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Review

Molecular ecology and biogeography of mangrove trees towards conceptual insights on gene flow and barriers: A review

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Abstract

In this review the most recent contributions to the field of molecular ecology and biogeography of mangrove trees are considered. Emphasis is on the obtained information of the different molecular marker methods used in mangrove genetics and on the potential to infer biogeographical patterns. Isozymes on average showed low or even no polymorphism in mangrove trees similar as known in seagrasses. The outcrossing *Avicennia* seems to be the most variable mangrove tree for isozymes. Both low amounts of interpretable allozymes and difficulties in maintaining the enzyme activity have reduced the number of successful studies during the isozyme era. Dominant marker methods (RAPD, AFLP and ISSR) were successful to demonstrate differences in amplified DNA products at large-scale geographical distances within *Avicennia* species and to estimate species relationships. Hybrid testing seldom revealed hybridization among tree species. The most promising markers (microsatellites or SSR) were only recently developed and will continue to provide evidence in future studies. SSR loci in *Avicennia* seem to show relatively low levels of polymorphism, though clearly demonstrating that populations located at the edge of the species range can be even more depauperated. Populations located more central in their native range and situated along the same coastline such as reported in *Rhizophora*, are expected to be only weakly differentiated due to increased levels of gene flow. Haplotypic chloroplast variants (PCR-RFLP) or sequences revealed strong genetic structuring between populations of *Avicennia*, *Kandelia* and *Ceriops* from different biogeographical oceanic regions. Recent views on long-distance dispersal and on gene flow across oceans as well as along the same coastline are discussed. A comparative analysis on genetic variables across species and regions indicated general trends in the partitioning of genetic variation. A conceptual map with a worldwide overview of those regions where high levels of gene flow were reported and of other regions that were considered as effective barriers, is presented. As an aim to increase the number of reliable comparisons of genetic variables across species or regions and to increase the relevance of mangrove genetics for local conservation issues, recommendations on the molecular markers and on the sampling design of individuals and populations are given within a conceptual context of evolutionary significant units.

Keywords: Mangrove; Biogeography; Dispersal; Genetic diversity; Genetic structure; Gene flow; Barrier; Conservation; Allozyme; RAPD; AFLP; ISSR; Chloroplast DNA; Microsatellite

Contents

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1. Introduction

The distribution of populations of mangrove tree populations is shaped by their response to colder climate and arid conditions at the limit of their ranges and therefore have been moving and expanding along changing coastal zones since the last glacial period, following a longer period of contraction of their ranges (Duke et al., 1998a,b; Saenger, 1998; Dodd et al., 2002). Detecting the patterns of such long-distance dispersal is a challenging research objective and allows to define evolutionary significant units and propagule dispersal routes, especially on basis of distinct chloroplast DNA variants. Such haplotypic chloroplast genomes were separated for a long period during the Pleistocene, but expanded along distinguishable routes on continents and islands. Understanding the historical factors that shaped the present-day populations is important for understanding the evolution of mangrove populations and predicting their likely response to climate change (Dodd et al., 2002). The extent and patterns of genetic diversity in natural mangrove populations are largely unknown across the species ranges except for a few *Avicennia* species (Maguire et al., 2002; Arnoud-Haond et al., 2006; Nettel and Dodd, 2007).

Several excellent and inspiring papers on the global biogeography and evolutionary aspects of mangrove trees appeared during the last decade. Patterns of genetic diversity, though at that time only available for a limited number of *Avicennia marina* (Forsk.)Vierh. populations (Duke, 1995) were discussed in the context of continental drift as a driver of tectonic gene dispersal of mangrove trees in geological times (>60 MYA). Measures of genetic identity, determined by enzyme electrophoresis were used by assuming that recent progenitor-derivatives have less variation than their progenitor. Saenger (1998) put forward the idea that the species composition of modern mangrove plants is largely a relict of historical processes, though these plants are subject to the climatic and geographical conditions of today. The modern mangrove flora on different continents shows a divergence between the Indo-Pacific and the Atlantic coastal zones. Paleontological studies shed more light on the possible time frame of mangrove evolution and distribution. Plaziat et al. (2001) estimated that the modern mangrove ecosystem and biogeographical split was established since the late Eocene (ca. 40 MYA). The discontinuity in the distribution of many mangrove species has become an attractive research subject and allowed interpretations on the origins of such unusual global patterns. Differentiation in epicuticular wax composition of *Rhizophora*, *Avicennia* and *Laguncularia* species from both West Africa and the South American Atlantic coast, gave evidence to suggest that mangroves from the latter region are a derivative of the former (Dodd et al., 1998). An inspiring essay on the disjunct nature of globally distributed mangrove trees raised many ideas on how to explain such an unexpected occurrence of restricted dispersal and gene flow, within widespread species of, e.g. *Rhizophora* (Duke et al., 2002).

At the range edges of a species (e.g. in *A. marina*) a decrease in allelic diversity was found, accompanied with a stronger genetic structure and inbreeding events when compared to populations in the core of the distribution range. This is suggested to be attributed to low effective population size, pollinator scarcity and higher environmental pressures at such range borders (Arnoud-Haond et al., 2006). Combined effects of founder events and enhanced local gene flow (e.g. in *Aegiceras corniculatum* (L.) Blanco; Ge and Sun, 1999) as opposed to low probability of long-distance dispersal (e.g. in *Avicennia germinans* (L.) Gaertn.; Nettel and Dodd, 2007) might be hypothesized as a more general pattern. Local deviations in gene diversities and differentiation of the averaged values in a species also might occur after disturbances of various origins, such as habitat fragmentation and isolation of estuaries in urban environments and associated pollution of sediments (Melville and Burchett, 2002), thereby altering locally the amount and distribution of genetic diversity. Local effects are custom, because highly significant actual gene flow (>30 migrants per generation) is usually within distances as short as a few tens of kilometres (Duke et al., 1998a,b) whereas effective barriers to gene flow (<1 migrant per generation) are at much larger distances. Historical gene flow, however, might have reached thousands of kilometers (Nettel and Dodd, 2007). Thus, the paradigm of mangrove tree distribution, namely the inferred ability of long-distance dispersal of well-adapted propagules in contrast to the accumulating data on sharp disjunct patterns of genetic diversity remains an attractive source of challenging hypotheses.

It is an intention of this review to summarize conceptually the recently published biogeographical considerations, opinions and thoughts, but it is highly recommended to read the original well-elaborated versions. The latest review on

molecular data in mangrove trees dates back several years (Schwarzbach and Ricklefs, 2001) and placed emphasis on both protein and DNA data. The future outlook as they stated it in 2001, was that molecular methods would play an expanding role in mangrove plant research. About 50 papers on the subject appeared during the period 2000–2007 which is more than a doubling as compared to 1986–1999 (<20 papers), herewith following on average the steady increase of articles on molecular ecology in general during that period (3-fold increase), but a much stronger increase than on mangroves in general (1.5-fold increase).

In this review, most – if not all – recent studies on the molecular ecology of mangrove trees are discussed in the light of usefulness of particular techniques and approaches for a thorough analysis of this combined field with ecological and genetical relevance. The objective of this review is to present in an analytical way the recent history of such studies in mangrove trees and shrubs, not only at larger scales but also at regional and local scales. The most convenient way to structure this review, comprising various approaches in molecular ecology, including different species from many parts of the world and using different methods for calculating genetic variability, was to consider first the different types of molecular information with an emphasis on mangrove papers and issues, and secondly highlighting the various kinds of biogeographical information.

2. Methodological and technical considerations

Case-studies on isozymes, dominant markers, haplotypes and codominant microsatellite markers will be subsequently discussed in a context of feasibility, usefulness and perspectives for improvement, including personal opinions and practical considerations. Acronyms are explained, but for more information on the different techniques, their advantages, disadvantages and explanations of the genetic terminology or abbreviations used, I hereby refer to the many recent textbooks available in the field of population genetics (Hartl and Clark, 2007), molecular ecology (Beebee and Rowe, 2004) and ecological genetics (Lowe et al., 2004).

2.1. Isozymes and the stressful marine environment

Isozymes are electrophoretic variants of an enzyme, expressed in the tissue (mostly leaves are used) at the very moment of collection. Much care is needed during collection and transportation to the lab to maintain the activity of the enzymes until their separation after electrophoresis and subsequent substrate-specific staining of all variants. In practice, this means that one either collects branches with leaves and tries to keep these alive (e.g. in plastic bags exposed to light but not direct sunlight) or to collect single leaves and store these in plastic ziplock bags on ice. Upon arrival, enzymes should be extracted from the leaves and analysed immediately. Alternatively, these must be frozen in liquid nitrogen and further stored, either in liquid nitrogen or at −80 °C (never as high as −20 °C and never unfreeze and freeze again). This is crucial because most allozyme variants will denature at different rates and might lose their activity necessary for detection on a relatively thick gel medium of acrylamide or starch, requiring high amounts of the active enzyme. Cellulose acetate plates are less commonly used but have an advantage of requiring less volume of extract. The abovementioned precautions hindered the development of knowledge on isozyme polymorphism in mangrove trees during an era where many such studies were conducted on plants, including many seagrasses (e.g. McMillan, 1982) and aquatic plants (e.g. Triest, 1991a). The field conditions in the tropics do not always allow such careful handling. Though not reported in literature, there most likely were attempts in several labs throughout the world to reveal active enzymes from mangrove tissues. However, another obstacle for successful analysis of enzyme polymorphism in mangrove species are the secondary metabolites which denature the enzymes during grinding in an extraction buffer, as internal membranes of cell compartments disintegrate and allow contact between those compounds (e.g. phenols, tannins, etc.) and the enzymes. Such secondary metabolites are found in all tissues of most mangrove species and are thus difficult to avoid by searching for alternative tissues than mature leaves. Generally in tree leaves, problematic compounds can be neutralized by adding products that prevent enzymes from oxidation, e.g. polyvinylpyrrolidone (PVP), however, this requires systematic analysis of different concentrations and combinations of such additives, albeit an empirical search for suitable conditions to keep allozymes active. Goodall and Stoddart (1989) reported on such techniques to assess variation within fourteen enzyme systems in *Rhizophora* species, revealing 28 putative loci. A further analysis of five widely separated populations of *Rhizophora stylosa* Griff. showed only little geographic variation. A uniform genetic structure also was observed in isozymes of *Ceriops tagal* (Pers.) C.B. Robinson var. *tagal*, var. *australis* C.T. White and *Ceriops decandra* (Griff.) Ding Hou in northern Australia (Ballment et al., 1988). Isozyme patterns in *A. germinans* were also used to indicate similarities between regions, e.g. western Gulf of Mexico and Texas or dissimilarities, e.g. between the latter regions with Florida and eastern Caribbean (McMillan, 1986). A large-scale isozyme study across Australia, New Zealand, New Caledonia and from western Australia towards Thailand revealed that each of the considered *A. marina* varieties also corresponded to a particular gene flow grouping (Duke et al., 1998a,b). Again, as mentioned above, there most likely were more labs involved in trials on isozyme variability of sufficient enzyme loci, however, experiencing major difficulties to interpret the enzyme patterns in terms of true genes and alleles or resulting in no polymorphism at all.

