For this marker, the length variation may have occurred elsewhere in the amplification product since the observed variation in fragment length indicated single nucleotide steps and not three bases as would be expected with a CTT repeat. Sequencing of the product would be required to obtain such information.

Genetic diversity within and among the *A. setosa* populations

In total, seven alleles were found in the *A. setosa* populations, three alleles were found in *TeaSSR3*, while *TeaSSR4* and *TeaSSR5* each generated two alleles. The average overall diversity (*h*) within *A. setosa* populations detected using each of the alleles was estimated, according to Nei (1973), to be 30.5%. *TeaSSR*3 detected 28.6%,

TeaSSR4 detected 24.9% and *TeaSSR5* detected 38% (Table 2). The extent of chloroplast diversity within and among *A. setosa* populations, estimated using H_S (within population genetic diversity), H_T (total genetic diversity = gene diversity) and G_{ST} (estimate of the genetic differentiation among the populations), is shown in Table 2. The mean value of H_S was 0.291 compared to 0.305 for H_T. Thus, most variation was explained by variation within populations and the mean value of G_{ST} (0.049) was correspondingly low. The mean estimation of the gene flow (N_M) was 10.65.

A summary of genetic diversity parameters for each population is given in Table 1. Phu Phan (A5) was the most diverse population among the 11 populations (N = 5, h = 0.267), followed by Nam Nao (A4) and Huai Kha Khaeng (A11). *Arundinella setosa* populations were divided into four groups according to floristic region (Northern, North-Eastern, South-Eastern and South-Western; Smitinand, 1958). The results of gene diversity between these *A. setosa* groups indicated that *A. setosa* from Northern Thailand was the most diverse group with 100% polymorphic loci and gene diversity of 0.34, compared to the least diverse (South-Eastern group) with 33.3% polymorphic loci and 0.16 gene diversity.

In total, seven haplotypes were detected and mapped and are illustrated in Figure 1. Nine of the 11 populations were polymorphic. The most common haplotype within the population was *Haplotype 1* which occurred at a frequency of 0.491. Ang Khang (A8) was found to be monomorphic for this haplotype. *Haplotype 2* was found in Suan Phung (A1) and Mae Rim (A9), with the latter population fixed for *Haplotype 2*. *Haplotype 3* was restricted to Khao Sung (A2). *Haplotype 6* was unique to Nam Nao (A4) and *Haplotype 7* was found to occur only in Ban Tak (A10) and Huai Kha Khaeng (A11). The H_S, H_T and G_{ST} values were estimated and compared for the regional groups (Northern, North-Eastern, South-Eastern and South-Western) to assess diversity and differentiation at a broader geographical scale (Table 3).

The structuring of *A. setosa* populations in Thailand was also examined using UPGMA based on Nei's (1978) unbiased genetic distance. The UPGMA tree is shown in Figure 2a. Four groups were formed. The PCA result was consistent with the UPGMA (Figure 2b) and divided the populations into the same major groups. Axis one and axis two accounted for 62.25 and 33.05% of the variance, respectively, with a

Table 2 Descriptive statistics, mean gene diversity estimates (Nei, 1973), Nei's (1987) genetic diversity statistics and estimates of overall gene flow per locus, of *A. setosa* and *G. tenella*.

Primer name	Sample size	Range of sizes detected (base pair)	Allele number	h	H_S	H_T	G_{ST}	N_M
A. setosa								
TeaSSR3	55	301–303	3	0.286	0.269	0.286	0.058	8.14
TeaSSR4	55	199–200	2	0.249	0.229	0.249	0.078	5.87
TeaSSR5	55	212–213	2	0.380	0.375	0.380	0.013	37.77
Mean	-	-	-	0.305	0.291	0.305	0.049	10.65
G. tenella								
TeaSSR3	40	300–302	3	0.524	0.440	0.524	0.160	2.627
TeaSSR4	40	199–201	3	0.184	0.160	0.184	0.129	3.368
TeaSSR5	40	211–213	3	0.659	0.555	0.659	0.158	2.675
Mean	-	-	-	0.455	0.385	0.455	0.155	2.734

h = Average overall diversity; H_S = Within population genetic diversity; H_T = Total genetic diversity (=gene diversity); G_{ST} = Estimate of the genetic differentiation among the populations; N_M = Estimate of gene flow.

cumulative variance of 95.29%. The geographic patterns of these groupings are illustrated in Figure 1. These groupings were tested for population structuring using AMOVA (Table 4). The majority of the variance was found within the populations (51%, $P \leq 0.001$), but there was also significant ($P \leq 0.001$) genetic structuring of *A. setosa* among regions, with 46% of the microsatellite variation.

Genetic diversity within and among the *G. tenella* populations

The genetic diversity within and among populations of *G. tenella* were also assessed using the three plastid microsatellite loci used for *A. setosa*. In total, nine different alleles were found in the Thai populations. Three alleles were found at each locus. The overall gene diversity (h) within *G. tenella* populations was estimated to be 45.5%. *TeaSSR3* detected 52.4%, *TeaSSR4* detected 18.4% and *TeaSSR5* detected 65.9% (Table 2). The extents of chloroplast diversity within and among *G. tenella* populations, estimated using H_S, H_T and G_{ST} are given in Table 2. The mean value of H_S was 0.385 and of H_T was 0.455. The mean value of G_{ST} was 0.155 and the mean estimation of N_M was 2.73.

A summary of the genetic diversity parameters is shown in Table 1 and Figure 3. Based on the number of alleles and h, Phu Kradueng (G7) was the most diverse population ($N = 6$, $h = 0.373$), followed by Khao Yai (G1) and Na Yong (G8). The results of the gene diversity values between *G. tenella* regional groups (Table 3) showed that *G. tenella* from the Northern group was the most diverse with 100% polymorphic loci and an H_T value of 0.52, compared to the two equally least variable regions (Eastern and Peninsular) with 66.67% polymorphic loci and an H_T value of 0.32.

