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## Species Occurrence, Hybridization and Speciation in Postglacial East and South Asia

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### 1. Introduction

Geological disjunction and the changing climate affect the distribution of plants and animals in East Asia. The species of postglacial East and South Asia have gone through the process of speciation. In the Northern Hemisphere, a disjunction of the distribution of closely related plants between eastern Asia and North America has been reported in many previous studies. These plants include *Rhus* (Yi et al. 2004), *Cornus* (Xiang et al. 2005), *Berberis* (Kim et al. 2004), *Chamaecyparis* (Wang et al. 2003), *Panax* (Wen and Zimmer, 1996), and *Pontica* (Milne 2004), *Fraxinus* (Jeandroz et al. 1997), etc. The modern distribution of plant species between eastern Asia and North America reflects migration, speciation, and extinction due to climatic exchange in past glaciating time periods (Milne 2004). Numerous case studies pertaining to eastern Asia and eastern North America have helped to reconstruct the relationships among morphologically similar species to explain the differences in species diversity among areas with similar environmental conditions (Wen et al., 1996, 1998; Wen, 1999, 2000, 2001; Qiu et al., 1995a, b; Soltis 2001). During the glacial and postglacial periods, climate and sea level changes affected the migration and distribution of plants and animals, which caused landbridge formations and a breakdown between islands and continents. The different species showed variant patterns of phytogeography in postglacial East and South Asia. For example, the *Cycas revoluta*, the insular species distributed from Kyushu to South Ryukyu, and *C. taitungensis*, the insular species restricted on South Taiwan, were allopatrically distributed but paraphyly due to ancient ancestral polymorphisms (Chiang et al., 2009). Likewise, *Amentotaxus* is restricted to Taiwan, to the west across southern China, to Assam in the eastern Himalaya and to the south in Vietnam. *A. argotaenia*, *A. formosana*, *A. poilanei* and *A. yunnanensis* are considered complex species and a result of speciation (Ge et al., 2005). In addition, most plant species in the Western Pacific island chain, including the islands from Ilyushin, Japan, and Taiwan to the Philippines, are derived from temperate and tropical Asia, particularly from the mainland of China. Due to the variable topography and the short distance from the continent of Asia, the island forest species are diverse and similar to their relatives that are found on continent of Asia. In addition, the tropical currents and the Kuroshio Current provide an ideal environment for long-distance colonization of seeds from Oceania to the circum-Pacific island arc. In the

past circles of glacial and postglacial time periods, the continental islands such as Taiwan, Japan, and the Philippines became melting pots for organisms, i.e., several taxa were likely to be multiple originated, showing the process of speciation. Here, we review various case studies to discuss the phylogeographic patterns and the process of speciation among related species in East and South Asia.

## 2. Case study: Diversification rate shift in SE Asian *Lithocarpus*

### 2.1 Introduction

The stone oaks (*Lithocarpus*, Fagaceae) originated from Southeast Asia (about 300 extant species) and underwent two range-expansion events: a westward dispersal to Europe identified by fossil records (no extant species) and an eastward colonization to western North America (one extant species) (Cannon & Manos, 2003). The diversification rate of *Lithocarpus* was primarily affected by the complicated geohistory, the geographic isolation, and the heterogeneous (micro-) habitats of Southeast Asia. Interspecific hybridization also affected the rate of speciation and the diversity of species of SE Asian stone oaks (Cannon & Manos, 2003). Early researchers had reported the break of reproductive isolation in the related taxa, Fagales (Hardin, 1975). Even later reports regarding the white oak group of *Quercus* indicated that the sympatric *Quercus* species shared identical or similar haplotypes with different species but had distinct haplotypes among different populations of the same species (Whittemore & Schaal, 1991; Petit et al., 1993; Petit et al., 2002).

The largest impact of the Pleistocene glacial time period on the geohistory in Southeast Asia was the emergence and submergence of Sundaland, the historic continent that once connected the Thai-Malay Peninsula to the Borneo and the Philippine Archipelagos. Several reports on the mangroves indicated that the Pleistocene glacial oscillations have affected the historical demography of the mangroves in this region (Liao et al., 2007; Liao et al., 2011). Postglacial range expansion of the *Ceriops tagal* (Rhizophoraceae) around the coasts of the South China Sea reflected the geohistory of the Sundaland submergence (Liao et al., 2009). The glacial-dependent change in population size, especially due to the postglacial expansion, has also been reported for several East and Southeast Asian organisms, such as *Castanopsis carlesii* (Fagaceae) (Cheng et al., 2005), *Machilus* (Lauraceae) (Wu et al., 2006), *Dyosma versipellis* (Berberidaceae) (Fu et al., 2009), and *Cinnamomum kanehirae* (Lauraceae) (Liao et al., 2010), etc. Another impact of the Pleistocene glacials pertained to the connection between the Borneo and Thai-Malay Peninsula (the continent of Asia). The land bridge of the Sundaland accelerated the migration (gene flow) of organisms between these two regions and increased the opportunity of interspecific hybridization, which may have resulted in the formation of new species (speciation by hybridization) (Comes & Kadereit, 1998). The postglacial isolation between Indochina and Borneo also provided opportunities for reproductive isolation and allopatric speciation. Both interspecific hybridization and geographic-dependent reproductive isolation can explain the high biodiversity of Southeast Asia, which includes the regions of Borneo, Java, Thai-Malay and the Indochina Peninsula (Swallow et al., 2005; Blattner et al., 2006; Sheldon et al., 2009).

Cannon and Manos (2003) explored the phylogeographic structure of the stone oaks of Southeast Asia and identified local molecular endemism in Southeast Asia. Significant fragmentation led to independent genetic diversification, and the independent evolutionary history of species in each locality increased the “rate of endemism”, which is an indicator of biodiversity. Such events corresponding to the high diversity of stone oaks in Southeast Asia

probably predated the Pleistocene Periods, which, therefore, suggests a relation to the glacial oscillations (Cannon & Manos, 2003). The changes in the phylogeographic patterns of endemic species caused by the catastrophic events may have resulted in a shift in the diversification rate. For example, the 1999 Chi-Chi earthquake in central Taiwan resulted in the extinction of populations of *L. konishii* and a loss of the genetic diversity (Hung et al., 2005). A loss of diversity can result in an increase in the genetic differentiation between extant populations due to the loss of shared haplotypes and the rapid drift of the small population size. In other words, such catastrophic events may play important roles in either extinction or speciation, *i.e.*, an alteration of the diversification rate.

In this case study, the systematic analysis of *Lithocarpus* (Fagaceae) based on the chloroplast *atpB-rbcL* spacer was used to explore the diversification rate shift in phylogeographic light. To resolve the question as to whether *Lithocarpus* evolved at heterogeneous speciation/extinction rates (*i.e.*, diversification rate) between distinct clades, the cpDNA was collected and used as the marker for phylogeographic inference.

## 2.2 Data collection

Partial sequences of the chloroplast *atpB-rbcL* spacer were obtained from the GenBank (NCBI) and were directly sequenced and collected in China and Taiwan (Table 1). In total, 110 sequences of *Lithocarpus* were obtained from the NCBI, and 21 sequences were obtained from direct sequencing. The alignment length of the sequence fragment used for systematic analysis was 623 bps. The species sampled in this study were distributed in the diversity center of Southeast Asia, *i.e.*, southern China, Japan, Taiwan, the Indochina Peninsula, the Thai-Malay Peninsula, Indonesia (Sumatra and Java), Borneo, the Philippines, and other areas (e.g., *L. densiflorus* of N. America). *Chrysolepis chrysophylla*, *Quercus suber*, *Castanopsis argyrophylla*, *Castanea crenata*, *Trigonobalanus vertic*, *Colombobalanus excels*, *Formanodendron doicha*, and *Fagus hyatae* were used as outgroups for the phylogenetic tree reconstruction.

Species	Source	Distribution
<i>L. amygdalifolius</i>	FBG	SE China & Taiwan
<i>L. bancanus</i>	NCBI	Malaysia & Borneo
<i>L. beccarianus</i>	NCBI	Borneo
<i>L. bennettii</i>	NCBI	Malaysia & Borneo
<i>L. blumeanus</i>	NCBI	Borneo
<i>L. brevicaudatus</i>	FBG	SE China & Taiwan
<i>L. bullatus</i>	NCBI	Malaysia & Borneo
<i>L. cantleyanus</i>	NCBI	Malaysia & Borneo
<i>L. clementianus</i>	NCBI	Malaysia & Borneo
<i>L. conocarpus</i>	NCBI	Malaysia & Borneo
<i>L. coopertus</i>	NCBI	Philippines, Malaysia & Borneo
<i>L. cornea</i>	FBG	Taiwan (endemic)
<i>L. cyrtocarpus</i>	NCBI	China & Vietnam
<i>L. dasystachyus</i>	NCBI	Borneo
<i>L. dealbatus</i>	NCBI	China
<i>L. densiflorus</i>	NCBI	W America
<i>L. dodoniifolia</i>	FBG	Taiwan (endemic)
<i>L. echinifer</i>	NCBI	Borneo & Malaysia
<i>L. echinophorus</i>	NCBI	Lao, Vietnam & China
<i>L. echinotholus</i>	NCBI	China & Vietnam
<i>L. edulis</i>	NCBI	Japan
<i>L. elegans</i>	NCBI	Borneo
<i>L. enclisocarpus</i>	NCBI	Thailand, Malaysia & Borneo