Similar low or absent enzyme polymorphism was found in seagrasses such as *Zostera* (e.g. Gagnon et al., 1980; De Heij and Nienhuis, 1992; Williams and Orth, 1998), *Posidonia* (Capiomont et al., 1996) and in saltwater tolerant *Ruppia* species (Triest and Symoens, 1991). These aquatic plant groups are thought to display rather low genetic variability, due to extensive clonal spread (McMillan, 1991; Triest, 1991b) and limited hydrophilous pollination (Les, 1988). Both explanations are unlikely for many mangrove trees that are known to be

highly sexual. Both the lack of resolving power and low genetic variability in genes coding for enzymes, actively expressed in leaf tissues, were at the basis of the general suffering to address questions on the genetic structuring of populations and the relationships with geographical and environmental factors. In general, aquatic plants have lower gene diversities than terrestrial plants when considering their enzymes (Hamrick and Godt, 1989; Triest, 1991a). Similar conclusions can be put forward, namely that mangrove trees are enzymatically very uniform when compared to their counterpart, the terrestrial tropical trees that exhibit the highest gene diversities in angiosperms (Hamrick and Godt, 1989). However, the number of case-studies in mangrove populations is not sufficient to allow a significant comparison. Such a more general hypothesis, as stated here – namely flowering plants from saline aquatic environments have lower enzyme polymorphism in their populations – should be further tested with DNA polymorphism, e.g. single nucleotide polymorphism (SNPs) in coding regions of enzyme genes. A comparative study of the genetic divergence of mangrove lineages from terrestrial relatives, already suggested that mangrove diversity is limited by evolutionary transition into the stressful marine environment (Ricklefs et al., 2006).

A particular stress factor for mangrove trees can be the rooting in contaminated sediments of estuaries close to urbanized areas. After assessing the allozyme variability within and among populations of *A. marina* in estuaries of Sydney, Australia, the resulting variability of different age-classes on clean and contaminated sediments was estimated by Melville and Burchett (2002) and Melville et al. (2004). Though it might remain difficult to interpret allozymes accurately in terms of genes and alleles, the presence–absence of each distinguishable allozyme allowed further multivariate approaches of both diversity and differentiation along the same coastline but under different habitat conditions. Allozyme distribution and patterns in leaf morphological attributes appeared to be very similar and allozyme differentiation corresponded to geographic distance (Melville and Burchett, 2002). Although only three groups at a distance of about 20, 60 and 80 km were available for comparison, it could be inferred that limitation in gene flow influenced the allozyme frequencies rather than the selection pressures imposed by the sediment characteristics. More diversity was observed in non-contaminated *A. marina* sites. Three age classes in clean and contaminated sediments showed greater allozymic differentiation among age classes than among sampling sites, however not related to the sediment metal or nutrient levels (Melville et al., 2004). They put forward that genetic distances within a mangrove habitat along a polluted river may reflect past fluctuations in pollution pressures, rather than age-classes as observed in subpopulations from a clean habitat.

Genetic variability analysis based on allozymes are extremely rare for mangrove trees and when achieved, relatively low levels of allelic polymorphism and heterozygosities were revealed, e.g. an expected heterozygosity *He* of 0.026 in *A. corniculatum* (Ge and Sun, 1999). Goodall and Stoddart (1989) found little polymorphism in Australian *R. stylosa* Griff. in only two out of the 28 enzyme loci and very low levels of heterozygosity ($He = 0.033$) were reported for *Kandelia candel* (L.) Druce in Hong Kong (Sun et al., 1998). A nearly complete lack of allozyme variation was found in four out of five investigated species of *Avicennia* (Duke et al., 1998a,b), with *A. marina* var. *marina* ($He = 0.0–0.132$) and related Australian varieties ($He = 0.025–0.217$) as an exception to this overall poverty of enzyme polymorphism in the genus. With expected heterozygosities $He = 0.0293$ and high gene flow levels of $Nm = 3.85$, *Bruguiera gymnorrhiza* (L.) Lamk. populations along the coast of China, present one of the few examples of outcrossing species that combine high rates of sexual reproduction with high amounts of propagule dispersal (Ge et al., 2005).

Allozymes remain reliable codominant markers of expressed genes (Table 1) and thus still have a future in mangrove genetics when field conditions allow careful handling of the collected tissues and when banding patterns of sufficient enzymes can be interpreted in terms of genes and alleles. This coding of unambiguous genotypes allows a whole spectrum of population genetic analysis, comparisons with other factors (morphology, geographical distance and environmental features) and multivariate techniques for the exploration of trends. At all times, one must avoid the interpretation of allozymes as merely phenotypes of banding patterns.

2.2. Dominant markers for identification purposes: is it a one or a zero?

Dominant markers can be defined as DNA fragments, amplified from any plant tissue, that allow to interpret their distribution only in terms of presence–absence coding. Heterozygosities are not readily detectable, though in particular cases, the intermediate intensity of an amplified fragment might indicate a heterozygous condition. This is somehow feasible for observation when dealing with related progeny of known parental origin or in cases of first generation hybrids (F1's). Dominant markers became very successful because these are relatively low cost and do not require knowledge of targeted sequences in the genome of an organism (Table 1). Thus, similar primers (a nearly unlimited series) can be tested on any species without the need for large investments of developing molecular markers. Additionally, DNA techniques are more popular than isozymes because they require only small amounts of leaf or other tissues to be dried on silica, which is a tremendous simplification of the logistics in the field, during transportation and for storage.

Dominant markers such as randomly amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) have their advantages in standard lab procedures, fast procedures on full genomic DNA extracts, but have a major disadvantage in showing no heterozygotes for estimating, e.g. levels of inbreeding (Table 1). An outcome to avoid misinterpretation from searching dominant markers is to combine these with either restriction polymorphisms (PCR-RFLP, polymerase chain reaction followed by restriction fragment length polymorphism, i.e. restriction enzymes that cut

Table 1
Characteristics of molecular marker methods as used in mangrove studies

Characteristic	Allozymes	RAPD	AFLP	ISSR[c]	Microsattelite (SSR)	cpDNA sequences
Level of polymorphism[a]	Low	Medium	Medium	Medium	High	Medium
Dominance[b]	Codominant	Dominant	Dominant	Dominant	Codominant	Haplotypic[c]
Sequence information needed[a]	No	No	No	No	Yes	Yes
Non-invasive sampling[b]	No	Yes	Yes	Yes	Yes	Yes
Start-up costs[a]	Medium[c]	Low	High[c]	Low	High	High
Development costs[a]	Low	Low	Medium	Low	High	Medium
Development time[b]	None	Limited	Limited	Limited	High[c]	Medium[c]
Reproducibility[a]	Medium/high	Low	Medium	Medium	High	High
Integration between labs[c]	Medium	Low	Medium	Medium	High	High
Allelic richness[c]	++	+	+	+	+++	++
Heterozygosity[c]	+++	+	+	+	+++	(++) pop level
Gene flow[a]	+++	(+)	(+)	(+)	+++	++
Inbreeding[c]	+++	–	–	–	+++	–
Individual genotyping[a]	(+)	(+)	+	(+)	+++	–
Population differentiation[a]	+++	++	++	++	++	++
Hybridization[a]	++	++	++	++	+	++
Polyploidy[a]	+++	–	–	–	+	(+)[c]
Phylogeography[a]	–	–	(+)[c]	–	(+)[c]	+++
Phylogeny	(+)	–	–	–	(+)[c]	+++

(+++), excellent; (++), good; (+), moderate; (+), has been used; (−), unlikely to be used or useless.
[a] Adapted from Lowe et al. (2004).
[b] Adapted from Frankham et al. (2002).
[c] Added or adjusted in this review.

the amplified products) or with other methods (e.g. PCR-RFLP of chloroplast DNA or mitochondrial DNA) to infer the maternal inheritance. The latter is necessary to detect the species that acted maternally (egg cell contribution to the formation of zygote) in hybrid formation or to detect the dispersal routes of different variants. RAPD and AFLP are often used for genotyping individuals but have more limitations in phylogeny and large-scale studies due to the possibility of increased product homology (i.e. amplified products of similar length but not similar in their sequence). Difficulties might occur when scoring according to the intensity of the amplified products and creating a data matrix of ones and zeros. The number of amplified fragments and the repeatability of AFLP is clearly higher than for RAPD (Table 1). Mostly no true genetic analysis is performed on RAPD and AFLP data as the scoring of presence–absence of amplified fragments do not allow to quantitatively measure the gene diversities. Estimations of expected heterozygosities are possible when assuming panmixis (Lynch and Milligan, 1994) or when *a priori* assigning a certain degree of inbreeding. Sharing amplified bands can be used to produce a cluster or an ordination plot to show interrelationships between individuals or populations.