In total, 11 haplotypes were identified. All populations were polymorphic. The most common haplotype within the population was *Haplotype 2* (frequency of 0.275). *Haplotype 6* and *Haplotype 7* were found to be unique to Um Phang (G4). *Haplotype 8* and *Haplotype 9* were

Table 3 Nei's (1987) genetic diversity and differentiation statistics and estimates of gene flow from four regions of *A. setosa* and from five regions of *G. tenella*.

Region	H_S	H_T	G_{ST}	N_M	N_A	P
A. setosa						
Northern	0.067	0.338	0.803	0.122	2.33	100
North-Eastern	0.240	0.247	0.027	18	1.67	66.67
South-Eastern	0.160	0.160	0*	0*	1.33	33.33
South-Western	0.167	0.298	0.441	0.633	2.33	100
Mean	-	-	-	-	1.95	75
G. tenella						
Northern	0.240	0.520	0.539	0.429	2.67	100.00
North-Eastern	0.240	0.373	0.357	0.900	2.33	100.00
Eastern	0.320	0.320	0*	-	1.67	66.67
South-Western	0.240	0.327	0.265	1.38	2.00	66.67
Peninsular	0.320	0.320	0*	-	1.67	66.67
Mean	-	-	-	-	2.07	80.00

H_S = Within population genetic diversity; H_T = Total genetic diversity (=gene diversity); G_{ST} = Estimate of the genetic differentiation among the populations; N_M = Estimate of gene flow; N_A = Mean observed number of alleles per locus; P = Percentage of polymorphic loci; * = Differentiation and gene flow are 0 for this group as it only contains one population and hence differentiation and gene flow cannot be calculated.

found only in Doi Suthep (G5) and *Haplotype 11* was restricted to Phu Kradueng (G7) as shown in Figure 3. Four groups were defined from the UPGMA based on the genetic distance (Figure 4a). PCA was consistent with the UPGMA (Figure 4b) and divided the populations into the same major groups as the UPGMA. Axis one and axis two accounted for 49.85 and 24.91% of the variance, respectively, with a cumulative variance of 74.77%. These haplotype groups were plotted on the map of Thailand to examine whether they had meaningful patterns from a geographical or ecological perspective (Figure 3). AMOVA analysis (Table 4) indicated that 40.81% of the variance was partitioned within population ($P \leq 0.001$) and 57.5% among regions.

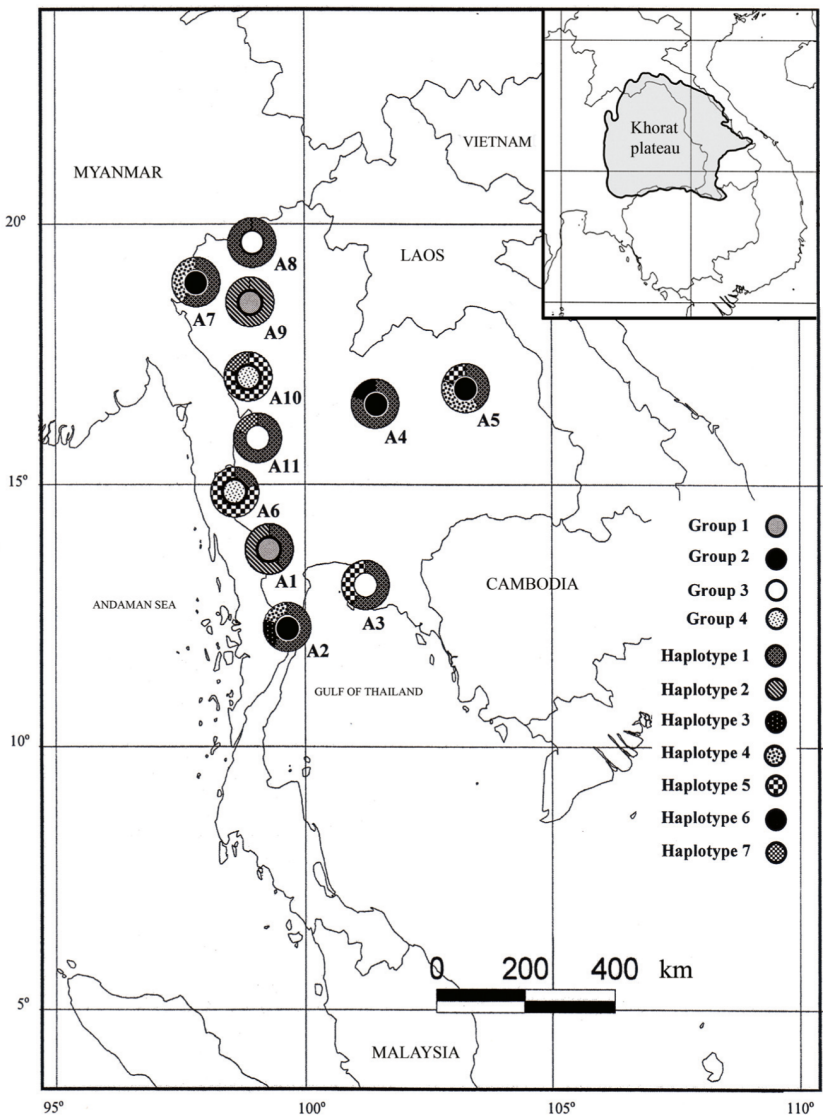

Figure 1 Map of Thailand with the proportions of haplotypes (outer circles), and overall patterns (inner circles) of *A. setosa* populations groupings according to Nei's (1978) unbiased genetic distance and unweighted-pair-group method of analysis. The inset shows the location of the Khorat plateau (shaded).