Species	Source	Distribution
<i>L. ewyckii</i>	NCBI	Malaya, Sumatera & Borneo
<i>L. fenestratus</i>	NCBI	China
<i>L. ferrugineus</i>	NCBI	Borneo
<i>L. formosana</i>	FBG	Taiwan (endemic)
<i>L. glaber</i>	FBG	China, Japan & Taiwan
<i>L. gracilis</i>	NCBI	Malaysia & Borneo
<i>L. grandifloius</i>	NCBI	China, Vietnam, Laos, Tibet & Assam
<i>L. hancei</i>	SCBG	S China
<i>L. hancei</i> var. <i>ternaticupula</i> f. <i>subreticulata</i>	FBG	Taiwan
<i>L. hancei</i> var. <i>ternaticupula</i> f. <i>ternaticupula</i>	FBG	Taiwan
<i>L. harlandii</i>	FBG	Taiwan (endemic)
<i>L. hatusimae</i>	NCBI	Borneo
<i>L. havilandii</i>	NCBI	Borneo
<i>L. jacobsii</i>	NCBI	Borneo
<i>L. kalkmanii</i>	NCBI	Borneo
<i>L. kawakamii</i>	FBG	Taiwan (endemic)
<i>L. keningauensis</i>	NCBI	Borneo
<i>L. konishii</i>	FBG	Taiwan (endemic)
<i>L. lampadarius</i>	NCBI	Borneo
<i>L. lepidocarpus</i>	FBG	Taiwan (endemic)
<i>L. lindleyanus</i>	NCBI	Thailand & Myanmar
<i>L. litseifolius</i>	SCBG	S China, Laos, Myanmar & Vietnam
<i>L. lucidus</i>	NCBI	Malaysia & Borneo
<i>L. luteus</i>	NCBI	Borneo
<i>L. meijeri</i>	NCBI	Borneo
<i>L. naiadarum</i>	SCBG	China (endemic to Hainan)
<i>L. nantoensis</i>	FBG	Taiwan (endemic)
<i>L. nieuwenhuisii</i>	NCBI	Borneo & Philippines
<i>L. pachylepis</i>	NCBI	S China & Vietnam
<i>L. pachyphyllus</i>	NCBI	China
<i>L. palungensis</i>	NCBI	Borneo
<i>L. papillifer</i>	NCBI	Malaysia & Borneo
<i>L. porcatus</i>	NCBI	Borneo
<i>L. pseudokunstleri</i>	NCBI	Borneo
<i>L. pulcher</i>	NCBI	Borneo
<i>L. rassa</i>	NCBI	Malaysia & Borneo
<i>L. revolutus</i>	NCBI	Borneo
<i>L. rhombocarpus</i>	SCBG	Taiwan & China
<i>L. rotundatus</i>	NCBI	Borneo, Java & Philippines
<i>L. ruminatus</i>	NCBI	Borneo
<i>L. sericobalanus</i>	NCBI	Borneo
<i>L. shinsuiensis</i>	FBG	Taiwan (endemic)
<i>L. taitoensis</i>	FBG	Taiwan & China
<i>L. truncatus</i>	NCBI	China & Assam
<i>L. turbinatus</i>	NCBI	Borneo
<i>L. variolosus</i>	NCBI	China & Vietnam
<i>L. wallichianus</i>	NCBI	Thailand & Sumatra
<i>L. xylocarpus</i>	NCBI	Lao, Vietnam, Assam & China
<i>L. xylocarpus</i>	NCBI	Assam, Tibet, Myanmar, Laos, Vietnam & China
<i>Castanopsis argyrophylla</i>	NCBI	China, Myanmar, Laos
<i>C. sieboldii</i>	NCBI	Japan & Korea
<i>Chrysopsis chrysophyllus</i>	NCBI	California
<i>Fagus hayatae</i>	FBG	China & Taiwan

Table 1. The sampling for the tree reconstruction and diversification rate shift analysis in this case study. FBG: Fushan Botanical Garden; SCBG: South China Botanical Garden; NCBI: National Center for Biotechnology Information.

### 2.3 Phylogenetic inference

Before reconstructing the phylogenetic tree, the substitution model was tested using MEGA 5.05 (Tamura et al., 2011), and the general-time-reversible model using a discrete Gamma distribution of the evolutionary rate among sites (GTR+G) was evaluated as the best model according to the corrected Akaike Information Criterion (AICc) ( $-\ln L = 1989.72$ ,  $AICc = 4549.56$ ). The phylogenetic relationships of *Lithocarpus* were reconstructed by Bayesian inference (BI) using MrBayes ver. 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), and the species tree was inferred by the Bayesian approach under the Yule's pure-birth model that was implemented using BEAST ver. 1.6.1 (Drummond & Rambaut, 2007). For reconstructing the gene tree (MrBayes), the GTR+G model was used, and two parallel runs of the Markov chain Monte Carlo (MCMC) searches were performed for ten million generations, sampling every 1000 generations for a total of 10,000 trees of each run. The first 10% of the generations were discarded (burn-in). Bayesian posterior probabilities were estimated as the proportion of the trees that contained each node over the number of trees that were sampled during runs. To reconstruct the species tree (BEAST), three independent pre-runs of ten million generations with the length of the MCMC were performed to obtain better parameter priors for the following five independent ten million generations of the MCMC process. Genealogies were sampled every 1000 generations, and the first 10% were discarded as burn-in. All the statistics of the output values of species-tree inference were summarized using TRACER ver. 1.5 (Rambaut & Drummond, 2003), and both the log files and the tree files of the last five runs were combined using LogCombiner ver. 1.6.1 (Drummond & Rambaut, 2007). TreeAnnotator ver. 1.6.1 (Drummond & Rambaut, 2007) and FigTree ver. 1.3.1 (Rambaut, 2008) were used to summarize and display the sampled trees, respectively. Both gene tree (reconstructed by MrBayes) and species tree (reconstructed by BEAST) have similar topologies except that there are more unresolved nodes in the gene tree. The reconstructed BI species tree is displayed in Figure 1.

The phylogenetic tree revealed six major clades, namely clades 1~6 (Figure 1). Three outgroup species, *Chrysolepis chrysophylla*, *Quercus suber*, and *Castanopsis argyrophylla*, are grouped into Clade 1: *L. pachylepi* is grouped with *Castanea crenata*, and *L. rotundatu* is grouped with *Colombobalanus excels* (Figure 1). These phylogenetic mal-positions are consistent with the chaos state of the taxonomy of *Lithocarpus*, and the non-monophyly (*i.e.*, poly- or paraphyly) of *Lithocarpus* also suggests (1) an inappropriate taxonomic treatment for *Lithocarpus*, (2) an incomplete lineage sorting of the cp *atpB-rbcL* marker, or (3) a reflection of incomplete sampling. In fact, a consensus of the taxonomy of the genera *Lithocarpus*, *Cyclobalanus*, *Synaedrys*, and *Pasania* was absent: the lumpers preferred combining the above genera as *Lithocarpus sensu lato*, but the splitters suggested that *Lithocarpus sensu stricto* should be separated from the others. In addition, the American species *L. densiflorus* was recognized as a novel genus, *Notholithocarpus*, which was based on recent morphological and phylogenetic evidence (Manos et al., 2008). Notwithstanding the taxonomic controversy of *Lithocarpus*, the occurrence of lineages in the phylogenetic tree is not affected by subjective classification, and it does not influence the following analysis of the diversification-rate shift because it only considers the "appearance" (speciation) and "loss" (extinction) of lineages instead of the taxonomic positions.

## 2.4 Inferring the diversification rate

The diversification rate shift of the clades of the *Lithocarpus* species were inferred based on a topological-based approach using the SymmeTree program (Moore & Chan, 2005). The asymmetric rate shift of the nodes of the species tree, which was inferred by the Yule's pure-birth model using BEAST, was estimated using the single-tree analysis that searched for the rapid evolving clades. Although Venditti et al. (Venditti et al., 2010) raised an objection that the Yule process in general may not explain the topology and branch-length distributions in real trees, the Yule process of diversification expects a constant rate of speciation with no extinction and a log-linear increase in the appearance of lineages. Therefore the deviation from the log-linear increase of lineage (i.e., rejecting the constant rate of speciation) can be used to infer the diversification rate variation (Rabosky, 2006). The polytomy was randomly resolved for sampling possible dichotomous resolutions using the taxon-size insensitive (TSI) model plus the equal-rate Markov (ERM) random branching model. The TSI-ERM model was selected due to its incomplete and biased sampling of taxa of the *Lithocarpus* species. One million random resolutions with 1000 TSI-ERM resolved trees under one million ERM simulations were performed to obtain the probability of a diversification rate shift of each node. A shift of the diversification rate of six clades of the *Lithocarpus* phylogeny was also tested separately to confirm the constant rate of diversification in these evolutionary lineages. The rate shift was also estimated using the gene tree inferred by MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Comparing the gene tree and the species tree aids in understanding whether the inferred rate variation is caused by the rapid substitution rate of the marker.