AFLP reveals an extremely large amount of polymorphic loci with amplified fragments, thereby increasing the probability that each individual lacks different series of fragments out of the nearly 1000 putative loci. The presence–absence way of interpretation allows to estimate average heterozygosities (mostly supposing an Hardy–Weinberg equilibrium and thus neglecting the reality of possible deviations due to inbreeding, drift or low sample sizes) at (sub)population and species level. The application of RAPD and AFLP, the latter developed for breeding studies, has been widely used. RAPDs are useful at initial stages of an investigation. Both RAPD and AFLP are controversial for use in phylogenetic and phylogeographic studies because the one-zero data matrix cannot be ordered. In gene diversity studies, problems of product homology determination exist and without detailed genetic analysis, the designation of a fragment to a locus may be equivocal (Lowe et al., 2004). Another type of dominant markers, Inter-simple sequence repeats (ISSRs) is increasingly applied since 2000, as it has the potential to show higher polymorphism than RAPD at lower costs than AFLP. However, ISSR have similar limitations for data analysis as the former dominant marker methods (Table 1). Basically, the method involves amplification of regions between adjacent, inversely oriented microsatellites using a single simple sequence repeat (SSR-) containing primer. RAPD, AFLP and ISSR are considered to be reliable methods in F1 hybrid detection or in confirming the absence of first generation hybrids (Table 1). The relevance of using dominant markers (RAPD, AFLP and ISSR) for assessing genetic diversity within and among individuals, subpopulations or populations within a considered area – usually much smaller than the species range – especially lies in providing ordination plots of individual genotype distances, clusters of (sub)populations on basis of their averaged genetic distances, analysis of molecular variance (AMOVA) within and between populations relative to the total, statistics and analogues tested by random permutation.

2.2.1. *Species characterisation and relationships: can order be obtained out of the unordened?*

Fingerprinting with dominant markers (RAPD, AFLP and ISSR) are elegant techniques when the studied species are much related and when these species occur in the same biogeographical region. Otherwise, the risk of encountering product homology increases and may underestimate the measures of

diversity due to amplified DNA fragments of similar length that are not homologous or contain substantial amounts of single nucleotide substitutions and insertion–deletions. To avoid this disadvantage, the amount of fragments is often increased, however this is not a real solution to the problem as it also increases the probability of touching upon more fragments showing product homology. Despite these disadvantages, it appears feasible to use dominant markers to confirm the existence of a taxon (at species level or lower) and to infer their degree of relationship to a certain level. However, the resulting phenograms as UPGMA clusters (unweighted pair-wise grouping method using averages) rarely can be considered as phylogenetically very accurate methods when compared to the potential of sequence data for phylogenetic analyses (Table 1).

AFLP proved to be useful in several case-studies to ascertain the status of a species. A large-scale study of *A. germinans* across the Pacific coast (from Baja California to Peru), the Atlantic coast (from Bahamas to Brazil) and western Africa, supported the justification of a single species across these biogeographical regions (Dodd et al., 2002), thereby rejecting the concept of a separate species *Avicennia africana* P. Beauv. along the eastern Atlantic coast or even any other lower taxon differentiation. AFLP characterisation of mangrove tree species and their relationship was performed for *Heritiera formes* Buch-Ham., *Heritiera littoralis* Dryand. and *Heritiera macrophylla* Wall. from India (Mukherjee et al., 2003). RAPD based relationships in legume species from mangroves in India were studied beyond species level (in fact rather distant genera) in *Dalbergia spinosa* Roxb., *Derris heterophylla* (Willd.) Backer, *Derris indica* (all three belonging to the subfamily *Papilinoideae*), *Caesalpinia crista* L. and *Cynometra ramiflora* L. (both of the subfamily *Caesalpinioideae*), which evidently clustered the subfamilies and subclustered the two *Derris* species, alongside with delivering the expected species-specific markers (Jena et al., 2004). Within family, relationships of eleven *Rhizophora* species using RAPD and AFLP also evidently showed a high degree of genetic divergence among the taxa and supported the morphologically based classification at tribe, genus and species level, except for *Bruguiera* and *Rhizophora* (Mukherjee et al., 2004). Additionally, attempts with RAPD and AFLP across families showed the expected relationships of 31 mangrove species as known from classical taxonomy, though at this level of higher taxonomic ranks, many unrelated mangrove species form clusters (Mukherjee et al., 2006). This is not surprising because the problem of product homology and the larger amount of non-shared amplified fragments might increase substantially. RAPD and PCR-RFLP (of nuclear DNA and chloroplast DNA) of the tribe *Rhizophoreae* in trees from India showed that the within-species variability was low (from RAPD data) and that species divergence was more elucidated with chloroplast gene regions than with ribosomal DNA repeat units of the nuclear DNA (Lakshmi et al., 2002). In my opinion, it is not recommended to use RAPD, AFLP or ISSR for constructing phylogenetic trees of taxa at species level and higher unless supplemented with sequences of chloroplast genes or other informative nuclear intron or exon sequences.

2.2.2. *Straightforward hybrid detection though F1's remain hard to find*

Hybrid mangrove trees and intermediate morphologies may present problems when there is a need to accurately identify for both field relevées as for *a posteriori* herbarium taxonomy. The existence of hybrids is mostly inferred from morphology by inventorying intermediate features or encountering putative hybrid vigour. Though hybridisation is supposed to occur between several mangrove tree species (Duke, 1984; Zhou et al., 2005), relatively few studies have concentrated on the identification of hybrids in populations. For this purpose, dominant markers can be applied effectively when the parents (or representatives of the parental species) are known, because the first generation hybrids must show a combined or additive pattern of amplified DNA products. This imperatively becomes less valid when introgressive hybridisation took place.

Hybrids between *Rhizophora apiculata* Blume and *Rhizophora mucronata* Lamk. were detected with both RAPD and PCR-RFLP of mitochondrial DNA at the eastern coast of Tamil Nadu, India (Parani et al., 1997). Hybrid detection is facilitated when the interpopulational diversity of each species (as spatially separated pure ones) and of the F1 population is low, thereby enhancing the probability to observe overall unique markers at species level for subsequent targeted hybrid genotyping. The use of dominant DNA markers at species level can be ideal to identify the hybrid status of populations and especially of the seedlings and young trees that lack sufficient diagnostic features in their morphology at that developmental stage. Lakshmi et al. (2002) found with PCR-RFLP of chloroplast genes that *R. mucronata* was the chloroplast donor for a natural hybrid (Pichavaram, India). Clear discrimination between two species and their hybrids was not only successful in the abovementioned *Rhizophora*, but also in *Sonneratia* × *gulngai* N.C. Duke (=*Sonneratia lanceolata* Blume × *Sonneratia alba* Smith) and *Sonneratia* × *hainanensis* W.C. Ko in Hainan, China. The latter putative hybrids showed little morphological variation and turned out to be all F1's, respectively with *S. alba* J. Smith and *Sonneratia caseolaris* (L.) Engl. as parents for *Sonneratia* × *gulngai* and *S. alba* and *S. ovata* for *Sonneratia* × *hainanensis*. Introgressive hybridisation was not observed and neither hybrid type deserved the species status because these were not self-sustaining populations (Zhou et al., 2005). Putative morphological hybrids at individual level also may turn out to be representatives of morphological variable species instead of true genetic hybrids. This was found in mixed populations of *Bruguiera sexangula* (Lour.) Poir. and *B. gymnorrhiza* along the western Sri Lankan coast (Abeysinghe et al., 2000). No hybrid *Bruguiera* individual was detected with RAPD, despite intermediate flower characteristics (Abeysinghe et al., 1999).

Similarly, enzymes that show uniform patterns within a taxon but high levels of genetic divergence among taxa, are very practical situations to detect whether or not hybridisation is involved in a morphological species complex e.g. *C. tagal* var. *tagal*, var. *australis* and *C. decandra*, that showed no sign of hybrid formation, even in sympatric areas (Ballment et al., 1988). On the other hand, closely related *Rhizophora* species are

supposed to hybridize in sympatric areas, without showing distinct morphological forms, but as ecotypes with differing flowering period and niche specialisation, e.g. between *R. stylosa* and *R. mucronata* in the region from South East Asia to the North West Pacific Ocean and Northern Australia (Duke et al., 2002). In general, one could question whether pollination barriers mostly prevent formation of hybrids among related mangrove tree species. True hybrids are most likely rare and difficult to observe.

2.2.3. Gene diversities in small sample sizes from distant areas

Species that are widespread across oceans and continents, may include evidence on genetic diversity, genetic differentiation and genetic distance to illustrate the relative effects of continental drift; barriers for dispersal eventually resulting in cryptic species boundaries within the range of a morphological species; regional differentiation as a result of lowering of sea level during the recent Pleistocene glaciations; and ultimately provide evidence for conservation priorities at a regional scale. An extensive study carried out by Dodd et al. (2002) and Nettel and Dodd (2007) on the genetic diversity of *A. germinans* using AFLP amongst other markers, revealed that long-distance dispersal remains a valid hypothesis for this species. Although the number of rare and unique AFLP fragments was significantly higher for populations along western Africa when compared to those of the eastern Atlantic and western Pacific, these authors found a closer relationship of the former with French-Guinean populations. This suggests historical gene flow events over long distances, even when low. UPGMA clustering and unrooted NJT (neighbour joining tree) between pairs of populations gave sufficiently high bootstrap supports for accepting a major division between Atlantic and Pacific populations. The close relationship between *A. germinans* from western Africa and the eastern Atlantic coast was supported better when adding populations from Brazil. In my opinion, this indicates that the choice of sampled populations has an important influence on the interpretation of dominant markers especially when using small sample sizes, ranging from 4 to 20 per site, for conducting large-scale studies across oceans.