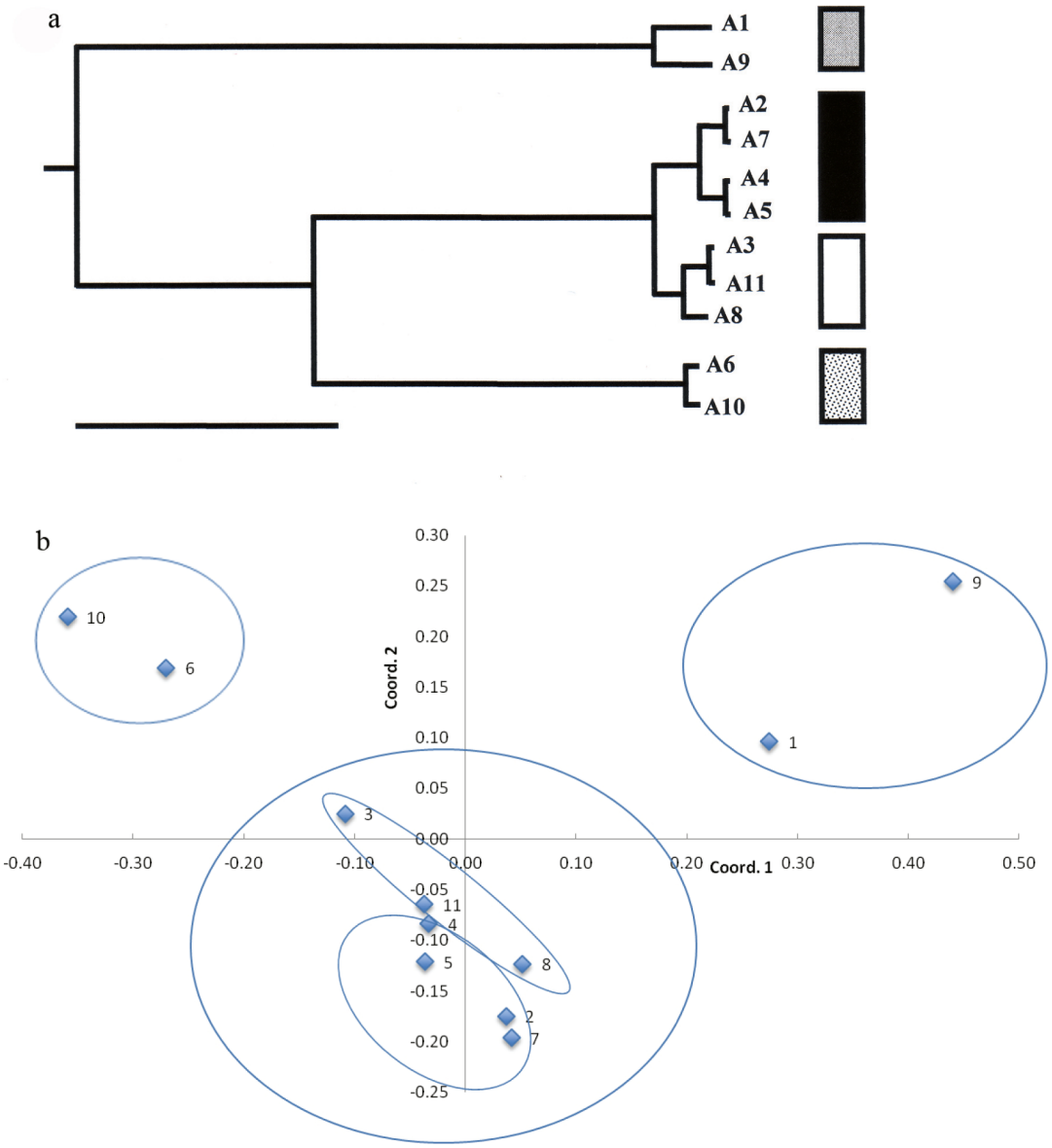

Figure 2 (a) Unweighted-pair-group method of analysis (UPMGA) dendrogram of *A. setosa* populations based on Nei's (1978) unbiased genetic distance. The scale bar below the tree indicates Nei's unbiased genetic distance of 5. Population numbers as in Table 1; (b) Principal component analysis results for *A. setosa* based on haploid population genetic distances. Axis 1 and 2 account for 62.25 and 33.05% of the variance, respectively. Numbers 1-11 represent the population numbers of *A. setosa* as in Table 1. The groupings are broadly congruent with the UPGMA analysis (Figure 2a).

DISCUSSION

Genetic diversity of *A. setosa*

The plastid DNA genotyping detected seven different haplotypes of *Arundinella* in Thailand. Despite the low number of loci screened, it was possible to detect considerable genetic variation within and among populations. It is important to interpret the findings with care due to the low number of markers but some conclusions can be made with respect to diversity, geography and putative clonality. According to population genetic theory which predicts that larger populations tend to maintain higher allelic diversity (Hamrick and Godt, 1996), the highest levels of genetic diversity in *Arundinella* based on the chloroplast microsatellite evidence were found in the Northern, South-Western and North-Eastern regions (Table 3), especially the Phu Phan and Nam Nao areas (Table 1), and would suggest that these might represent the largest effective population sizes of the species. However, within the Northern group, the Ang Khang and Mae Rim populations were the least diverse containing only one haplotype. Small, isolated populations may lose genetic diversity and become increasingly differentiated from other populations (see for example, Young *et al.*, 1996; Young and Brown, 1999). Ang Khang and Mae Rim are small isolated fragment areas. Ang Khang is located in an ecotone of deciduous dipterocarp forest and lower montane oak forest. The Mae Rim population is located in a small and isolated forest fragment of deciduous dipterocarp forest. Plastid DNA variation reflects the pattern of plant dispersal via seed. It is possible that the lack of genetic diversity in these small and more isolated populations could have resulted from a lack of gene flow (via seed dispersal) and increased genetic drift.