The result of the diversification rate shift in the species tree indicated that the inconstant diversification rate of the topology of the whole tree was determined by a significant deviation ( $P < 0.05$ ) from the constant-rate hypothesis by indices IC,  $M_{\Pi}^*$ ,  $M_{\Pi}$ ,  $M_{\Sigma}^*$ ,  $M_{\Sigma}$ , and B1 (Table 2). We further detected the diversification rate shift based on the Bayesian tree using the  $\Delta$ -shift statistic, which assessed the probability of a rate shift along an internal branch by calculating a function of likelihood difference between the homogeneous model (one diversification rate parameter) and the heterogeneous model (two-rate parameter). Three nodes, A, B, and C, were detected to have asymmetric diversification rates for the derived clades, in which the constant diversification rate was rejected ( $P = 0.014, 0.001, \text{ and } 0.013$ , respectively) by the  $\Delta 1$  statistic (Figure 1). Three deriving clades of the nodes A, B, and C, which had relatively higher diversification rates than the sister clades, were named clades  $A_R$ ,  $B_R$ , and  $C_R$ , respectively (R signifies "radiation clade"). However, the homogeneous model was rejected at two nodes (indicated by the stars in Figure 1) in the gene tree: one node was located within Clade 1, and the other was identical to node B of the species tree (Figure 1). This result might suggest that the inference of evolutionary radiation in clade  $B_R$  could be due to a rapid evolutionary (substitution) rate of cp *atpB-rbcL*, although more evidence is needed to confirm this speculation.

## 2.5 Diversification rate shift and biogeographic inference

The formation of the diversity center of *Lithocarpus* in Southeast Asia is a serial process that is composed of fragmentation, dispersal, and hybridization, etc. Although the climate change in Southeast Asia was not as severe as temperate regions, the rise and fall of the sea level severely altered the area of landmass of Southeast Asia, i.e., the emergence and

submergence of the Sundaland during glacial and interglacials, respectively. Such geographic processes might result in the differentiation of the composition of species in various areas. Three of the six clades of *Lithocarpus*, Clades 4~6, are mainly composed of species distributed in Borneo and the nearby regions (Indochina). In contrast, the species distributed throughout continental Asia (China) are primarily found in Clades 1~3. However, the species composition of the Clades 1~3 is not as simple as that for Clades 4~6;

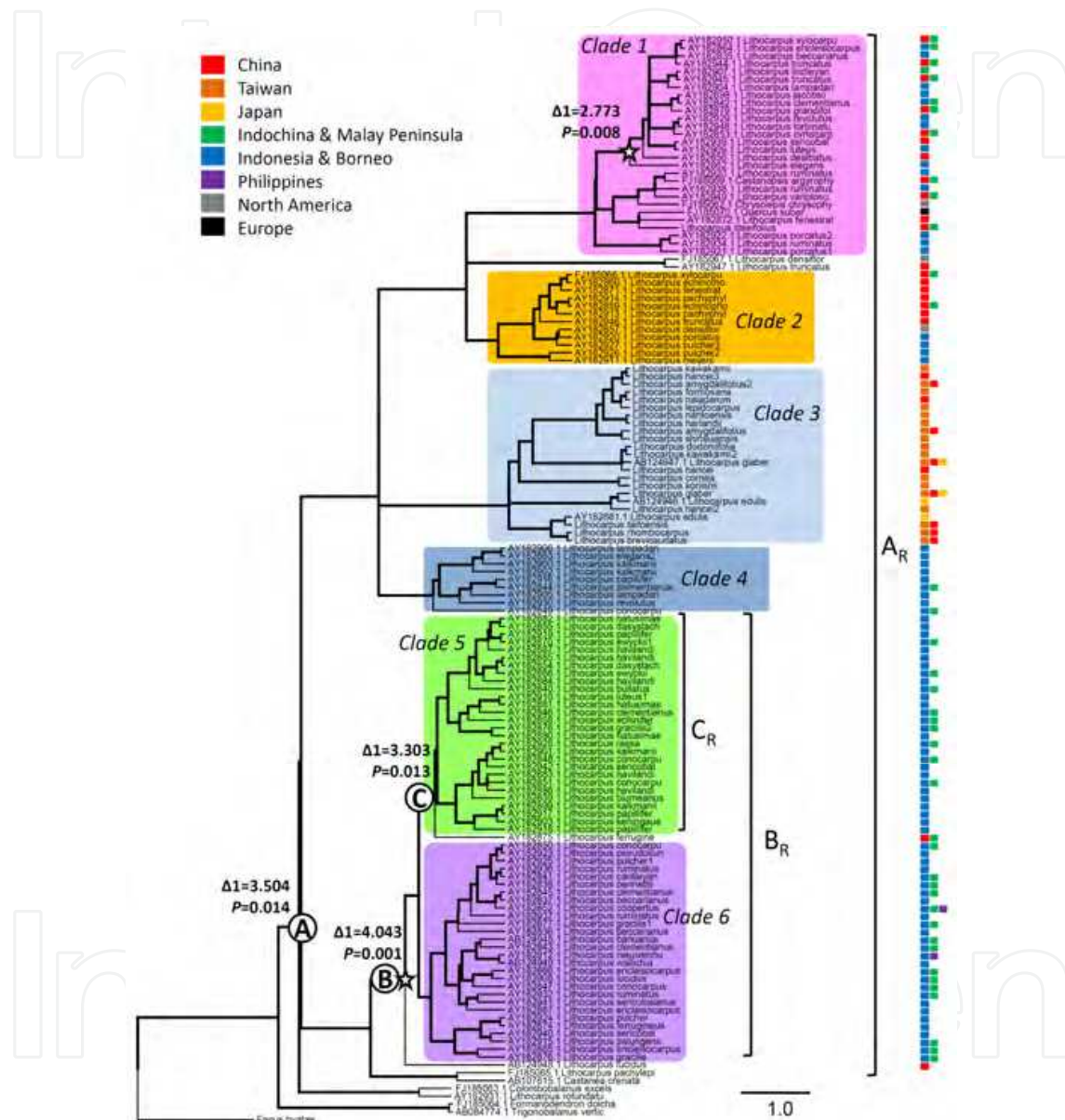


Fig. 1. The Bayesian inference species tree inferred by the Yule's pure-birth model. The  $\Delta$  statistic ( $\Delta 1$ ) and its tail probability are indicated in the nodes of the significant diversification rate shift (A~C). The nodes marked by the stars are significantly shifted in the diversification rate that was estimated in the gene tree. Clades  $A_R$ ,  $B_R$ , and  $C_R$  are the derived clades with high diversification rates of nodes A, B, and C, respectively, namely radiation clades. Six major clades of *Lithocarpus* (Clades 1~6) are indicated in colored blocks. The colored boxes to the right indicate the distribution area of the species. The bold lines represent branches with >90% statistical support by posterior probability.



Clade	IC	$M_{\Pi}^*$	$M_{\Pi}$	$M_{\Sigma}^*$	$M_{\Sigma}$	B1
Whole tree	0.0012	0.0001	0.0012	0.0076	0.0768	0.2193
Clade 1	0.0986	0.1064	0.1690	0.2039	0.3229	0.1771
Clade 2	0.3322	0.3396	0.4043	0.3425	0.4236	0.5100
Clade 3	0.1675	0.2450	0.3973	0.1859	0.3900	0.8635
Clade 4	0.3111	0.2611	0.2873	0.3202	0.2754	0.1937
Clade 5	0.3329	0.2434	0.2357	0.4177	0.3300	0.1384
Clade 6	0.5280	0.4005	0.4886	0.5365	0.6662	0.8031

Table 2. The tail probabilities of the phylogenetic uncertainty of the species tree on inferences of among-lineage diversification rate variation that was assessed using one-million random resolutions with the TSI-EMR algorithm.

the Bornean species and the continental species are phylogenetically interlaced in Clade 1 but distinctly positioned at different subclades in Clade 2, and most of species of Clade 3 are distributed in the continental islands of Taiwan and Japan (Figure 1). Based on the phylogenetic position and geographic distribution of the species of *Lithocarpus*, Borneo is suggested as their origin and as the diversity center, and the Indochinese and Chinese species are probably allopatrically derived due to vicariance or long-distance colonization. Such biogeographic inference is consistent with Cannon & Manos's phylogeographic inference of the network by the separation of a widespread clade and the Bornean clade (Cannon & Manos, 2003). Introgression and bottleneck events of Pleistocene climatic change were therefore used to explain the unapparent genetic structure and the homogeneity of the species composition of the widespread species of *Lithocarpus* that was distributed in Indochina and Borneo (Cannon & Manos, 2003). The Pleistocene glacial/interglacial oscillations largely affected the route of species colonization and their evolution, which was very similar to the European Fagaceae (*Quercus* and *Fagus*) (Comes & Kadereit, 1998). The influence of Quaternary climate change on the Asian tropical forests has been mainly reported on the spatial/demographic expansion and long-distance dispersal of mangroves (Liao et al., 2007; Liao et al., 2009; Liao et al., 2011) or the tropic forest shift at elevational gradients (Colwell & Rangel, 2010). However, the emergence/submergence of the Sundaland also played an important role in the range shift of the SE Asian Fagaceae (*Lithocarpus*), such as in the contraction and expansion of the European *Quercus* and *Fagus* during the Quaternary period.