ISSRs have often been applied for the comparative study of genetic variability of mangrove populations across large geographical ranges. Despite the often low sample size of a population (10–20 individual mangrove trees), when pooled into regions, significant differences in genetic diversity estimates between regions were obtained. When the distribution area of a species is not fully covered (or with a non-representative subsampling) and only very distant populations across continents are compared, then the obvious and mostly *a priori* expected outcome with dominant markers is that clearly divided clusters per geographical region will be obtained from the calculated genetic distances, e.g. in *H. littoralis* Dryand. from China and Australia with sample sizes 10–20 per site (Jian et al., 2004); in *C. decandra* (Griff.) Ding Hou from East Malaya, West Malaya, southernmost Malaya and North Australia with sample size 7–22 per site (Tan et al., 2005); in *Lumnitzera racemosa* Willd. from the South China sea, East Indian ocean and North Australia with sample sizes 6–16 (Su et al., 2006) and similar areas (sample size 16–24) plus Sri Lanka (sample size 2) for *Lumnitzera littorea* (Jack) Voigt (Su et al., 2007); *C. decandra* (Griff.) Ding Hou (sample size 6–22), *C. tagal* (Perr.) C.B. Robinson (sample size 8–20) from the east and west coast of Thailand and the distant island Hainan (Ge and Sun, 2001), *C. tagal* (sample size 8–16) from the South China sea, East Indian ocean and North Australia, with sufficient bootstrap values in the NJT (Huang et al., 2007). Similarly for RAPD and ISSR, many studies were on low sample sizes, e.g. RAPD of *Acanthus ilicifolius* L. (sample size 5–7 in eight populations) and *Excoecaria agallocha* L. (sample size 12 in seven populations) along the east and west coast of peninsular India (Lakshmi et al., 1997; Lakshmi et al., 2000); *R. apiculata* Blume (10 samples in six populations) from India with one deviating population showing low polymorphism, most likely due to small sample sizes (Lakshmi et al., 2002); and ISSR in *S. alba* J. Smith from five populations in Hainan Island, China (Li and Chen, 2004).

The use of dominant markers is less appropriate for inferring reproductive strategies, outcrossing rates and local patterns of gene flow, due to the absence of heterozygote detection—a prerequisite for calculating deviations from the equilibrium. Nevertheless, a few attempts were made on mangrove trees to explore such possibilities. *A. corniculatum* from Hong Kong and other sites in southern China, showed substantial genetic differentiation in ISSRs between populations despite the relatively high levels of polymorphism (sample size of 15 individuals in 10 populations). This species has a mixed-mating to outcrossing system and the observed patterns might indicate the rare success of dispersal, however with sufficient gene flow through water-dispersed seedlings, thereby maintaining high diversities in the local populations (Ge and Sun, 1999). High levels of RAPD polymorphism were observed in *B. sexangula* (sample size 18–23 in three populations) from Southwestern Sri Lanka (Abeysinghe, 2000). Five populations of *A. germinans* along the coast of Mauretania (sample size 18–22) also showed high levels of polymorphism with only a moderate differentiation ($F_{ST} = 0.186$) at 60 km distance (Abeysinghe, 2000). At a very local scale, e.g. the disjunct zonation pattern of *A. marina* in Gazi bay (Kenya) RAPD allelic frequencies were used to observe significantly deviating frequencies between these two subpopulations. This fine-scaled approach allowed to demonstrate that seaward and landward populations (sample size 37) may have significantly different allele frequencies – four out of 48 – in each habitat, suggesting that restricted gene flow is possible at distances as short as 300 m (Dahdouh-Guebas et al., 2004). At a much shorter distance of only 100 m, *R. mucronata* showed no significant differences between a seaward sand ridge and a somewhat more landward site within Gazi bay (Abeysinghe, 2000).

Avicennia is the most studied genus among mangrove trees whereas the gene diversity assessment in other tree species was approached mainly once in a case-study. A thorough AFLP study combined with codominant SSR markers (see further) was achieved on *A. marina* in Australia (Maguire et al., 2002). AFLP was considered as a reliable and fast technique for delivering a large amount of marker fragments (nearly 1000) to distinguish individuals, thereby rendering AFLP useful in

applied programs such as the monitoring of propagation in nurseries and identifying duplicates in collections. Besides providing a huge number of multilocus genotypes at individual tree level, AFLP also allowed to separate (sub)populations because of lower amounts of variance at higher geographical levels. AFLP thus revealed a large amount of putative loci of which a large proportion is polymorphic, i.e. the absence of an amplified fragment, indicating strong genetic structure with one group of populations in close vicinity being more related to each other than to other groups at larger geographical distances, sometimes including a "deviating" population due to lower sample size, e.g. *A. marina* in northern (sample sizes of 24–25), central (11) and (11–27) southern Vietnam (Giang et al., 2003); *A. germinans* along the Colombian coast with sample sizes of 10–12 in four populations though corrected for small sample sizes (Cerón-Souza et al., 2005); *Pelliciera rhizophorae* Triana & Planchon along the Colombian coast with samples sizes of 8–10 in six populations (Castillo-Cárdenas et al., 2005).

A meta-analysis of total gene diversities estimated from sufficient AFLP markers in *A. marina* (Maguire et al., 2000a,b; Giang et al., 2003), *A. germinans* (Dodd et al., 2002; Cerón-Souza et al., 2005; Nettel and Dodd, 2007) and *Pelliciera rhizophorea* (Castillo-Cárdenas et al., 2005) reveals that, on average, groups of central populations have *He* around 0.2 or higher, whereas a group of peripheral populations has *He* <0.1. Large-scale studies including both central and peripheral populations show intermediate values (Fig. 1). When adding more peripheral populations to a study, the total gene diversity of the species tend to become lower. There also seems to be a relationship (no significant positive correlation) between the considered percentage of polymorphic loci and their respective gene diversities (Fig. 2). A low proportion of polymorphic loci as well as a low gene diversity was found in a group of populations at the edge of a species range.

In my opinion, and based on the sampling strategies as argued by Lowe et al. (2004), dominant marker studies to infer long-distance dispersal in mangrove trees should be conducted with a sufficient large sample size (e.g. 20 or more individuals per population) because the mean number of alleles per gene is low to very low in mangroves trees. The sampling design should cover as much as possible the geographic range of the considered species because the aim is to detect unique alleles that are often at very low frequencies. Alternatively, a larger

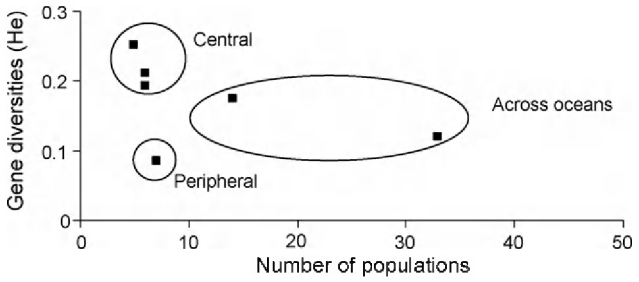

Fig. 1. Gene diversities for the total population in relation to the number of populations studied from central, peripheral and global ranges on basis of AFLP data from Dodd et al. (2002), Maguire et al. (2002), Giang et al. (2003), Cerón-Souza et al. (2005), Castillo-Cárdenas et al. (2005) and Nettel and Dodd (2007).

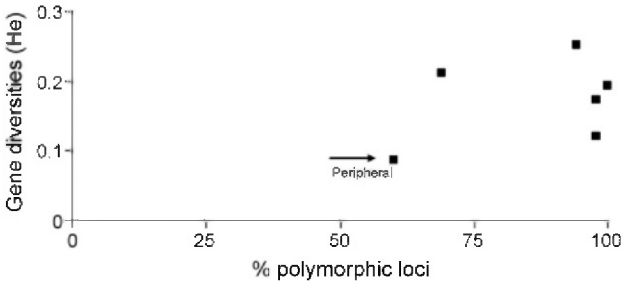

Fig. 2. Gene diversities for the total population in relation to their polymorphic loci on basis of AFLP data from Dodd et al. (2002), Maguire et al. (2002), Giang et al. (2003), Cerón-Souza et al. (2005), Castillo-Cárdenas et al. (2005) and Nettel and Dodd (2007).

sample size per population (e.g. 30–50) for a coastal transect, covering only a part of the species range, is required for genetic structure analysis, local patterns of diversity, differentiation and inferring gene flow within metapopulations at a few hundreds of kilometers distance. Much criticism is necessary when trying to estimate gene diversities and genetic structuring of mangrove tree populations from few individuals in few populations at large distances (>1000 km), though such exploratory studies may evoke some new ideas to be tested.

2.3. Codominant microsatellite markers: highly variable but not in all species

Microsatellite markers, also named SSR (simple sequence repeats) are short tandem repeats of mono- to tetra-nucleotide repeats, which are assumed to be randomly distributed in the nuclear genome. Such SSRs are relatively abundant and have high mutation rates in comparison to other markers, which make them useful for various types of population studies (Lowe et al., 2004). An enormous advantage is that the exact designation of alleles (their length) to a known locus allows to standardise information between laboratories thereby making worldwide studies fully integrative (Table 1). This is not feasible with RADP, AFLP and ISSR. In mangrove trees, only recently the development of SSRs in a few species could facilitate studies on the molecular ecology of their populations with a much greater accuracy than allozymes and with much more analytical power than the dominant markers at both the individual level and for the *a priori* grouped assemblages of (sub)populations. The sample size needed to have a 95% probability of encountering at least one copy of each allele in a gene depends heavily on the frequency of the rarest allele in the population. At sample sizes of 50 individuals, this probability is reached in a two-allelic system if the most common allele has a frequency of 0.95 (Lowe et al., 2004). In most SSR loci, the most common allele has a lower frequency, thereby making it possible to detect more than two alleles at sample sizes as low as 50 individuals per population.