In addition, the *A. setosa* populations in the Northern group showed low H_S values (6.7%), high G_{ST} (80.3%) and low N_M (0.122), as shown in Table 3. The results are therefore consistent with a low level of seed migration between the populations. However, according to H_T, the total genetic diversity of the Northern group was the highest, which may have been because Northern Thailand has the largest forest area of all the regions (RDF, 2003). However, a considerable amount of geographical population genetic structuring has occurred. Differentiation between populations can be affected by many factors such as gene flow, genetic drift, vagility and dispersal, natural selection, physical barriers and environmental selection factors (Lowe *et al.*, 2004).

Two factors that may cause high population differentiation (G_{ST}) in the Northern group are topographical barriers (such as mountains) and habitat fragmentation. Although the presence of topographical barriers in Northern Thailand may have an effect on the gene flow in some

Table 4 Analysis of molecular variance (AMOVA) partitioning of genetic variation in 11 populations of *A. setosa* and 8 populations of *G. tenella* among regions, according to Nei's (1978) unbiased genetic distance, among populations within groups and within populations.

Source of variation	*Arundinella setosa*				*Garnotia tenella*			
	df	SS	% of total variance	P-value	df	SS	% of total variance	P-value
Among Regions	3	10.87	46.41	<0.001[a]	3	22.24	57.50	<0.001[a]
Among Pops/Regions	7	2.33	2.28	0.245[b]	4	2.5	1.68	0.39[b]
Within Pops	44	12	51.31	<0.001[c]	32	16.8	40.81	<0.001[c]
Total	54	25.2			39	41.5		

df = Degrees of freedom; SS = Sum of squares; [a] = ϕGT; [b] = ϕPG; [c] = ϕPT (significance of F_{ST} analogs (Phi values) where G=UPGMA/PCA defined groups, P=populations, T=total).

local regions, it may not be of major significance to *Arundinella* that is found throughout the mountainous region and which is not restricted to particular sites or altitudes. In Northern Thailand, many areas were first fragmented over 50 years ago due to shifting cultivation and hunting and other forms of development (for example, roads and human settlements, Dearden, 1996). Many areas in Northern Thailand are now protected, though the remaining forests are more highly fragmented and isolated. The effects of habitat fragmentation on wildlife in Northern Thailand were studied by Pattanavibool and Dearden (2002), and Pattanavibool *et al.* (2004). The studies indicated that the variation in the species abundance and species composition of wildlife, including birds

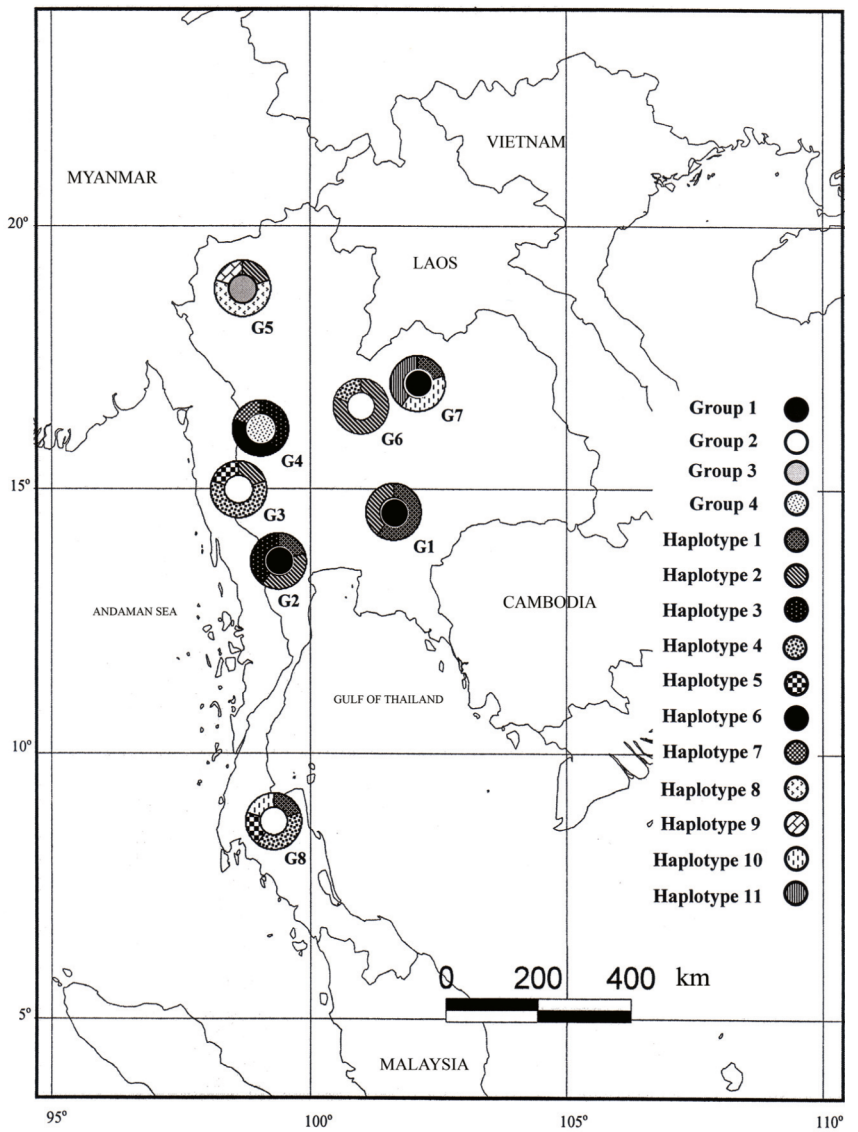

Figure 3 Map of Thailand with the proportions of haplotypes (outer circles), and overall patterns (inner circles) of *G. tenella* populations groupings according to Nei's (1978) unbiased genetic distance and unweighted-pair-group method of analysis.