A serial event of radiative evolution (diversification rate shifts at nodes A, B, and C) was detected in the evolutionary history of *Lithocarpus* (Figure 1). The rate shifts at these three nodes indicate that three catastrophic events caused the burst of appearance of three species: (1) the occurrence of the core *Lithocarpus* (Clade A<sub>R</sub>), (2) the rapid evolution of the core Bornean *Lithocarpus* (Clade B<sub>R</sub>), and (3) one subclade of B<sub>R</sub> with a high speciation rate (Clade C<sub>R</sub>). In addition, it has been inferred that the subclades of Clade 1 and the Clade B<sub>R</sub> evolve relatively more rapidly than the sister groups in the cp *atpB-rbcL* sequences in the gene-tree analysis (the nodes marked as stars in Figure 1). The rate shift in the gene tree fails to exclude the influence of local molecular clocks of selected markers on the inference of the diversification rate shift in the species tree. In other words, we cannot exclude the possibility that the inference of the evolutionary radiation of Clade B<sub>R</sub> was caused by the rapid substitution rate of the cp *atpB-rbcL* spacer. The use of multilocus markers will hopefully resolve this ambiguity.

In addition to these three rate shift events, the other diversifying events (*i.e.*, the other nodes) derived descendents in the saturating phases of speciation rate and the extinction

rate. None of six major clades (Clades 1~6) had a significant rate shift in their descendents (Table 2). This indicated that the diversification rate shift may not have occurred in the recent past but rather a long time ago. However, by performing a temporal analysis of each clade using lineage-through-time (LTT) plots (with the assistance of Mesquite ver. 2.6 (Maddison & Maddison, 2009)), we observed that the rates of lineage appearance were varied at the early stage of Clade 1, at the late stage of Clade 3, and at the middle stage of Clade 6 (Figure 2). In contrast, the growth rate of the lineage appearance is constant in Clades 2, 4, and 5, which indicated that the appearance (speciation) and loss (extinction) of lineages of these three clades reached balanced phases. Clade 1 was composed of species distributed in diverse areas (China, Indochina, Thai-Malay Peninsulas, Indonesia, and Borneo). Species diversification (which involved in the integration of speciation and extinction) in this clade was probably accomplished through multiple processes, such as repeated colonization and fragmentation. Severe climate change is adverse to the survival and reproduction of organisms but accelerates the speciation rate by natural selection (in strict habitats), isolation (between refugia), and rapid drift (in small populations) (Rieseberg & Wendel, 2004; Rieseberg & Willis, 2007; Pagel et al., 2010). The past climate fluctuations (for example, the Pleistocene glacial/interglacial oscillations) that resulted in the connection and disconnection of Indochina and Borneo (*i.e.*, the emergence/submergence of the Sundaland) may be responsible for the early increase in the diversification rate in Clade 1.

Sea-level changes during the Pleistocene glacials were also responsible for the composition of species around the coasts and the continental islands of East Asia and the genetic diversity of species, including, for example, gobioid fishes in the northeastern Pacific coasts (Fu et al., 2010), lilies in Taiwan and Ryukyus (Hiramatsu et al., 2001), the genus *Kirengeshoma* in East China and South Korea and Japan (Comes et al., 2009), and monkeys in China, Japan, and Taiwan (Wu et al., 2007). The varied diversification rate through the time period of Clade 3 (Figure 2) that primarily encompassed the East Asian continental islands (Taiwan and Japan) was probably a consequence of repeated fragmentation and secondary contacts of populations between the islands (Japan and Taiwan) and the continent (China) during the Pleistocene glacial oscillations. The diversification rate variation of lineages in these continental islands was also involved in the ragged topographies, which provided multiple heterogeneous microhabitats and resulted in niche differentiation of these species. Therefore, the integration of colonization and both adaptive and non-adaptive radiation is responsible for the species endemism (diversity) of these continental islands.

The diversification rate changes in Clade 6 inferred by the LTT analysis are relatively more complicated than those of other clades: the diversification rate increased initially and was followed by a phase of decreased rate, but then ended with a rapid rate recovery (Figure 2). Clade 6 is mostly composed of species from Borneo, the Indochina Peninsula and the Philippines (Figure 1), which has been suggested as the diversity center of this genus (Cannon & Manos, 2003). The factors that caused the change of the speciation rate of Clade 6 are not known yet. Several factors may have separately or jointly affected the diversification rate of this clade in regions such as the diversity center. In actuality, the violation of the constant rate may not necessary involve natural selection or any biotic effects but only stochastic processes (Venditti et al., 2010). Notwithstanding the unknown causal effects of diversification rate variations of the Bornean species, the fluctuated rate reveals an unbalanced speciation-extinction process of *Lithocarpus* in Borneo and the

nearby regions (e.g., Indonesia and Malay Peninsula), which may explain the high diversity of these regions.

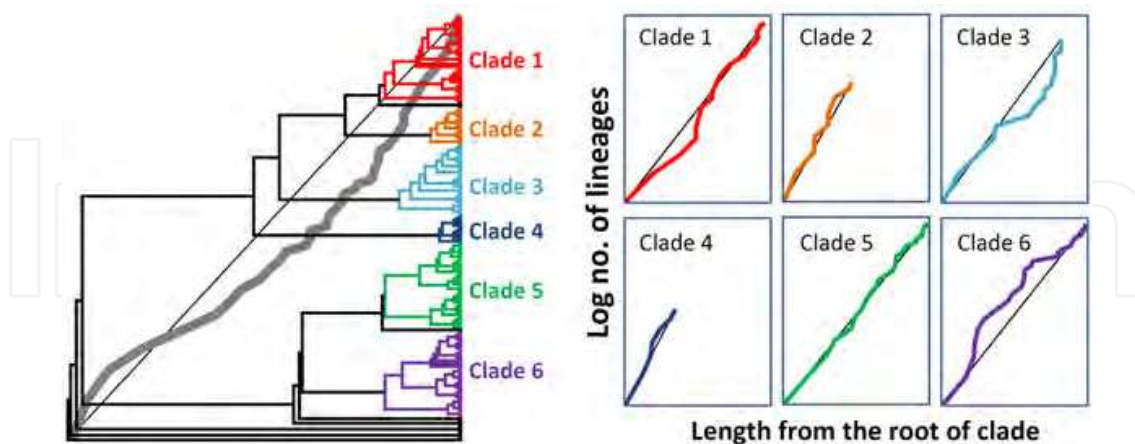


Fig. 2. Plot of the lineage-through-time (LTT) analysis of *Lithocarpus* using the species tree inferred by the Bayesian-based Yule's pure birth model. The left panel is the speciation tree inferred by Yule's pure-birth model, and the gray curve indicates the LTT curve (scaled by the log of the number of lineages). The right panel is the LTT curves of the specific clades (Clade 1~6, indicated in different colors). Straight lines under the LTT curves indicate the averaged LTT in constant growth rate.

## 2.6 Brief summary

Pleistocene climate changes predominately affected the migration, isolation, and local extinction of species and resulted in diversification rate variation (Comes & Kadereit, 1998; Willis & Niklas, 2004). Major increases in the vascular plant diversification rate occurred at least three times in the past 50 Mya (Willis & Niklas, 2004), during which the climate dramatically changed. The glacial refugia and the postglacial expansion routes of the European and American trees are not unequivocal. Although the glacials affected the climates in the Asian tropics and subtropics less, they changed the SE Asian topography, *i.e.*, in the Sundaland. The phylogenetic and geographic association of *Lithocarpus* (Figure 1) implied (1) its origination in Borneo and its subsequent northward colonization and (2) independent evolution of *Lithocarpus* in the continental islands (Taiwan and Japan). In addition, based on estimates of diversification rate variation, radiation events were detected in the Bornean species by topological analysis (SymmeTree, Figure 1), and an inconstant growing rate of speciation was detected using temporal analysis (Figure 2) in the lineages of the colonization clade (Clade 1), the continental-island clade (Clade 2), and one derived clade in Borneo (Clade 6). The causal event of diversification rate variation is likely related to the emergence and submergence of Sundaland by Pleistocene glacial/interglacial oscillations. However, we cannot exclude other possibilities, such as intrinsic or stochastic factors.

## 3. Case study: Hybridization and fern speciation

### 3.1 Introduction

Hybridization is an important mechanism for speciation. Most studies of fern hybrids are noticed by marked morphological variation, which appears to be more than the

morphological variation within a species. The ferns have continuous morphologies between two species. Consequently, the plants with intermediate morphologies are regarded as hybrids, such as some *Pteris* species (Walker, 1958; Wagner & Nauman, 1982; Pangtey et al., 1990). This continuous morphology also results in possible complicated phylogenies, and it is often difficult to identify specific species (Baack & Rieseberg, 2007; Chapman et al., 2007; Rieseberg & Willis, 2007). The earliest reported could be the reticulated evolution of *Asplenium* species in the Appalachian, which was supported by the mispairing of chromosomes (Wagner, 1954). Together with morphological characters, a network relationship constructed using multiple and recurrent hybridizations is revealed.