2.3.1. Allelic and genetic diversity in Avicennia microsatellites

Sixteen SSR primers were developed for *A. marina* by Maguire et al. (2000b). Three of these were used in a large-scale

study with gene diversities *He* ranging from 0 to 0.85. Reduced values were towards the extremes of the species range, e.g. southern Africa, southern Australia and Japan (Maguire et al., 2000a,b). Such lower levels can be the result of founder effects and environmental constraints. Additional studies on *A. marina* from the northern edge of its range in Vietnam, showed a narrower range of *He* values (0.23–0.40 with population sizes from 11 to 30) in four SSR loci (Giang et al., 2003) and lowered *He* values (0.09–0.35), with population sizes from 19 to 34) when using seven SSR loci (Arnoud-Haond et al., 2006), thereby supporting the previous launched hypothesis on reduced gene diversity at range edges. Additional development of 10 microsatellite primers in *A. marina* and preliminary testing on 40 individuals in a population from Hainan, showed *He* values of 0.096–0.767 at locus level (Geng et al., 2007). The availability of 26 SSRs will allow even more accurate estimations of the allelic and genetic diversity in the near future. Despite this large amount of loci, the number of alleles per locus is often as low as two or three, thereby confirming the general opinion of low allelic diversity raised from earlier allozyme studies in mangrove trees. There is a high probability of encountering unique alleles combined with low or no polymorphism in some parts of the distribution range of *A. marina*. As the variable microsatellite regions were developed and initially selected for polymorphism in 15 individuals from three populations of Australian source material (Maguire et al., 2000b) and more recently from a Chinese source (Geng et al., 2007), this choice might potentially skew the resolution of revealing microsatellite polymorphism along other coastal areas such as South Africa (Dheopursad and Lamb, 2006). The absence of polymorphism in SSRs of Japanese populations might be indicative of this phenomenon resulting from biased initial screening. Out of the six polymorphic SSR loci, only one could be cross-amplified in four *Avicennia* species (*A. marina*, *Avicennia alba*, *A. rumphiana* Hallier f. and *A. officinalis* L.) but none in *A. germinans* (Maguire et al., 2000b), illustrating their limitation for a direct comparative analysis between several species, even when morphologically related. Six polymorphic microsatellite loci were *de novo* developed and made available for *A. alba* Blume which were tested on 36 individuals from the Mekong delta in Vietnam. Cross-amplification with *A. marina* revealed either monomorphic loci or no amplification at all (Teixeira et al., 2003). Ten polymorphic SSR loci were selected for *A. germinans*, of which nine also yielded amplification products in *Avicennia schaueriana* Stapf & Leech., five in *A. alba* and three in *A. marina* (Nettel et al., 2005) while six additional primers for *A. germinans* (Cerón-Souza et al., 2006) were developed from a source population in Puerto Rico. All these attempts clearly illustrate the extremely specific nature of microsatellites in the genomes of this genus and the need for additional microsatellite loci in search of sufficient polymorphism.

2.3.2. Microsatellite primer developments in several genera

Three out of the 10 SSR primers developed for *Rhizophora mangle* L. by Rosero-Galindo et al. (2002) were used by Arbeláez-Cortis et al. (2007) in five Colombian populations (populations sizes of 16–21). *He* values were high (0.601–0.725) but no unique alleles were detected. Genetic differentiation along the Colombian coast was low (R_{ST} values—analogous to F_{ST} were only 0–0.16), suggesting high amounts of gene flow, even over 400 km distance. SSRs for >10 species were recently published giving way to obtain more information on the diversity and genetic structuring of populations. Seven SSRs are available for *B. gymnorrhiza*, developed from Japanese source material, of which five SSR primers cross-amplified with *Bruguiera cylindrica* (L.) Blume and *Bruguiera parviflora* (Roxb.) Wright & Arnold ex Griff. (Sugaya et al., 2003); a single SSR with six alleles for *Kandelia obovata* Sheue, Liu & Yong from Japan (Harada et al., 2005); eight SSRs for *P. rhizophorae* Triana & Planchon from Colombia (Castillo-Cárdenas and Toro-Perea, 2007); five SSRs for *R. stylosa* (Islam et al., 2004), eight SSRs for *K. candel* (Islam et al., 2006a) and four SSRs, revealing 54 alleles in six populations of *K. obovata* and *K. candel* (Giang et al., 2006); and 10 additional primers for *B. gymnorrhiza* (Islam et al., 2006b); eight SSRs for *A. corniculatum* and nine SSRs for *S. caseolaris* from China (Chen et al., 2007a,b). Development of SSRs on these genera were achieved during the last years and still need to be validated in case-studies with ample materials to solve hypothesis driven research questions in the field of mangrove genetics.

2.4. Chloroplast DNA and mitochondrial DNA as clearcut haplotypic markers

Organel DNA mostly is maternally inherited but this should however not just be assumed but be tested for each species because exceptions to the "rule" might exist. Chloroplast DNA (cpDNA) is especially informative in phylogeny (e.g. maturase sequences of *matK* in Rhizophoraceae; Shi et al., 2002), species identification, phylogeography and hybrid detection (Table 1). Few population studies on mangrove trees involved chloroplast DNA, either sequencing variable intron regions or applying restriction enzymes (PCR-RFLP) to detect site variability. Lakshmi et al. (2002) found that *R. mucronata* was the chloroplast donor for a natural hybrid from Pichavaram, India. PCR-RFLP of cpDNA was applied for distinguishing species and estimating relationships in a few *Rhizophora*, *Ceriops* and *Bruguiera* species (Lakshmi et al., 2002). No differences in size and restriction patterns of cpDNA were found for *B. gymnorrhiza* and *B. sexangula* populations in distant sites of Southwestern Sri Lanka (Abeysinghe, 2000). PCR-RFLP of cpDNA was especially successful in a large-scale study of *A. germinans*, clearly separating phylogeographical regions such as Pacific coasts of Panama, Mexico, Costa Rica, Atlantic coasts along Central America, Florida, Caribbean coasts and the strikingly related haplotypes of the East Atlantic (French Guyana, Brazil) with those from western Africa (Nettel and Dodd, 2007).

Sequences of cpDNA were helpful in revealing phylogeographical patterns in *K. candel*, namely two distinct lineages – one in South China, Vietnam and East China Sea region

(Taiwan, Japan) and another in the southern China Sea region (Sarawak) – with low levels of genetic differentiation within each phylogeographical unit, indicating long-distance dispersal of maternal haplotypic variants across oceans between continents as well as island populations (Chiang et al., 2001). A study on a *matK* region of about 1500 bp length in *A. marina*, revealed that four indels (insertion–deletions) and two nucleotide substitutions distinguished Vietnam populations from those of Okinawa, Japan, whereas only one indel and one substitution separated populations from northern and southern Vietnam (Kado et al., 2004). *K. candel* also showed a clear haplotype discontinuity between northern and southern Vietnam (Kado et al., 2004), so there might be more mangrove species showing such distinct seed dispersal routes. Intrar-egional or intrapopulational cpDNA variation appeared to be low or absent. In some species there is no genetic variation in the *matK* region, e.g. *L. racemosa* from Vietnam and Japan (Kado et al., 2004). Rare and recently evolved cpDNA variants in *K. candel* were restricted to marginal populations in the northern part of Southwest Asia. CpDNA sequences in *C. tagal* revealed very different haplotypes (28 changes in nucleotides of two introns of totally 855 bp) on each side of the Malay Peninsula, a land mass considered as an actual and historical barrier to gene flow. The Indian Ocean haplotypes appear to be derived from the haplotypes present in the South China sea (Liao et al., 2007), though sample sizes were low (2–10 per population) and therefore is rather indicative than conclusive. Equally low sample sizes of <10 in a very similar study on *C. tagal* and *C. decandra*, using another intron of cpDNA revealed a similar pattern, namely the occurrence of different haplotypes from southern China, the South China Sea (Borneo, Malay Peninsula), the East Indian Ocean and northern Australia (Huang et al., 2007). Such a highly significant structure was attributed to the geological events during and just after the recent Pleistocene glaciation, when the maximum sea level dropped down to the Sunda Shelf. This land bridge separated the East Indian Ocean from the South China Sea refugia and allowed accumulation of different mutations in the cpDNA introns. It can be hypothesized that the closer relationship between *Ceriops* populations from Borneo and the Eastern Malay Peninsula could be the result of a gradual dispersal of propagules along the changing coastline of the gradually flooded Sunda Shelf at the end of the glaciation period. Additional phylogeographical information from cpDNA of mangroves across land or oceanic barriers most likely will be obtained in the near future.

3. Biogeographical patterns unravelled with molecular markers

The large range studies with AFLP and ISSR markers show that long dispersal distribution is possible (at least for *A. germinans*) and that genetic differences might correspond to a large biogeographical oceanic unit. Rather surprising is the observation that the ISSR and AFLP studies of populations from very distant locations – thousands of km – are not suffering from product homology and continue to provide evidence for the geographical separation at regional and oceanic level. This constancy can be attributed to the rather low regional genetic diversity within mangrove trees, thereby placing more emphasis on the interregional differences than the interpopulational levels. Up to now, none of such studies could demonstrate that pairwise genetic distances obtained from AFLP or ISSR were significantly correlated with the geographic distances.