and animals, are affected by forest fragmentation. Many animals such as ants, termites, birds and deer are considered as means of distributing grasses (Ridley, 1930; Chapman, 1996). Although there is no direct evidence, field observations strongly suggest that the natural dispersal agents of *A. setosa* seed are also animals, especially birds, rodents and mammals (A. Teerawatananon, pers. obs.). The awn-bearing lemma remains attached to the seed in *A. setosa* and forms an important part of the dispersal unit. Seeds can attach themselves to hooves, feathers and wool mechanically by

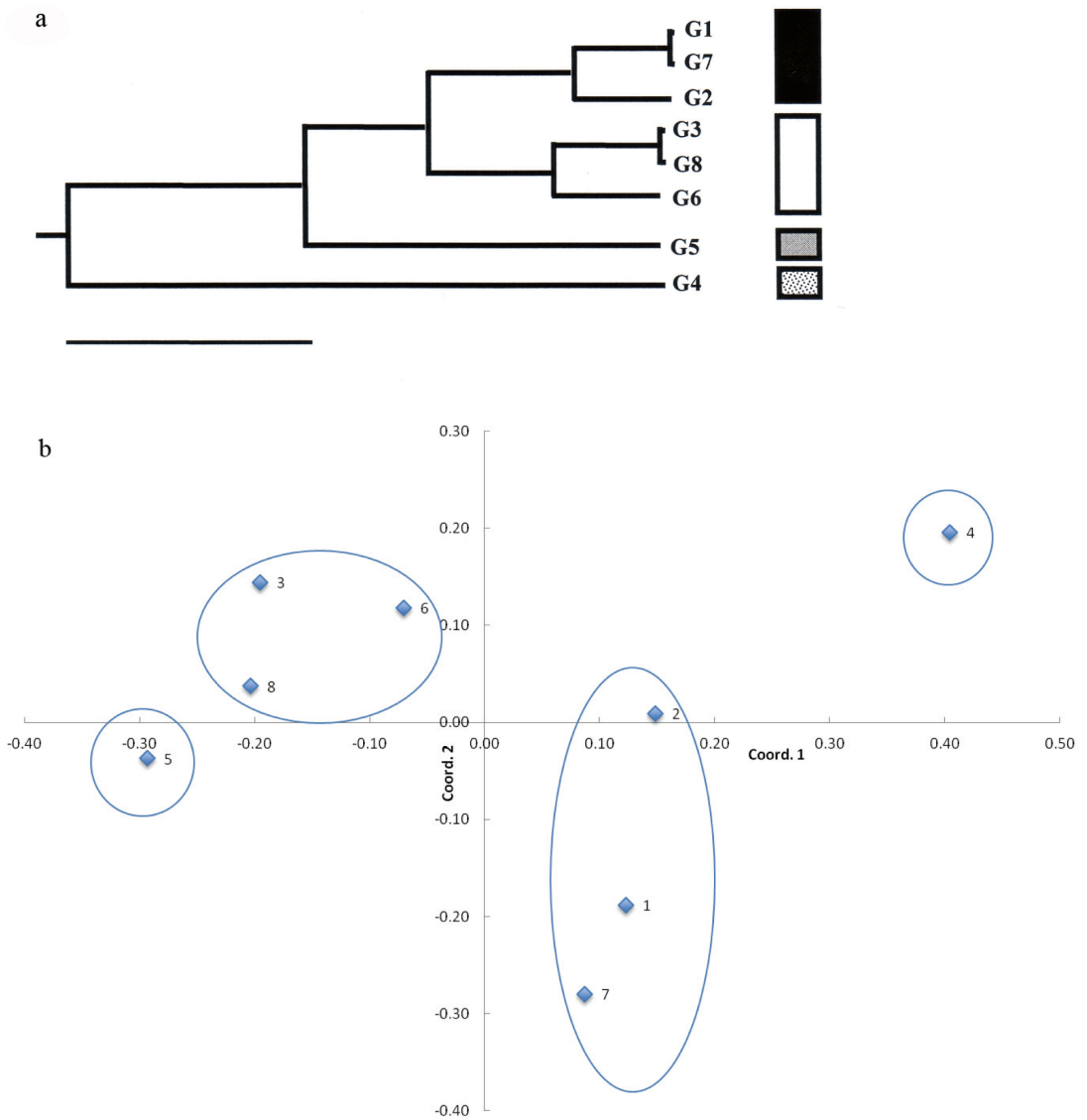

Figure 4 (a) Unweighted-pair-group method of analysis dendrogram of *G. tenella* populations based on Nei's (1978) unbiased genetic distance. The scale bar below the tree indicates Nei's unbiased genetic distance of 5. Population numbers as in Table 1; (b) Principal component analysis results for *G. tenella* based on haploid population genetic distances. Axis 1 and 2 account for 49.85 and 24.91% of the variance respectively. Numbers 1–8 represent the population numbers of *G. tenella* as in Table 1.

bristles, awns or trichomes (Razi, 1950) and can be transferred to new areas. Therefore, habitat fragmentation can reduce the dispersal ability of seed via reduced movement of wildlife species and hence this can become a limiting factor to seed-mediated gene flow. In the current study, this was considered to be a major contributor to the high G_{ST} values observed.