### 3.2 Fern speciation under hybridization

Hybridization is a kind of sympatric speciation. After a hybridization event, reproduction isolation is an important mechanism for reinforcement (Turelli et al., 2001). Hybrids are able to maintain sexual function by means of direct genome addition during crossing or genome duplication; the offspring could have successful chromosome pairing during meiosis. For example, two diploid species can cross and produce an allotetraploid, or first have a diploid (homoploid) hybrid which then undergoes chromosome doubling to become an allotetraploid, that is, an amphidiploid. For example, *Isoetes sinensis* is an amphidiploid formed from the hybridization of two diploids, *I. taianensis* and *I. yunguiensis* (Taylor et al., 2004). The amphidiploid species can have sexual function and further hybrid with other species. It has been suggested that *Isoetes sinensis* may backcross with its parental species, *I. taianensis*, and produce another species, *I. japonica* (Kim et al., 2010).

In another case, hybrids can be apogamous, such as hybrids that maintain homoploid speciation, or hybrids that have an odd number of chromosome sets (e.g., allotriploids and allohexaploids) (Park & Kato, 2003). For some hybrid ferns, apogamy increases their fitness by providing a mechanism by which failed meiosis due to chromosome mispairing can be avoided (Mehra & Singh, 1957; Braithwaite, 1964; Bretagnolle & Thompson, 1995).

Strictly speaking, it would be untrue to think that fern hybrids should have morphological variation. Apogamy is common in ferns. Apogamous hybrids and their progenies have almost the same morphologies due to the same genetic characters. If an apogamous hybrid species indeed has genetic variation, it may be caused by: (1) the recurrent origins of apogamous races from the same sexual species (Lin et al., 1995; Takamiya et al., 2001), (2) the recurrent hybridization between apogamous races and the same sexual species (Walker, 1962; Suzuki & Iwatsuki, 1990; Lin et al., 1992), (3) somatic mutations (Stebbins, 1950), (4) unequal segregation during meiosis (Lin et al., 1992), or (5) homoeologous pairing at meiosis (Klekowski, 1973).

For apomictic hybrids, the lack of recombination preserves their heterozygosity (Normark et al., 2003). As a result, apogamy creates reproductive barriers that prevent gene flow among closely related taxa, which facilitates sympatric speciation. Excepting some possibilities that can increase the genetic variation of apogamous ferns, apogamy can actually be thought of as reproductive cloning. It is doubtful that the apogamous hybrid is only alive for a short time period and would be soon extinct. Recently, it has been revealed that the asexual *Astrolepis* taxa exist 7 to 47 times younger than sexual parents (Beck et al., 2011). Could this signify that the apogamous ferns have much shorter lives than sexual ferns? This idea requires more research for clarification.

Introgression is led by backcrossing and has been frequently reported in angiosperms. Although the mechanism is discussed in fern hybridization, it is mostly limited in presumption. The limited reports may be because most fern hybrid offspring immediately gain apogamy. Possible introgression cases are found in genus *Asplenium* (Polypodiaceae) and *Serpocaulo* (Aspleniaceae) (Van den heede et al., 2003; Kreier et al., 2008; Hunt et al., 2011). A hybrid population of *Polystichum munitum* and *P. imbricans* may be the only study to provide strong and direct evidence of introgression (Kentner & Mesler, 2000). The hybrids have intermediate morphologies in the hybrid zone, which maintain environmental gradients. It is suggested that the hybrids backcross with parental species, then exhibit clinal patterns of variation.

Although the morphological gradient due to backcrossing is seldom recorded in ferns, a kind of “hybrid swarm” is found in ferns. Limited to a specific geographic area, several parental species cross with one other and produce a morphological gradient, which is formed by a mass of hybrid offspring. In New Zealand, seven tetraploid *Asplenium* species cross with each other to produce 10 octoploid hybrids (Shepherd et al., 2008). One of the parental species, *A. hookerianum*, even devotes its genetic element to seven hybrid species. In this morphological gradient, it seems impossible to understand the phylogenetic relationship without molecular evidence.

### 3.3 Species concept in fern hybridization

In light of the high frequency of hybridization, the concept of a biological species does not appear appropriate to all fern species. The high frequency of hybridization has been explained by ferns’ relative lack of pre-zygotic reproductive isolation mechanisms. Many hybrid species are sexual and cross with other species, especially *Asplenium* species (Trewick et al., 2002; Van den heede et al., 2003; Shepherd et al., 2008). However, as we discussed above, apogamy is common in ferns and is maintained in absolute reproductive isolation. Asexual organisms are not discussed with respect to the biological species concept (Arnold, 1997).

In practice, the species boundaries in ferns are often problematic (Haufler, 2008). Comparatively, “to define a species” is easier. The morphological species concept is more commonly accepted in ferns than are other species concepts. In other words, a fern taxon with identical morphologies could be treated as a species. Most hybrid ferns, such as *Acrorumohra subreflexipinna* (Ogata) H. Ito., *Archangiopteris itoi* Shieh, and *Adiantum meishanianum* F. S. Hsu ex Y. C. Liu & W. L. Chiou (Chang et al., 2009; Liu et al., 2009), were considered as species before hybridization was evident.

Ideal morphological analyses provide the independent clustering of taxa (Thomson, 2000). However, these analyses are not always sufficient, especially for a species complex. Taxa in a species complex seem to exhibit continuous morphologies with one other, and the gaps, for species delimitation, are difficult to identify. In some cases, taxa have clear hybridization patterns but have poorly differentiated morphologies, and it is difficult to determine their taxonomic ranker. Three cryptic species in *Ceratopteris thalictroides* species complex have been isolated based on crossing tests, that is, the biological species concept instead of the morphological species concept (Masuyama et al., 2002). Sequential studies have provided evidence that they are of hybrid origin and have a different number of chromosomes (Adjie

et al., 2007; Masuyama, 2008). Although a detailed diagnosis of the characters of the species is analyzed for the final taxonomic treatment, the continuous variation in morphologies remains elusive (Masuyama, 2008; Masuyama & Watano, 2010).

However, it is still necessary to face the problem: should the hybrid taxon be thought of as a hybrid species or just a hybrid offspring? It is a more difficult issue during the taxonomic treatment of a reticulate phylogeny arising from hybridization. The biological species concept could be applied, although there is high frequency of hybridizations in ferns. In the reticulated relationship of the *Vandenboschia radicans* complex, the fertile amphidiploid taxa are treated as species, whereas the taxa are treated as hybrids (Ebihara et al., 2005; Ebihara et al., 2009).

### 3.4 Abundant hybridizations in the genus *Pteris* (Pteridaceae)

Genus *Pteris* L., belonging to Pteridaceae, is a cosmopolitan genus with more than 250 species and is diverse in Asia. Many hybridizations of *Pteris* have been reported (Walker, 1962). According to a review of the breeding systems of 99 *Pteris* species, 31 species are apogamous (Chao et al., unpublished). This ratio is higher than the previously estimated value of 10% (Walker, 1984). Apogamous fern species are usually considered potential hybrids. However, this does not mean that the speciation of 31 species from the 99 *Pteris* species are of hybrid origin. As chromosomal mispairing of some autopolyploids could also cause apogamy, such as in autotriploids and autopentaploids, it is difficult to distinguish before a more detailed investigation. A species can have several cytotypes. If we consider the frequency of the reproductive mode combined with cytotypical data, the frequency of apogamous triploids is 21%, and we still have 11% apogamous diploid and 6% apogamous 4X. In total, there is at least a 17% frequency of hybrids and apogamy in the *Pteris* species.

In addition, there are many species complex in *Pteris*. One of the most famous of *Pteris* is the *P. quadriaurita* complex. Species complex may involve multiple mechanisms, including hybridization, polyploidy, morphological plasticity, gene expression, geographical divergence, and reproductive traits (Rosenthal et al., 2002; Guo et al., 2004; Lai et al., 2005; Zhuang & Adams, 2007; Guggisberg et al., 2009). As all of these are closely associated with speciation, species complex could be considered a group of taxa undergoing speciation.

In this study, we supplied more species in the sampling for the genus *Pteris*. We sequenced the *rbcL* gene of cpDNA from 25 *Pteris* species. Relevant sequences were collected from the previous studies and via NCBI BLAST (<http://blast.ncbi.nlm.nih.gov/>) searches, including 17 *Pteris* species and related species belong to other genera. Maximum likelihood calculations were performed for phylogenetic relationships (Zwickl, 2006).

First, within the maximum likelihood tree (Figure 3), which includes 40 *Pteris* species, *Pteris* is a paraphyletic genus. We provided more samplings than previous studies, and our analysis still supports previous studies (Jefferson et al., 2007; Schuettelpelz et al., 2007). Two genera are clustered in the major clade of *Pteris*, *Neurocallis* and *Ochropteris*. Furthermore, there is a strange species, *Pteris vittata*, which is not closely allied to the other *Pteris* species. That could be another issue about taxonomy (Christenhusz et al., 2011). *P. vittata* could arise from multiple hybridizations. The species shows considerable morphological variation and diverse cytotypes, including sexual diploids, triploids, tetraploids, pentaploids, and

hexaploids (Srivastava et al., 2007). Furthermore, the chromosomes of the spore mother cells show varied multivalence, such as 20I+26II+5III, 9I+45II+3III+2IV, 29II+29I, and 29II+87I (Khare & Kaur, 1983; Wang, 1989), which indicates its hybrid origin.