A comparison of the gene diversity components *Ht* (total gene diversity), *Dst* (gene diversity between populations) and *Hs* (gene diversity within populations) of *C. decandra* (Huang et al., 2007), *L. racemosa* (Su et al., 2006), *L. littorea* (Su et al., 2007), *H. littoralis* (Jian et al., 2004), *C. tagal* (Huang et al., 2007) and *Ceriops australis* (Huang et al., 2007; Ge and Sun, 2001) revealed that most of the dominant marker based genetic diversity is between the populations for most cases (Fig. 3), except for *H. littoralis* and *C. australis*, most probably as a result of too low sample sizes or too restricted area of sampling in the latter. The importance of the sampled range is demonstrated with a meta-analysis of the molecular variance (AMOVA) and considered for three sets of literature data, i.e. the variance within a population, the variance between populations from the same coastline and the variance between distant populations across seas (Fig. 4). *Ceriops* species appear to have most of their variance across seas. Case studies with distant populations but from the same coastline show a large proportion of their molecular variance within the local populations, e.g. *C. australis* (Huang et al., 2007), *H. littoralis* (Jian et al., 2004), *P. rhizophorae* (Castillo-Cárdenas and Toro-Perea, 2007), *B. gymnorrhiza*, *B. sexangula* (Abeysinghe, 2000) and *A. germinans* (Abeysinghe, 2000; Dodd et al., 2002).

Though not conclusive with dominant markers for only a few species, these findings nevertheless indicate a more general pattern appearing in the distribution of genetic diversity of mangrove trees. It can be further hypothesized that when considering populations from the same coastline, the largest amount of genetic variation will be within the populations rather than between, which most likely is related to the dispersal routes of propagules along with prevailing water currents. When considering populations from different continents, opposite sides of a continent or a peninsula, and from islands then it can be hypothesized that the largest amount of genetic variation will be between the populations.

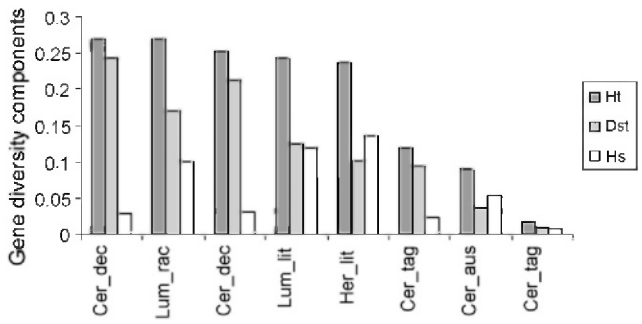

Fig. 3. Gene diversity components for total (*Ht*), between (*Dst*) and within (*Hs*) diversity of ISSR and AFLP markers from eight cases on six species—data from Ge and Sun (2001), Jian et al. (2004), Tan et al. (2005), Huang et al. (2007) and Su et al. (2007).

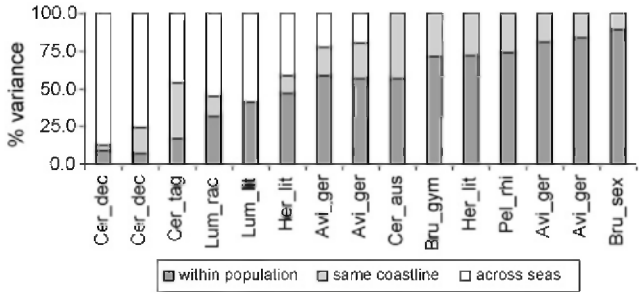

Fig. 4. Comparison of analysis of molecular variance results of ISSR and AFLP markers from 12 cases on nine species at three geographical levels—data from Dodd et al. (2002), Jian et al. (2004), Tan et al. (2005), Cerón-Souza et al. (2005), Castillo-Cárdenas et al. (2005), Su et al. (2006, 2007), Nettel and Dodd (2007) and Huang et al. (2007).

3.1. Do large oceanic barriers exist in AEP and IWP?

Two major disjunct patterns of mangrove distribution can be recognized. The Atlantic-East Pacific (AEP) with low species diversity (<10) and the Indo-West-Pacific (IWP) with higher species diversity in the Indo-Malaysian region (up to 40), though lower in the Western Indian Ocean (<10). The evidence of this difference between AEP and IWP is further supported by the absence of shared species, e.g. within the genera *Avicennia* (Duke, 1995) and *Rhizophora* (Duke et al., 2002). The richer biodiversity of the Indo-Malaysian region generally is explained by the more complex drift of tectonic fragments. The historical changes in the IWP are therefore considered to be more complex than in the AEP (Briggs, 1987). This hemispheric disjunction and the richer biodiversity in the IWP is generally accepted and no matter of debate. However, there are differing views on the importance of long-distance dispersal (LDD) to explain the actual distribution of species.

One view is that LDD remains limited because of major dispersal barriers, both land and water barriers (Duke et al., 2002), though this is species-dependent, with, e.g. *Rhizophora* being more mobile and having longer surviving propagules than *Avicennia*. Especially the idea that dispersal limitations can be finite across open water evoked an interest to study individual taxa across their entire distributional ranges for a better understanding of the earlier, historical dispersal of modern mangrove species. LDD ability is expected to vary with each taxon and there is a plea for coordinating genetic and morphological sampling (Duke et al., 2002). Establishment is primarily temperature limited whereas propagule dispersal is determined by ocean circulation patterns with cold water currents skewing the species range towards the equator and warm water currents towards or beyond the tropic of cancer or capricorn (Fig. 5). North equatorial ocean currents did not bring mangrove propagules to the Eastern Pacific Ocean islands despite available niche habitats. Land barriers such as the African continent and the Caribbean Atlantic Isthmus (CAI) are

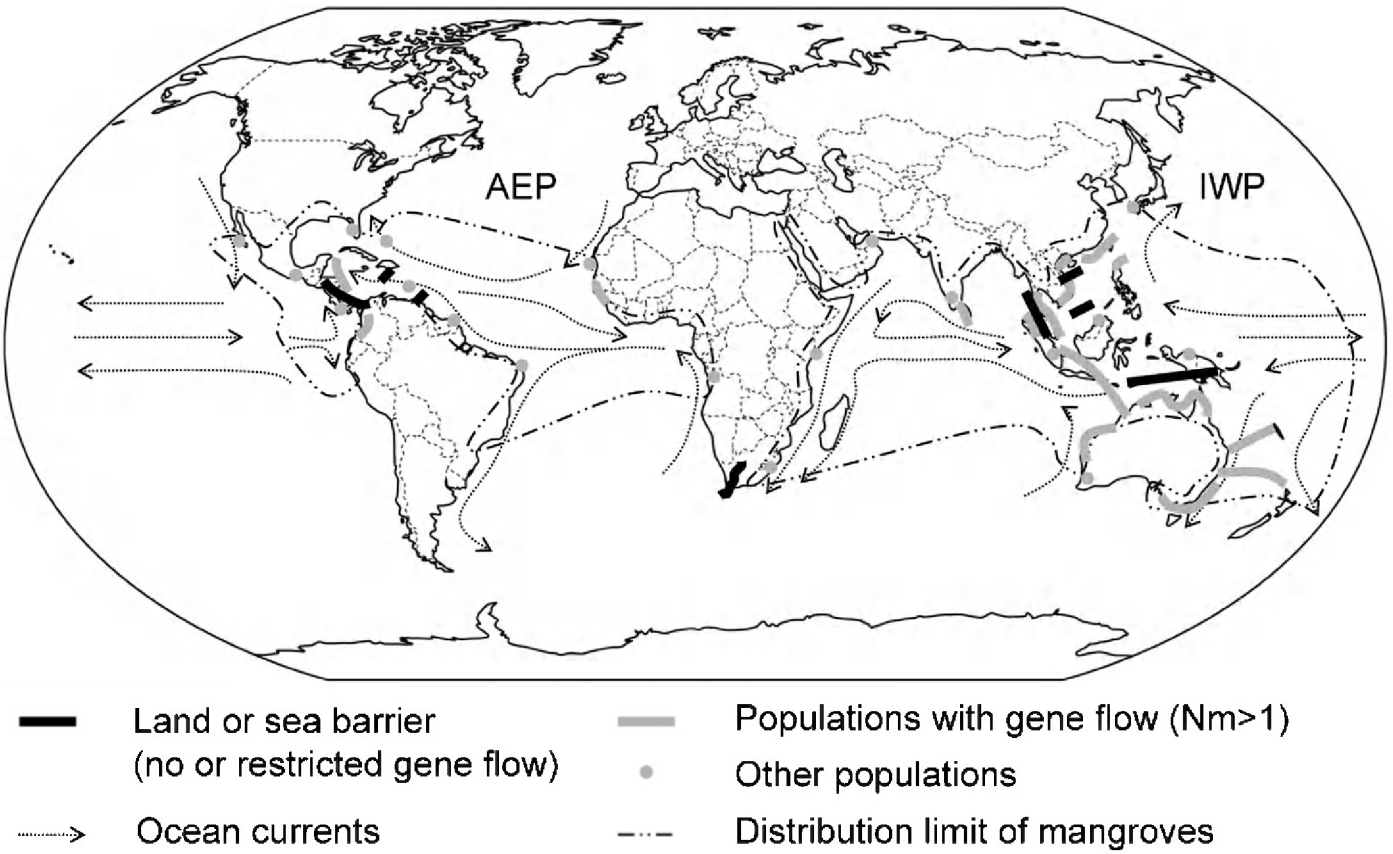

Fig. 5. Conceptual overview of land and oceanic barriers and of coastal zones with high levels of gene flow within the Atlantic East Pacific region and the Indo West Pacific (values and references are given in Tables 2 and 3).

generally accepted (Dodd et al., 2002; Duke et al., 2002; Nettel and Dodd, 2007). However, different views on basis of different taxa were raised on the importance of oceanic barriers in both AEP and IWP, i.e. the Atlantic Ocean separating West Africa from South America; the Western Indian Ocean and arid Middle East from the rest of the IWP; Australasia from the rest of the IWP (Fig. 5).