However, polyploidy is another explanation. *Arundinella setosa* was reported to show polyploidy variation by Larsen (1963) who discovered that there are three races of chromosomes within the species. The 32-chromosomed race is the group of specimens collected from the dry deciduous dipterocarp forest on Doi Sutep at altitudes between 450 and 500 m (Herbarium specimens of *Sørensen et al*. no. 4591 and 4810, housed in the Herbarium, University of Copenhagen, Denmark). The inflorescences are large and diffuse with dark violet spikelets. The others are 48- and 54-chromosomed races. Both races are represented by the specimens that were collected from higher altitude (for example, specimens of *Sørensen et al.* no. 4395 and 5415, also in the Herbarium, University of Copenhagen, Denmark). The inflorescences are shorter and less diffuse than the 32-chromosomed race and the spikelets are brown or light brown. Larsen (1963) also suggested that more work was required before the 32-chromosomed plants could be classified. However, *A. setosa* has been shown in other studies (Bor, 1955; Sun and Phillips, 2006) to be a variable species in terms of morphology (for example, the morphological key to the species reveals that *A. setosa* is a variable species). The differences in the types of inflorescence and spikelet are not good enough to separate taxa. In the current study, Mae Rim was the only population which has the morphological characters of the 32-chromosomed plant and it is monomorphic at all three loci. However, the population from Ang Khang which has the morphological characters of a 48- and 54-chromosomed plant, is also monomorphic, but is a different haplotype. It is not known what breeding barriers exist between the chromosome races reported by Larsen (1963), but it would be interesting to obtain plants for the future chromosome counts or ploidy estimation via flow cytometry.

The seven haplotypes of *A. setosa* detected were widely distributed across Thailand. The most common haplotype within *A. setosa* populations was *Haplotype 1* with a frequency of 0.49. This haplotype occurred in all populations except Ban Tak (A10) and Mae Rim (A9). The average number of haplotypes per population was two, and the maximum was three in the Phu Phan and Khao Sung populations. The genetic variation detected within *A. setosa* populations suggested that the populations consist of multiple genets. *Arundinella setosa* is a perennial species capable of extensive vegetative reproduction. The populations are not, therefore, composed of predominantly asexually reproduced individuals.

Overall, *A. setosa* showed values for H_S of 29.1%, H_T of 30.5% and G_{ST} of 4.9%. The estimate of N_M was 10.65 (Table 2). The results therefore revealed an overall low level of population genetic differentiation (most variation in haplotypes was distributed within and not among populations). This might be explained by a high level of migration between populations but also by the low sampling intensity and the low number of loci screened. McGrath *et al.* (2007) used chloroplast microsatellite markers to examine the genetic diversity of Irish *Lolium perenne* (perennial ryegrass) ecotypes, and recorded a low G_{ST} value (23.8%) among ecotypes. Their result indicated a high level of gene flow, via seed, had occurred. In the current study, the results were similar to the study on *Lolium*, but demonstrated an even lower level of differentiation. This would suggest that the movement of seed between the populations was relatively high. Grasses are wind pollinated and hence the potential for pollen-mediated gene flow is also high (not measured here). Based on the current study, it is predicted that population differentiation measured by nuclear markers would

infer less differentiation than the plastid markers (because they are biparentally inherited, Lowe *et al.*, 2004).

Genetic diversity of *G. tenella*

The total gene diversity values (and percentage of polymorphic loci per total number of alleles) of *G. tenella* showed that the Northern region populations sampled here were the most diverse (Table 3) and also indicated that Phu Kradueng, Khao Yai and Na Yong were particularly variable (Table 1); they could, on the basis of population genetic theory, represent the populations with the largest effective population size. The Northern group had the highest value for population differentiation (53.9%) and hence the lowest N_M value (0.429). As with *A. setosa* (discussed above), it is possible that habitat fragmentation might explain much of this pattern (Table 3).

A total of 11 haplotypes were detected. The most common haplotype within the *G. tenella* populations was *Haplotype 2*. This haplotype occurred in all populations except the Um Phang population (G4). The average number of haplotypes per population was three and the maximum was four in the Na Yong population (G8). All 11 haplotypes were widely distributed across Thailand with no obvious geographic trends in their pattern. The H_S value of the *G. tenella* populations was 38.5% (Table 2). H_T was estimated to be 45.5% and G_{ST} was estimated to be 15.5%, while N_M was 2.73. Therefore, as with *A. setosa*, most variation in haplotype was distributed within populations and not among populations. Seed of *G. tenella* is dispersed by wind, water, and animals (A. Teerawatananon, pers. obs.) and especially birds (Carlquist, 1967).

The overall G_{ST} values were, however, three times higher for *Garnotia* than *Arundinella* (15.5% versus 4.9%, respectively). This would suggest that there was less gene flow between *Garnotia* populations or that they were more susceptible to the effects of genetic drift. Perennials tend to have lower levels of G_{ST} than shorter-lived perennials or annuals (Hamrick and Godt, 1996; Godt and Hamrick, 1998; Nybom, 2004). This expected pattern was also found here. The highest overall value of differentiation among populations was found for the annual species, *G. tenella*. However, care is required with these broad comparisons as they are not being made between two closely related species (or groups). It is also not known if *Arundinella* and *Garnotia* can self fertilize. Furthermore, there is a high degree of regional variation in G_{ST} for both species (Table 3). For example, the Northern population of *Arundinella* has a much higher G_{ST} value than the Northern population of *Garnotia* (which is therefore opposite to the pattern seen over all populations).