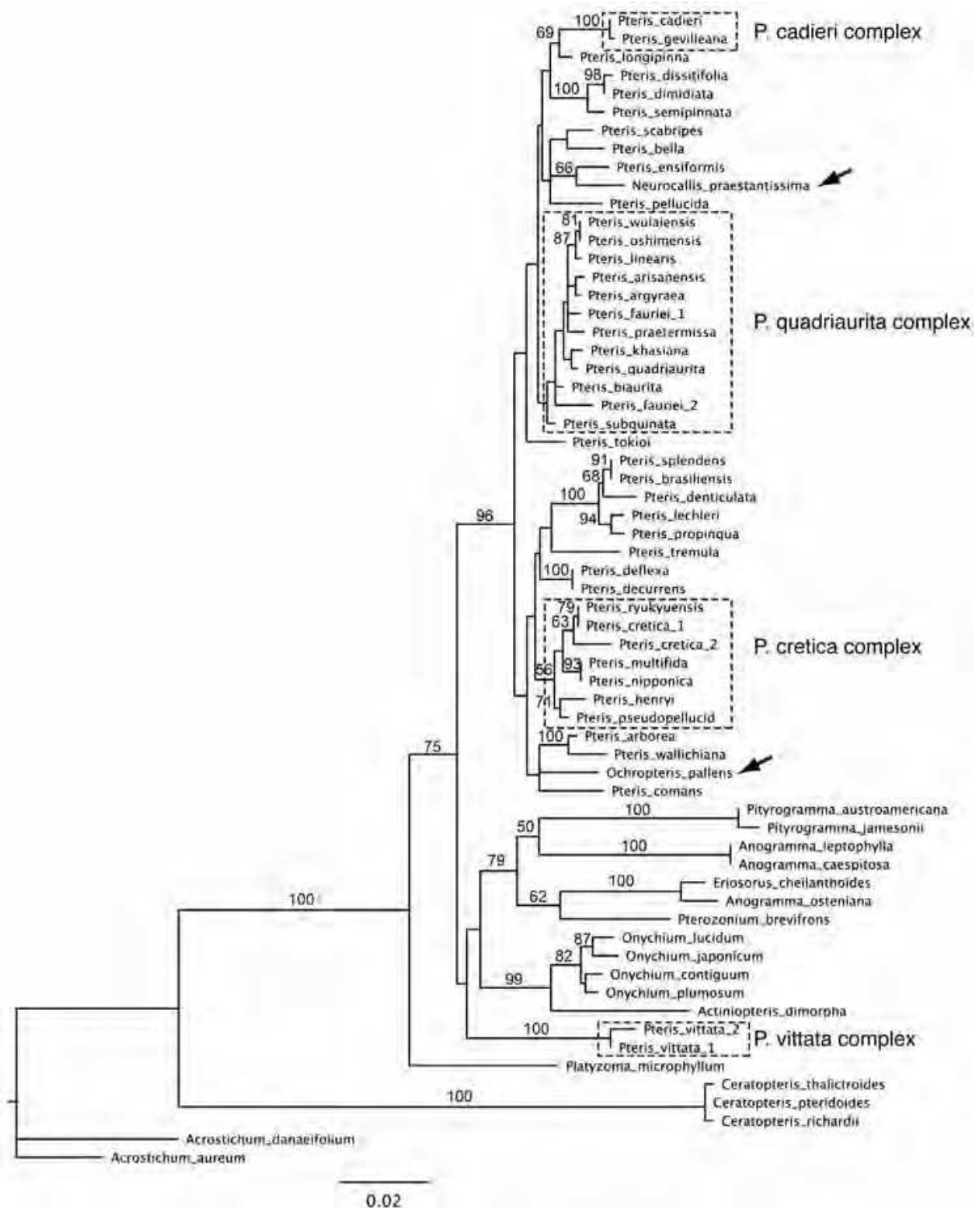


Fig. 3. Chloroplast DNA tree based on the *rbcL* gene and assessed using maximum likelihood (ML) analysis. Arrows indicate two genera included in the clade of genus *Pteris*. Numbers above the branches are ML bootstrap (> 50%).

### 3.5 Species complex in the genus *Pteris* (Pteridaceae)

Division of the lamina has been considered as key characters for section classification of *Pteris* (Shieh, 1966). These characters, simple pinnate, bipinnatifid, or semi-bipinnatifid (irregular bipinnatifid) were mapped on the ML cladogram, using the trace character function and ACCTRAN optimization as implemented in MacClade (Maddison & Maddison, 2000) (Figure 4). It is indicated that the character is not so consistent in a single clade and could be not a synapomorphic character.

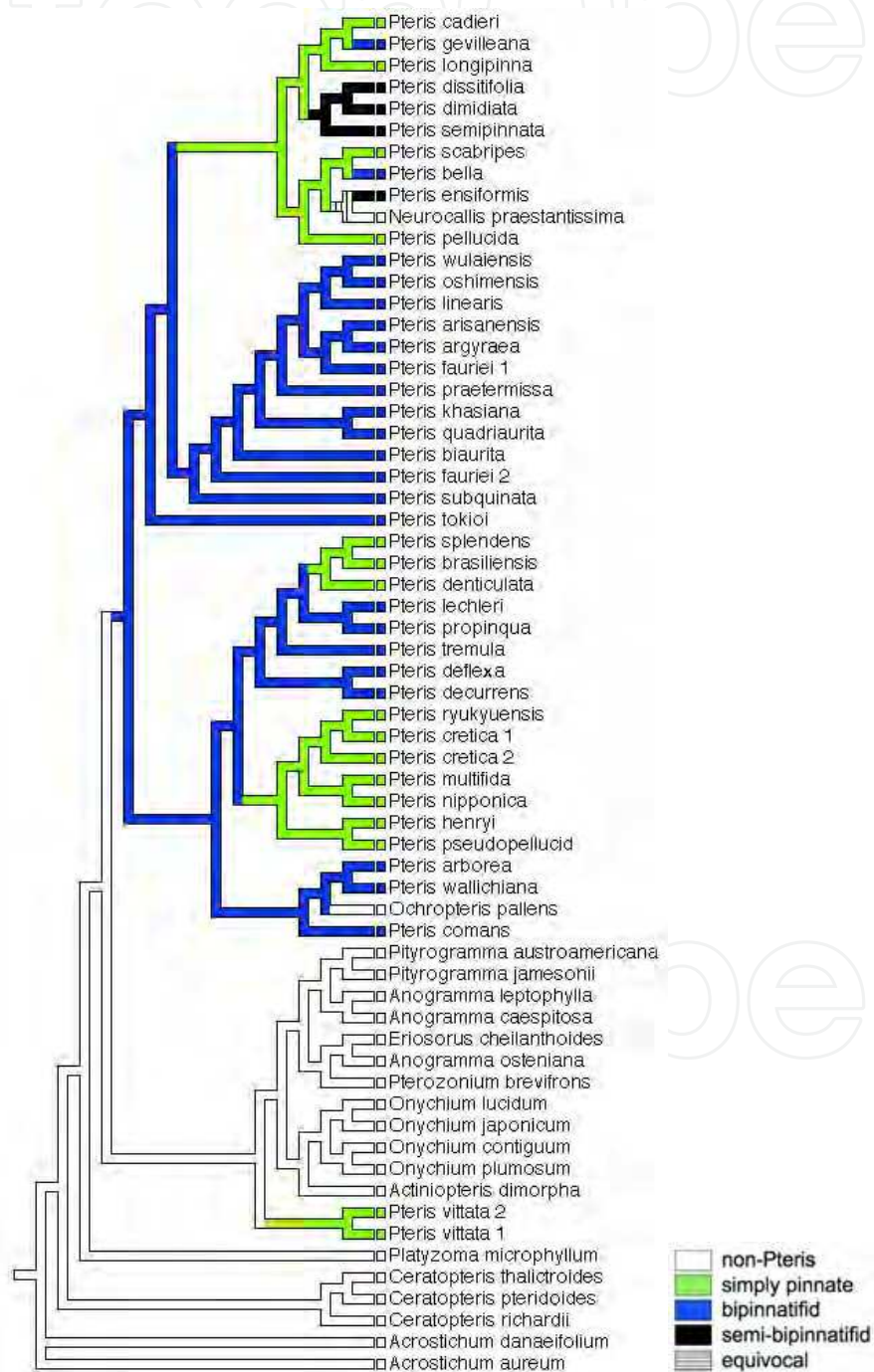


Fig. 4. Division of the lamina are mapped onto the MacClade optimization tree inferred by the maximum likelihood analysis.



Several species complexes can be found in the tree, and which indeed prove the species within the particular species complex having close relationship. They are *P. cadieri* complex, *P. cretica* complex, *P. quadriaurita* complex, and *P. vittata* complex (Figure 3). *Pteris cadieri* complex is distributed throughout eastern Asia, from including Japan, China, Taiwan, Indochina and Malaysia. The species complex exhibits markedly morphological variations, from simply pinnate to bipinnatifid fronds. Previous studies suggested that *P. grevilleana* was parental species of *P. cadieri* (Chao et al., 2010). However, it has been found that the two species are apogamous. Both of them could arise from hybridization.