3.1.1. Trans-Atlantic dispersal

Duke et al. (2002) hypothesized both the Atlantic Ocean, though common species assemblages occur in the three subregions of the AEP (East Pacific, West Atlantic and East Atlantic) and the Indian Ocean to be an effective current barrier. Recent findings on *A. germinans* in the AEP were conclusive about the historical gene flow across the Atlantic. Dodd et al. (2002) and Nettel and Dodd (2007) found clear evidence for a close genetic relationship between populations from West Africa and South America using AFLP, cpDNA haplotypes and ITS sequences. Though Dodd et al. (2000) revealed closer similarities between populations of Atlantic South America and those of West Africa than with those of Atlantic North America, the resulting geographic pattern was still considered as ancient vicariance events (as put forward by Tomlinson, 1986; Duke, 1995). In the latter scenario, a higher degree of genetic differentiation should be observed after continental drift. When assuming historically more recent trans-Atlantic dispersal, only a low degree of genetic divergence should be expected. The large scale phylogeographical study of Nettel and Dodd (2007) confirmed such a scenario of historical LDD, most likely due to the strength and direction of the equatorial Atlantic ocean current during the Quaternary.

3.1.2. Barriers to dispersal and gene flow

Whereas the historical LDD across the Atlantic appears to be evident for *A. germinans*, such a LDD does not seem to hold for *A. marina* in the IWP (Maguire et al., 2000a,b; Duke et al., 2002). The Indian Ocean is considered as the only effective present-day barrier on basis of both species composition (East African mangrove communities are a subset of more species-rich mangroves in the East Indian Ocean and beyond) and of genetic evidence on *A. marina* across its range (Maguire et al., 2000a,b). A high number of unique alleles in each of the distant populations from South Africa, United Arabic Emirates, India and the Malaysian-Australasian region, allowed to put forward the idea of the Indian Ocean as a historical and present-day barrier. The historical changes of continental drift were more complex in the IWP than in the AEP (Briggs, 1987). Duke et al. (2002) suggested that current gene flow might exist between the Southeast Asian archipelago to Australasia and that exchange of genes through dispersal might occur in the EIO via India and Middle East. This then might correspond to a discrete metapopulation model to be expected along the coastal zone of the Middle East and Africa, following the southward Aguilhas ocean current. The existence of discontinuities in the distribution of taxa is used to support the idea that propagules are not so well adapted for long-distance dispersal (Duke et al., 1998a). However, this reasoning should not exclude the ability of LDD, but that sufficient gene flow (in fact seed flow) across the coastal areas is limited by the strength of particular equatorial counter currents and by the influence of large oceanic catchments.

On basis of the recent literature, one may conclude that several discontinuities exist in the IWP. Supported by evidence of absent gene flow (maternal seed flow) as detected especially with cpDNA, in addition to AFLP, ISSR and isozymes, the following barriers can be considered for further testing: between the EIO and Northern Australia; between New Guinea and Australia; between EIO and the Southern China Sea (SCS) due to the Malay peninsula land barrier; between SCS and Sarawak; between northern and southern Vietnam (Table 2, Fig. 5). Although the discontinuity in the Indian Ocean is supported by only one SSR study on *A. marina* (Maguire et al., 2000a,b), there is now ample evidence for restricted gene flow in the other Southeast Asian barriers for a number of species

Table 2
Evidence from literature on barriers and much restricted gene flow between regions (CAI = Central American Isthmus; CAR = Caribbean; EIO = East Indian Ocean; EP = East Pacific; ME = Middle East; NA = North Australia; SCS = South China Sea; WA = West Atlantic; WIO = West Indian Ocean)

Barrier	Gene flow	Species	Marker	Reference
CAI	Absent	*Avicennia germinans*	AFLP	Dodd et al. (2002)
CAI	Absent	*A. germinans*	cpDNA	Nettel and Dodd (2007)
WA vs. CAR	Restricted	*A. germinans*	cpDNA	Nettel and Dodd (2007)
EP (Mexico vs. Panama)	Absent	*A. germinans*	cpDNA	Nettel and Dodd (2007)
Malay Peninsula	Absent	*Ceriops tagal*	cpDNA	Liao et al. (2007)
Malay Peninsula	Absent	*Ceriops decandra*	ISSR	Ge and Sun (2001)
New Guinea vs. Australia	Restricted	*Avicennia marina*	Isozymes	Duke et al. (1998a,b)
EIO vs. NA vs. SCS	Absent	*C. tagal*	cpDNA	Huang et al. (2007)
EIO vs. NA vs. SCS	Absent	*C. decandra*	cpDNA	Huang et al. (2007)
EIO vs. SCS and Sarawak	Absent Restricted	*Kandelia candel*	cpDNA mtDNA	Chiang et al. (2001)
EIO vs. NA vs. SCS	Absent	*C. decandra*	ISSR	Tan et al. (2005)
EIO vs. NA vs. SCS	Restricted	*Lumnitzera racemosa*	ISSR	Su et al. (2006)
EIO vs. NA vs. SCS	Restricted	*Lumnitzera littorea*	ISSR	Su et al. (2007)
North vs. South Vietnam	Restricted	*A. marina*	cpDNA	Kado et al. (2004)
WIO vs. ME vs. EIO	Restricted	*A. marina*	SSR, unique A	Maguire et al. (2000a,b)

such as *A. marina*, *C. tagal*, *C. decandra*, *K. candel* and *L. racemosa* (references are in Table 2). Also in the AEP, besides the CAI as a land barrier, there is evidence of restricted gene flow on basis of cpDNA in *A. germinans* (Nettel and Dodd, 2007) along the coast of the East Pacific between Mexico and Panama; along the coast of the West Atlantic towards the Caribbean region; and along the Atlantic Central American coast (Table 2, Fig. 5).

3.1.3. Higher levels of gene flow along the same coastline

The present-day distribution of mangroves is the result of albeit recent shifts in ranges and range expansions after the last glacial maximum. These events undoubtedly account for the currently detected high levels of gene flow along the same stretch of a coastline. Much evidence on high levels of gene flow (i.e. values of $F_{ST} < 0.2$ or $Nm > 1$) became available with allozymes, ISSR and SSRs (occasionally with AFLP, RAPD or cpDNA sequences) on *A. germinans*, *A. marina*, *R. mangle*, *L. racemosa*, *B. gymnorrhiza*, *B. sexangula*, *H. littoralis*, *K. candel* and *A. corniculatum* (values and references are given in Table 3). High levels of gene flow could be observed along relatively short stretches along the same coast or across islands in the same region. In the AEP on average a moderate genetic structuring can be observed in distance classes <1000 km (Dodd et al., 2002), which suggests higher levels of gene flow at shorter distances, e.g. for *A. germinans* in the Caribbean sea between Mexico and Costa Rica; in the East Pacific along the Colombian coast; and along West Africa (Fig. 5, values and references in Table 3). In the IWP more species were studied than in the AEP and several showed at least a particular stretch with higher levels of gene flow: *B. sexangula* in southwestern Sri Lanka; *A. marina* in northern Vietnam or the northern Philippines; *B. gymnorrhiza*, *K. candel* and *A. corniculatum* along the South China coast; *L. racemosa* in northern Australia and *A. marina* in several neighboring regions of Australia or islands at the eastern distribution limits (Fig. 5, values and references in Table 3).

Up to now, there is no real evidence for an isolation by distance model, most probably due to either founder effects and range expansions following the last glaciation (Dodd et al., 2002) or due to higher levels of inbreeding in more isolated peripheral populations (Arnoud-Haond et al., 2006).

4. Molecular ecology in service of conservation and management

Despite their unique status as intertidal forests, hosting numerous faunal organisms (Nagelkerken et al., 2008; Cannicci et al., 2008) and providing essential functions and services to tropical and subtropical zones and their populations (Kristensen et al., 2008; Walters et al., 2008), mangroves are one of the world's most threatened ecosystems (Duke et al., 2007). Retrospective studies document how mangroves have been degraded over time (Dahdouh-Guebas and Koedam, 2008; Ellison, 2008). Not only direct or indirect anthropogenic degradation (Farnsworth and Ellison, 1997; Alongi, 2002) but

Table 3
Evidence from literature on high levels of gene flow (F_{ST} or Nm) between populations of the same biogeographical area

Coastline	F_{ST}	Nm	Marker	Species	Reference
West Africa	0.192 0.177 (ϕ_{ST})	–	AFLP	*A. germinans*	Dodd et al. (2002)
West Africa (Mauretania)	0.186	–	RAPD	*A. germinans*	Abeysinghe (2000)
Caribbean (Mexico-Costa Rica)	0.154 (ϕ_{ST})	–	AFLP	*A. germinans*	Dodd and Afzal-Rafii (2002)
New South Wales-New Caledonia	0.086	2.6	SSR	*A. marina*	Maguire et al. (2000a,b)
New South Wales–New Caledonia	–	>2–30	Allozymes	*A. marina*	Duke et al. (1998a,b)
Queensland-Northern Territory (Australia)	0.115	1.9	SSR	*A. marina*	Maguire et al. (2000a,b)
Queensland-Northern Territory (Australia)	–	>2–30	Allozymes	*A. marina*	Duke et al. (1998a,b)
Victoria-New South Wales (Australia)	0.154	1.4	SSR	*A. marina*	Maguire et al. (2000a,b)
Victoria-New South Wales (Australia)		1–2	Allozymes	*A. marina*	Duke et al. (1998a,b)
Victoria(Australia)-New Zealand	0.049	–	SSR	*A. marina*	Maguire et al. (2000a,b)
New South Wales (Australia)-New Zealand	0.100	2.2	SSR	*A. marina*	Maguire et al. (2000a,b)
New South Wales (Australia)-New Zealand		1–2	Allozymes	*A. marina*	Duke et al. (1998a,b)
Northern Territory (Australia)-Unit. Arab. Emir.	0.150	1.4	SSR	*A. marina*	Maguire et al. (2000a,b)
W. Australia-Thailand		1–2	Allozymes	*A. marina*	Duke et al. (1998a,b)
Northern Vietnam	0.06–0.20	–	SSR	*A. marina*	Arnoud-Haond et al. (2006)
Northern Philippines	Low	–	SSR	*A. marina*	Arnoud-Haond et al. (2006)
Northern Vietnam	Low	–	SSR	*A. marina*	Giang et al. (2003)
Southern Vietnam	Low	–	SSR	*A. marina*	Giang et al. (2003)
South China	–	High	cpDNA	*K. candel*	Chiang et al. (2001)
Australia (Daintree river)	–	1.92	ISSR	*Heritiera littoralis*	Jian et al. (2004)
South China (Hainan-Taiwan)	–	3.85	Allozymes	*Bruguiera gymnorrhiza*	Ge et al. (2005)
South China (Hong Kong-Hainan)	–	2.08	Allozymes	*Aegiceras corniculatum*	Ge and Sun (1999)
South China (Hong Kong-Hainan)	–	1.16	ISSR	*A. corniculatum*	Ge and Sun (1999)
Northern Australia	–	2.9	ISSR	*L. racemosa*	Su et al. (2006)
Southwestern Sri Lanka	0.105	–	RAPD	*Bruguiera sexangula*	Abeysinghe (2000)
East Pacific, Colombia	–	1.18–12.96	SSR	*Rhizophora mangle*	Arbeláez-Cortis et al. (2007)
East Pacific, Colombia	0.162	–	AFLP	*A. germinans*	Cerón-Souza et al. (2005)