Geographic distribution of *A. setosa* and *G. tenella* chloroplast haplotypes

In order to try and interpret the complicated geographical patterns recorded in the haplotypes of *A. setosa* and *G. tenella* in Thailand, the haplotypes were grouped according to Nei's (1978) unbiased genetic distance and UPGMA dendrograms constructed and PCA performed. The results of UPGMA and PCA were congruent. The populations can be divided into 4 major groups among 11 populations of *A. setosa* (Figure 2) and 4 major groups among 8 populations of *G. tenella* (Figure 4). For *Arundinella*, 51% of the total variance was partitioned within populations ($P \leq 0.001$) compared to 46% among regions of UPGMA/PCA-defined groups ($P \leq 0.001$). A similar pattern was found for *Garnotia* with 41% within the total variance partitioned population ($P \leq 0.001$) and 58% among the UPGMA/PCA-defined groups ($P \leq 0.001$). The overall patterns of *A. setosa* and *G. tenella* populations were compared to their geographic locations. In this study, there was no obvious relationship of biogeographical distribution in *A. setosa* and *G. tenella* with the Thai floristic regions (Smitinand, 1958) or UPGMA/PCA-defined regions. This

could have been because of the small number of samples per population used in this study. Another possibility could have been because there are only a few major geographical or ecological barriers to gene flow in Thailand. The studies by McGrath *et al.* (2007) on *Lolium* indicated that gene flow via seed was extensive over the geographic scale of Europe. The data presented here indicate that such gene flow is also likely in *Arundinella* and *Garnotia*. These two species are not restricted to Thailand and have a wide geographic distribution. This also supports the hypothesis that they are good dispersers which can tolerate a broad range of ecological conditions.

However, there is weak evidence from the groupings to suggest an east-west divide, with only Group 2 of *A. setosa* and Group 1 of *G. tenella* predominant in North-Eastern populations. On the other hand, some haplotypes were restricted to Northern, South-Western and Peninsular Thailand, and were not found in the North-Eastern region (Groups 1 and 4 of *A. setosa* populations, Group 2 of *G. tenella* populations). Although, the populations from the North-Eastern region were in the same group with the Northern and South-Western populations, the North-Eastern populations were always grouped together and separated from the others. This may have been because the Nam Nao (A4), Phu Phan (A5), Khao Yai (G1) and Phu Kradueng (G7) populations are located in the Khorat plateau basin which is a large area of Mesozoic continental sedimentary rocks of the Khorat group covering approximately 200,000 km^2 (Bunopas, 1981; Charusiri *et al.*, 2006) as shown in Figure 2. The Khorat group is broader than the Khorat plateau and consists of North-Eastern and Eastern Thailand, Laos, Cambodia, some parts of Vietnam and some parts of Malaysia (Bunopas, 1981; Metcalfe, 1996; Charusiri *et al.*, 2006). However, more sites from Thailand and neighboring countries need to be tested using these primers or additional primers to test the proposed genetically distinct Khorat plateau populations of *A. setosa* and *G. tenella*.

CONCLUSION

Seven and eleven haplotypes of *Arundinella* and *Garnotia*, respectively, were detected in Thailand using three plastid markers. Although it is premature to make conclusive statements about the phylogeography of the species, a number of other conclusions can be drawn. The populations are not monomorphic for the plastid haplotypes and there is, in fact, considerable plastid DNA diversity within and among populations. In the case of the perennial *A. setosa*, this indicates that many ramets exist in each population and that the extent of clonal spread is limited. In the annual *G. tenella,* this indicates that the population is not fixed for any particular plastid type and supports the hypothesis that seed-mediated geneflow is occurring among populations. Higher G_{ST} values were found in the annual compared to the perennial species and this may also be explained by the breeding system, as the annuals generally showed higher G_{ST} than perennials. Some population genetic differentiation was detected and there was weak evidence for an east-west geographical divide among populations. However, more loci and more populations will need to be included to thoroughly test that hypothesis.

ACKNOWLEDGEMENTS

The authors are much indebted to the director and staff of the Herbarium, Copenhagen, for the use of and information on grass collections, and especially to D. Lupton, M. Delêtre and M. Thomasset for their kind support. This work was supported by the TRF/BIOTEC Special Program for Biodiversity Research and Training Grant BRT_148026, the Trinity College Dublin Postgraduate Travel Reimbursement Fund, the Natural History Museum, the National Science Museum, Technopolis, Pathum Thani, Thailand.

LITERATURE CITED

Bor, N.L. 1955. Notes on Asiatic grasses XXIV: The genus *Arundinella* Raddi in India, Burma and Ceylon. **Kew Bull.** 1955(3): 377–414.

Bunopas, S. 1981. **Paleogeographic History of Western Thailand and Adjacent Parts of Southeast Asia: A Plate Tectonics Interpretation. Geological Survey Paper** Vol. 5. Department of Mineral Resources of Thailand, Bangkok. 810 pp.

Carlquist, S. 1967. The biota of long-distance dispersal: The biota of long-distance dispersal V: Plant dispersal to Pacific islands. **Bull. Torrey Bot. Club.** 94(3): 129–162.

Chapman, G.P. 1996. **The Biology of Grasses.** CAB International, Oxon, UK. 288 pp.

Charusiri, P., S. Imsamut, Z. Zhuang, T. Ampaiwan and X. Xu. 2006. Paleomagnetism of the earliest cretaceous to early late cretaceous sandstones, Khorat group, Northeast Thailand: Implications for tectonic plate movement of the Indochina block. **Gondwana Res.** 9(3): 310–325.