The clade consisted by species with simple pinnate frond is *Pteris cretica* complex, including 6 species in this tree (Figure 3). *Pteris cretica* is widely distributed in warm-temperate and tropical parts of the Old World (Kramer & McCarthy, 1998). The two "*P. cretica*" have different molecular traits. The first study on *P. cretica* has revealed its apogamy and different cytotypes (Manton, 1950). Although sexual diploid *P. cretica* was reported, apogamous diploids, triploids, and tetraploids suggested possible hybridization (Walker, 1962; Roy et al., 1971; Nakatô, 1975; Huang et al., 2011) and supported by mispairing during meiosis (Verma & Khullar, 1965; Jha & Sinha, 1987). Based on the evidence of allozyme, triploid *P. cretica* may be produced by diploid apogamous *P. cretica* and diploid sexual *P. kidoi* (Suzuki & Iwatsuki, 1990). In *P. cretica* complex, some ferns with intermediate morphologies are suggested hybrid origin. For example, *Pteris namegatae*, recorded in Japan, is thought as a hybrid species between *P. multifida* and *P. ryukyunesis* (Kurata, 1969).

In the earliest reports, it was proposed that *P. quadriaurita* complex is consisted by *P. quadriaurita sensu stricto*, *P. multiarita*, *P. confusa*, and *P. otaria* in Ceylon (Walker, 1954; Walker, 1958). In Dr. Walker's hybridization experiment, the four plants were from hybridizations among tree parental species. Practically, there are more bipinnatifid ferns difficult to distinguish from *P. quadriaurita*. In figure 3, 10 species clustered together with *P. quadriaurita* complex, are so similar and difficult to identify. For this great group of *Pteris quadriaurita* complex, it is unsure if the problem is just taxonomical.

Totally, the difficulty of studying *Pteris* taxonomy and phylogeny could be species complexes, hybridization. Serious hybridizations form reticulate evolution. Different methodologies and synthetical analysis are in need.

### 3.6 Brief summary

Hybridization is an important mechanism for speciation. It increases the opportunity for hybrids to reproduce and maintain their lineages. Beginning with recurrent hybridizations that were revealed in the genus *Asplenium*, more complicated depictions have been reported in the years of the previous decade, including genus *Equisetum*, *Isoetes*, *Cheilanthes*, and *Vandenboschia*. The genus *Pteris* L., which belongs to Pteridaceae, is a cosmopolitan genus with more than 250 species and is diverse in Asia. It has been found that plants with different morphologies have the same scientific name in different local floras. Meanwhile, species complexes have confused morphologies and are often distributed across different geographical areas. Based on phylogenetic evidence, some taxa have been shown to be of hybrid origins. We intended to reconstruct the evolutionary history of the *Pteris* in Asia and to investigate the role of hybridization in fern speciation.

## 4. Case study: Divergence of *Toxicodendron radicans* ssp *hispidum* and *orientale* in East Asia

### 4.1 Introduction

In the past 2 million years, climate changes have often been interpreted as the primary driver of range fragmentation and the speciation of plants and animals (Hewitt 2000). Tree species have responded by migrating to regions where the environmental conditions allowed them to survive (Hewitt 1996). Phylogeography investigates the spatio-temporal dynamics of populations. The field relies on inferences that are made from macrofossils and pollen in sediment profiles and molecular evidence that can reveal aspects of history, such as the location of cryptic refugia (cf. Ge et al., 2005). Few paleoecological studies have identified the pollen and fossils of tree species. The molecular evidence provides an effective method for inferring the number of genetically distinct ancestral lineages, their location during the last glacial maximum, and the postglacial migration routes for tree and plant species (Chiang et al. 2006; Naydenov et al. 2007; Cheng et al. 2008). Altogether, phylogeography and genetic characteristics of populations/species are dictated by the interplay between historical vicariance and recurrent genetic exchanges (cf. Chiang & Schaal 2006). These evolutionary events leave evolutionary footprints in the spatial apportionment of genetic polymorphisms within and among populations across the distributional range of *P. massoniana*, which in turn provides information for recovering their demographic histories (Schaal & Olsen 2000). Of the geological events, historical regular glacial cycles in the Eurasia Continent have had prevalent influences on the survival (extinction) and recolonization of populations/ species. According to geological evidence, during the early Pleistocene period, ice ages occurred at regular intervals of 100,000 years followed by a 20,000-year warm period (Milankovitch cycles) (cf. Bennett 1990).

In the continental island of Taiwan and Southwest China, only one endemic subspecies, *Toxicodendron radicans* ssp. *hispidum*, is present with an extended distribution over all of the Taiwan middle high mountain area and Yunnan, Szechwan, Guizhou, Hunan, Hubai and Fujian. However, in the continental islands of Japan, another endemic subspecies, *T. radicans* ssp. *orientale*, is present with an extended distribution over all of Japan. The close sister for both subspecies is distributed in eastern North America and is divided into several subspecies (Gillis 1975). The total world distribution of the genus *Toxicodendron* includes eastern Asia and North America south to Colombia in South America. Poison ivy is found only in North America and eastern Asia, which is a classical distribution pattern that was reported by a different study and included species and genus levels (Yi et al. 2004; Xiang et al. 2005; Kim et al. 2004; Wang et al. 2003; Wen and Zimmer, 1996; Milne 2004; Jeandroz et al. 1997). Population genetic variation of *T. radicans* using molecular evidence is important for the reconstruction of the distribution patterns of poison ivy between continents and islands and to rebuild the evolutionary paths of these subspecies. *T. radicans* in East Asia is a good model system to use the population genetics approach to explain the effects of the land bridge disconnection after the ice age.

### 4.2 Data collections

*Toxicodendron radicans* ssp. *hispidum* is distributed in Southwest China and between the island of Taiwan and the continental mountains of Southwest China, including Yunnan, Szechwan, Guizhou, Hunan, Hubai and Fujian. One-hundred and eighty individuals from

Taiwan and 174 from China were sampled for *spp. hispidum*. *Toxicodendron radicans ssp. orientale* is restricted to Japan, ranging from Hokkaido to Kyushu; a total of 259 samples were collected from 35 locations from Hokkaido to Kyushu. More than 500 individuals were sampled in two subspecies to compare the population differentiation by plate disjunction affected by the landbridge formation and breakdown. Young and healthy leaves were collected from the field, rinsed with tap water and dried in silica gel. Leaf tissue of the above materials were ground to powder in liquid nitrogen and stored in a  $-70^{\circ}\text{C}$  freezer. Genomic DNA was extracted from the powdered tissue according to a CTAB methodology (Doyle and Doyle 1987). For ISSR fingerprinting, a total of 200 primers from UBC were pretested, and 11 primers were chosen for population genetic analyses. PCR amplification was carried out in a total volume of 20  $\mu\text{l}$  reaction using 2  $\mu\text{l}$  of 10X reaction buffer, 2  $\mu\text{l}$  of  $\text{MgCl}_2$  (25 mM), 2  $\mu\text{l}$  of dNTP mix (8 mM), 10 pmole of ISSR primer, and 2 U of Taq polymerase (Promega, Madison, USA). The reaction was optimized and programmed on a MJ Thermal Cycler (PTC 100). The reaction was performed as follows: one cycle of denaturation at  $95^{\circ}\text{C}$  for 4 min, 40 cycles of 45 s denaturation at  $94^{\circ}\text{C}$ , 1 min 15 s annealing at  $37^{\circ}\text{C}$ , 1 min 30 s extension at  $72^{\circ}\text{C}$ , and 10 min extension at  $72^{\circ}\text{C}$ . A negative control, in which no template DNA was added, was included in the PCR reaction to test for contamination. The PCR products were separated on 2.0% NuSieve® 3:1 Agarose gels using 1X TAE buffer. The gel was stained with ethidium bromide for 30 min, and DNA fragments were identified using the Bio-Rad Molecular Imager Gel Doc XR+ System for gel documentation. Each sample was run three times to obtain repeatable results. The ISSR bands were scored by dominant markers using 1 (present) or 0 (absent) binary characters. Analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA) were performed to estimate the hierarchical apportionment of variation using GenAlEx v. 6.1 (Peakall and Smouse 2006).

#### 4.3 Population differentiation based on ISSR molecular markers

Population differentiation based on ISSR dominant markers was estimated by AMOVA and PCoA analyses. Both showed the high differentiation among populations of islands and continents. In total, the analyses showed 36.67% variation among populations and 63.33% variation among individuals within the populations. Based on the AMOVA results, the mean  $\Phi_{\text{ST}}$  was 0.367, and the result was significant ( $p < 0.010$ ). The PCoA result showed three groups separated in Japan, Taiwan and China (Figure 5). Almost 38.42%, 18.76, and 12.86 of the variation is explained by the first, second, and third axes in this test, respectively. Based on the results, populations of *spp. hispidum* and *spp. orientale* were differentiated among the three regions of the islands and the continent. According to a hierarchical AMOVA analysis, significant genetic differentiation was detected among these populations, with values of  $\Phi_{\text{ST}}$  ranging from 0.021 to 0.850. Using three geographic regions, with values of  $\Phi_{\text{ST}}$  ranging from 0.198 to 0.224, significant genetic differentiation was detected that was much higher than the average  $\Phi_{\text{ST}}$  of 0.17 for outcrossing plant species (Nybom and Bartish 2000; Nybom 2004). The mating system and gene flow were probably two of the main factors that determined the levels of genetic variability and the spatial distribution of the genetic polymorphisms in *Toxicodendron radicans ssp. hispidum* and *spp. orientale*. In general, outcrossing plants maintained the most genetic variation within the populations, whereas predominantly selfing, short-lived species harbored comparatively higher variation among populations (Hamrick and Godt 1989).