also cryptic ecological degradation (Dahdouh-Guebas et al., 2005) and the increasing pressure of climatic change such as from sea-level rise (Gilman et al., 2008) jeopardises the survival of individual mangrove trees and of mangroves as a system. It becomes increasingly more important to understand the early drivers in mangrove establishment (Krauss et al., 2008), adult mangrove growth and development (Komiyama et al., 2008), and vegetation dynamics (Berger et al., 2008) in order to draft mangrove recovery programmes (Bosire et al., 2008).

A reasonable number of attempts were made in the field of molecular ecology of mangrove trees. Despite the tremendous efforts in collecting tree samples, analysing and treating the molecular data, only several outcomes became interpretable and hold promise for further hypothesis testing such as the phylogeographical patterns in *A. marina* and *A. germinans*. The genetic structuring of *A. marina* populations over its entire range is characterized by high overall F_{ST} values per SSR locus (0.25–0.52), supporting the idea that discrete populations are mostly differentiated but some of which show little differentiation, indicating some gene flow with $Nm > 1$ (Maguire et al., 2000a,b). In *A. marina*, most of the variation is partitioned among the populations of a large-scale distribution than among individuals within subpopulations (Maguire et al., 2000a,b). A similar partitioning of the variation could be observed at regional scale such as the coastal zone of Vietnam (Giang et al., 2003). *A. marina* populations located at the edge of their distribution area in North Vietnam were found to be strongly structured for SSR loci, combined with low gene diversities, indicating that high inbreeding levels occur (Arnoud-Haond et al., 2006). The gene flow is supposed to be low between such peripheral *A. marina* populations. Loss of genetic diversity may occur in heavily impacted areas and the transfer of germplasm can be envisaged for too fragmented and isolated populations (Su et al., 2007).

Allozymes, though still proven to be useful codominant markers in local studies on a few species (especially on the outcrossing *B. gymnorrhiza*), might be replaced by microsatellite markers in the near future, despite all encountered difficulties in the very specific and *de novo* development of suitable primer regions and screening for polymorphic loci. Sufficient SSRs are now available for *A. marina*, *A. germinans*, *S. caseolaris*, *A. corniculatum*, *B. gymnorrhiza*, *P. rhizophorae* and *K. candel* to perform detailed studies in the field of molecular ecology and testing hypothesis about reproductive strategies, age-class differences, pollination systems (pollen flow), mating systems (inbreeding, mixed, outcrossing), dispersal of propagules (seed flow) at various distance classes, considering putative historical dispersal routes and phylogeographical patterns that originated in changing coastal landscapes since the last glaciation period.

Schwarzbach and Ricklefs (2001) predicted a large impact of mangrove genetics, especially for conservation and management issues. Molecular data would provide insights in the genetic structure of populations for conserving and protecting genetic variation. Seven years later, my conclusion is that there still is a long way to go before reaching these practical goals on conservation and management of populations despite the outstanding studies on hemispheric and oceanic level that gave new insights on biogeographical and distributional patterns. Defining ecological significant units (ESU's), potentially to be considered also as management units for conservation, to detect hotspots of genetic diversity, primarily based on the haplotype

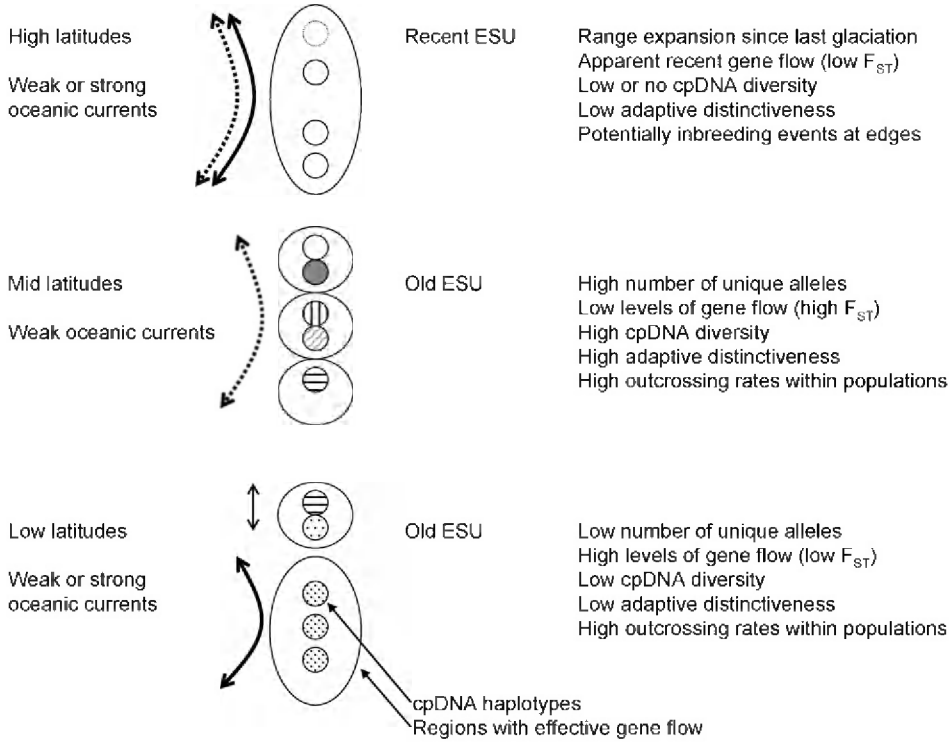

Fig. 6. Conceptual model for latitudinal distribution of evolutionary significant units (ESU) and putative features of genetic variables in mangroves along coastlines.

diversity reflecting historical seed dispersal across regions, remains an important task. The extent of each distinguishable ESU might depend on the latitudinal position of the populations within a species range and is supposed to be influenced by ocean currents, thereby potentially stretching or mixing the ESU's with unique alleles. An attempt to conceptually summarize the idea of ESU's could inspire future studies (Fig. 6). ESU's should be tested for distinctiveness in evolutionary timeframe, local (or regional) adaptation and local inbreeding events.

As can be inferred from this review, several studies considered low numbers, either a low number of individuals per population, low amounts of interpretable polymorphic loci and low amounts of populations or comparisons between few and distant mangrove populations. The low sample sizes in several studies certainly are related to the difficult conditions for collecting leaves of distant trees in hardly accessible inner parts of mangrove forests. For reasons of both scientific rigidity and potential opportunities in making generalisations, it is recommended in the field of mangrove genetics to give also priority to targeted research – thus less explorative – on few, well-known species, using 5–10 highly polymorphic SSR loci (>10 if only two to three alleles per locus); >10 populations when two groups are compared and up to 50 individuals per population when local dynamics such as pollen flow, paternity testing or inbreeding are envisaged. This design would allow testable hypotheses on, e.g. significant differences of gene flow between populations or inbreeding events within a restricted part of the species range but highly relevant for local conservation at province or country level.

Large-scale investigations should aim to collect the geographical and ecological range of the species, preferably a few from the centre and more from the periphery. Increasing the number of populations and sampling fewer individuals per population is an option (Lowe et al., 2004). Nevertheless, placing emphasis on either within or among populations must be determined by the life strategy of the species being studied and the problem investigated. Though very attractive and meaningful, large-scale analysis of a species should not be the scientific goal of every researcher as this requires concerted efforts to be successful. Well-designed local studies are equally challenging and are easier to achieve from a logistic point of view. Mangrove genetics will then become even more acceptable and applicable in discussions and negotiations on mangrove conservation and management with various stakeholders. Clearance of mangroves for other land uses, wood extraction and shrimp pond operation are causing threats to the maintenance of sufficient gene diversity in outcrossing or mixed mating species. This concern for conservation, both for maintenance of existing variation and considering indigenous source materials for replantation projects will be of major importance in future mangrove management plans. Reforestation programmes (Bosire et al., 2008) should take into account at least the possibilities of distinct cpDNA haplotypes and the existence of evolutionary significant units to be considered as management units (Liao et al., 2007), still visible through the maternally inherited features. Careful protection is needed against deliberate plantations across distant geographical locations using unknown source materials with an unknown genetic background. Such plans need consideration of breeding and naturalness for reasons of adaptiveness to local conditions of climate, flooding, sediment type, species interactions etc. Sufficient genetic variation should be maintained in smaller mangrove areas and in marginal populations of species at the edge of their natural range, as these appear to be subject to genetic erosion. Additionally, detection of gene diversity hot spots (many alleles, much heterozygosity) or unique genotypes (unique alleles), either within a region, country or worldwide, will be a necessary argument, among many others, to convince decision makers to preserve and protect such unique biogenetic reserves.

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