Chase, M.W. and H.H. Hills. 1991. Silica gel: An ideal material for field preservation of leaf samples for DNA studies. **Taxon.** 40(2): 215–220.

Dearden, P. 1996. Development, the environment and social differentiation in northern Thailand, pp. 111–130. *In* J. Rigg, (ed.). **Counting the Costs: Environmental Growth and Economic Change.** ISEAS, Singapore.

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue**. Phytochem. Bull.** 19: 11–15.

Excoffier, L., P.E. Smouse and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distance among DNA haplotypes: Application to human mitochondrial restriction data. **Genetics** 131: 479–491.

Gao, L.Z. 2004. Population structure and conservation genetics of wild rice *Oryza rufipogon* (Poaceae): A region-wide perspective from microsatellite variation. **Mol. Ecol.** 13: 1009–1024.

Godt, M.J.W. and J.L. Hamrick. 1998. Allozyme diversity in the grasses, pp. 11–29. *In* G.P. Cheplick, (ed.). **Population Biology of the Grasses.** Cambridge University Press. Cambridge, UK.

Hamrick, J.L. and M.J.W. Godt. 1996. Conservation genetics of endemic plant species, pp. 281–304. *In* J.C. Avise and J.L. Hamrick, (eds.). **Conservation Genetics.** Chapman and Hall. New York, NY, USA.

Hodkinson, T.R., M.W. Chase, C. Takahashi, I.J. Leitch, M.D. Bennett and S.A. Renvoize. 2002. The use of DNA sequencing (*ITS* and *trnL-F*), AFLP, and fluorescent in situ hybridization to study allopolyploid *Miscanthus* (Poaceae). **Am. J. Bot.** 89(2): 279–286.

Hodkinson, T.R., S. Waldren, J.A.N. Parnell, C.T. Kelleher, K. Salamin and N. Salamin. 2007. DNA banking for plant breeding, biotechnology and biodiversity evaluation. **J. Plant Res.** 120(1): 17–29.

Larsen, K. 1963. Studies in the Flora of Thailand 14: Cytological studies in vascular plants of Thailand. **Dansk Bot. Ark.** 20(3): 213–244.

Lowe, A.J., S.A. Harris and P. Ashton. 2004. **Ecological Genetics: Design, Analysis and Application.** Blackwells. Oxford, UK. 344 pp.

McGrath, S., T.R. Hodkinson, N. Salamin and S. Barth. 2006. Development and testing of novel chloroplast microsatellite markers for *Lolium perenne* and other grasses (Poaceae) from de novo sequencing and in silico sequences. **Mol. Ecol. Notes.** 6(2): 449–452.

McGrath, S., T.R. Hodkinson and S. Barth. 2007. Extremely high cytoplasmic diversity in natural and breeding populations of *Lolium* (Poaceae). **Heredity** 99(5): 531–544.

Metcalfe, I. 1996. Gondwanaland dispersion, Asian accretion and evolution of eastern tethys. **Australian J. Earth Sci.** 43(6): 605–623.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. **Proc. Natl. Acad. Sci. USA.** 70(12): 3321–3323.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. **Genetics** 89(3): 583–590.

Nei, M. 1987. **Molecular Evolutionary Genetics.** Columbia University Press. New York, NY, USA. 512 pp.

Nybom, H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. **Mol. Ecol.** 13(5): 1143–1155.

Pakkad, G., C. James, F. Torre, S. Elliott and D. Blakesley. 2004. Genetic variation of *Prunus cerasoides* D. Don, a framework tree species in northern Thailand. **New For.** 27: 189–200.

Pattanavibool, A. and P. Dearden. 2002. Fragmentation and montane evergreen forests in northern Thailand. **Biol. Conserv.** 107(2): 155–164.

Pattanavibool, A., P. Dearden and U. Kutintara. 2004. Habitat fragmentation in north Thailand: A case study. **Bird Conserv. Int.** 14: 513–522.

Peakall, R.O.D. and P.E. Smouse. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. **Mol. Ecol. Notes.** 6: 288–295.

Razi, B.A. 1950. A contribution towards the study of the dispersal mechanisms in flowering plants of Mysore (south India). **Ecology** 31(2): 282–286.

Ridley, H.N. 1930. **The Dispersal of Plants Throughout the World.** L. Reeve & Co, Ltd., Kent, UK. 744 pp.

Royal Forest Department (RFD). 2003. **Forestry Statistics of Thailand 2007.** Planning Division, Royal Forest Department, Bangkok, Thailand. [Available from: http://www.forest.go.th/stat/stat50/TAB7.htm]. [Sourced 2 March 2009].

Smitinand, T. 1958. The genus *Dipterocarpus* Gaertn.f. in Thailand. **Thai For. Bull. (Bot.).** 4: 1–26.

Sun, B and S.M. Phillips. 2006. *Arundinella*, pp. 563–570. *In* W. Zhengyi and P.H. Raven, (eds.). **Flora of China Vol 22 (Poaceae).** Science Press, Beijing, People's Republic of China and Missouri Botanical Garden Press. St. Louis, MO, USA.

Yeh, F.C., R.C. Yang and T. Boyle. 1999. **Popgene: Microsoft Windows-based Freeware for Population Genetic Analysis.** University of Alberta and Centre for International Forestry Research, Canada. [Available from: http://www/ualberta.ca/_fyeh]. [Sourced 2 March 2009].

Young, A.G., T. Boyle and T. Brown. 1996. The population genetic consequences of habitat fragmentation for plants. **Trends Ecol. Evol.** 11(10): 413–418.

Young, A.G. and A.H.D. Brown. 1999. Paternal bottlenecks in fragmented populations of the grassland daisy *Rutidosis leptorrhynchoides*. **Genet. Res.** 73(2): 111–117.