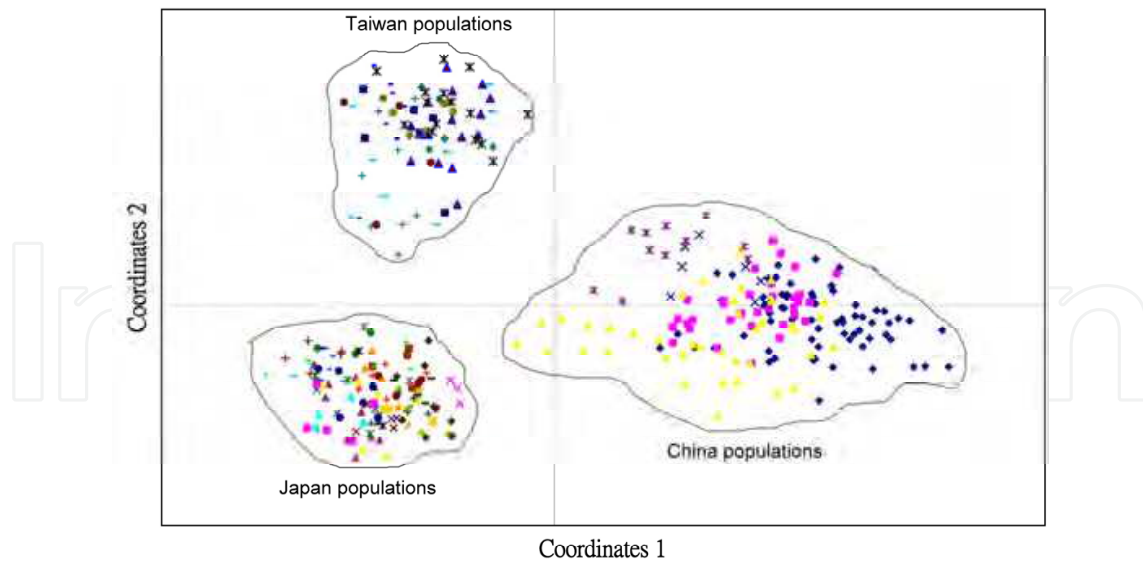


Fig. 5. Distribution of varieties in a two-dimensional PCoA space based on the ISSR data in *Toxicodendron radicans* spp. *hispidum* and spp. *orientale*. According to the ISSR result, the three groups were separated by three different regions, including Japan, Taiwan, and China.

An UPGMA topogram based on the pairwise Nei genetic distance among the populations identified three major clusters that corresponded to the three geographic regions (Figure 6). A close phenetic relationship between the populations of spp. *hispidum* from Taiwan and China was recovered. Populations of Taiwan and populations of China were clustered together in the UPGMA dendrogram. All of the populations of spp. *orientale* from Japan were grouped together within a mono cluster. Geographically, the distribution of spp. *hispidum* in Taiwan is close to that of spp. *orientale* in Japan. Nevertheless, the genetic affinity between spp. *hispidum* of Taiwan and China was identified in the UPGMA topogram, which reflected the different historical migratory route. According to the geological evidence, Japan and Taiwan, now continental islands 150 km east of the Chinese mainland, attained their modern shapes via collisions between the continent and the oceanic tectonic plates only several million years ago (Sibuet and Hsu 1997, 2004). During the Pleistocene glacial expansion, the drops in the sea levels of the East and South China Sea to about 120 m formed the land bridge that emerged from the oceans, which linked Taiwan (Chiang et al. 2001) and Japan to the mainland. Meanwhile, plants and animals migrated from north to south because of the dramatic climatic changes (Comes and Kadereit 1998; Hewitt 1996). The ancestral populations of *T. radicans* were able to migrate across the Bering Straits via a land bridge connection between North America and Asia. After that, the ancestral population separated into two paths: one from Sakhalin to Japan or via the Korean Peninsula to Japan, and one from the north to the south via Siberia which then separated into several paths of scattered refugia in Yunnan, Szechwan, Guizhou, Hunan, Hubai, Fujian and Taiwan (cf Lu et al. 2001; Ge et al. 2005). The genetic drift effected those isolated populations and promoted the process of speciation in various geographic regions.

#### 4.4 Brief summary

The phylogeographic pattern of *Toxicodendron radicans* in East Asia is indicated by the population differentiation and the process of speciation. The spp. *hispidum* were distributed

in the middle high mountain area of Taiwan and the areas of southern China. Their populations were restricted only to refugial regions, which was most likely due to the refugial effect of the Quaternary glaciations. The spp. *orientale* were distributed in lat and hilly areas in Japan and were affected by the bottleneck event in the founder populations, such that they expanded their range and spread to the entire area of Japan. ISSR results

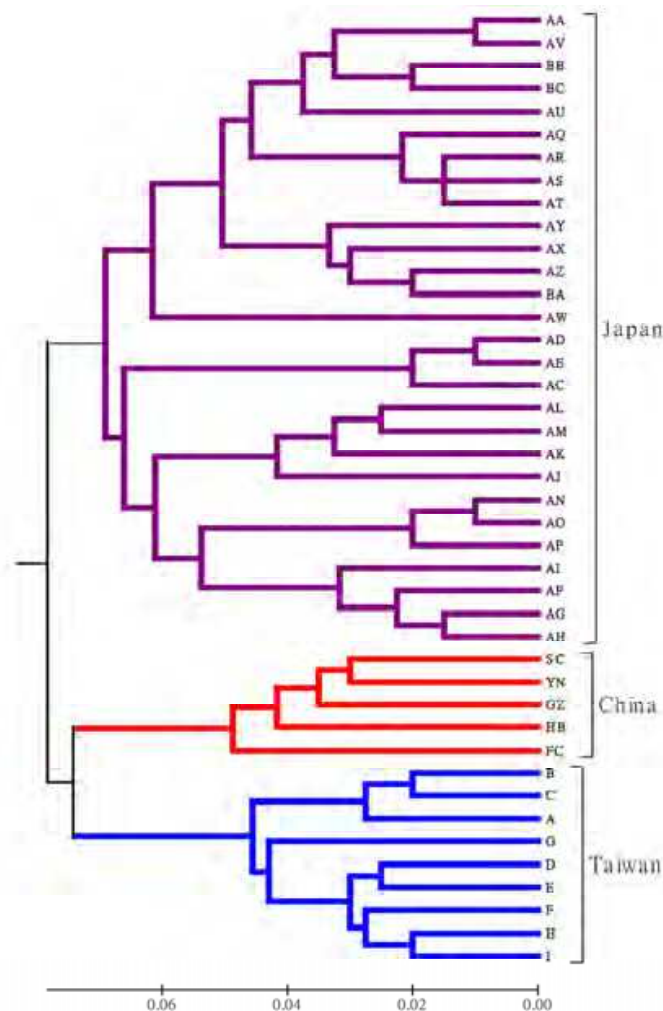


Fig. 6. UPGMA dendrogram of the populations of *Toxicodendron radicans* spp. *hispidum* and spp. *orientale* based on ISSR fingerprints.

showed population differentiation between islands to continents. Different independent lineages with separate clusters are indicative of genetic differentiation among geographic regions and should be considered as evolutionary significant units for plant migration and speciation study.

## 5. Conclusion

The maintenance of genetic diversity is critical for the long-term survival of species (Frankel and Soulé 1981) because loss of variation may largely limit the adaptability of populations to changing environments. In glacial and postglacial periods, climate and sea level changes affected the distribution of plants and animals. At the same time, isolated populations within

refugial regions might be affected by bottlenecks, which cause genetic variation loss. Meanwhile, the different species showed variant patterns of phylogeography in the postglacial East and South Asia. The process of species occurrence and speciation in the past geologic history was caused by isolation, adaptation, and even hybridization in different cases.

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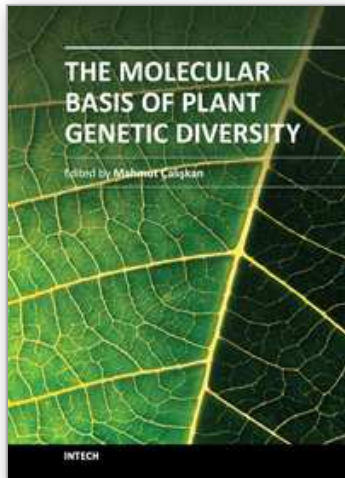
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## **The Molecular Basis of Plant Genetic Diversity**

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The Molecular Basis of Plant Genetic Diversity presents chapters revealing the magnitude of genetic variations existing in plant populations. Natural populations contain a considerable genetic variability which provides a genomic flexibility that can be used as a raw material for adaptation to changing environmental conditions. The analysis of genetic diversity provides information about allelic variation at a given locus. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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