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The Use of DNA Molecular Techniques for Mangroves Studies

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Abstract : Biological and ecological studies of mangroves have provided limited insights in relation to the evolutionary processes in mangroves. However, with the development of molecular genetic techniques, a much better understanding of such evolutionary processes can be obtained. This review briefly introduces some of the molecular techniques available to researchers and then summarises their main applications. From an evolutionary perspective, genetics can be used in a hierarchical approach in order to appraise differences between species (phylogenetics), groups of populations (phylogeography) and individuals (population genetics). Some of the main theoretical and practical issues concerning each of these research areas will be briefly reviewed and illustrated with mangrove examples.

Key words : mangrove, phylogenetics, phylogeography, population genetics, polymorphism

1. Introduction

Biological and ecological studies of mangroves have provided limited insights in relation to the evolutionary processes in mangroves, i.e. the development, diversification or extinction of species. Understanding the evolutionary patterns and relationships between economically important species and their close relatives has long been recognised as a management and conservation priority. With the development of molecular genetic techniques, a much better understanding of such evolutionary processes can be obtained. These techniques are particularly useful to solve systematic and evolutionary questions as well as clarifying the extent and distribution of genetic variation within single taxa. As DNA technologies become more informative and less complex, an increasing number of laboratories are able to access them and benefit from their use.

Regardless of which specific molecular technique is used, the naturally occurring genetic variation at the population level is a useful tool for many applications, including conservation studies. Genetic investigations are useful in inferring the ancestral history of a group of organisms and, in some cases, merely the amount of genetic variation can be revealing. For instance, quantifying and qualifying the overall genetic pool available to a species can be useful for assessing its evolutionary potential. Genetic polymorphisms can also be useful for investigating genetic relationships among subpopulations and identify possible links between geographic distribution and environmental adaptation. The principle is that alleles (different forms of the same gene) are shared among subpopulations because of migration, and as a result similarities in allele frequencies can be used to estimate migration rates. The absence of shared alleles between populations can either indicate the lack of movement between population or the presence of selective forces. From a more practical application, genetic polymorphisms can also be used to study mating systems in plants. Such studies rely on changes in genotypic frequencies to differentiate between random mating and self-fertilising individuals or populations.

The purpose of this review is to briefly introduce some of the molecular techniques available to researchers and then summarise their main applications. From an evolutionary perspective, genetics can be used in a hierarchical approach in order to appraise differences between species (phylogenetics), groups of populations (phylogeography) and individuals (popula-

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tion genetics). Some of the main theoretical and practical issues concerning each of these research areas will be briefly reviewed and illustrated with mangrove examples.

2. Molecular Techniques: the Options Available

Recent advances in molecular biology, especially the development of polymerase chain reaction (PCR) technology, have produced new and powerful techniques useful for the screening, characterisation and evaluation of genetic diversity. Some techniques can be more suited to specific purposes than others and when selecting the right tool for a genetic investigation it is important to consider various issues such as informativeness, simplicity, cost and ease of interpretation.

Molecular techniques can be broadly classifyed within three main groups: non-PCR based, targeted PCR based (including sequencing), and arbitrary PCR based. Some of these are briefly described below.

Non-PCR technology is principally limited to allozyme and restriction fragment length polymorphism (RFLP) analyses. The former methodology relies on protein rather than DNA polymorphisms. Small structural mutations within selected enzyme systems modify their electric charge and, as a consequence, their electrophoretic mobility across a gel. Allozyme analysis has been in use for many years, thus providing a large amount of comparative data to practitioners. Despite being simple and universally applicable, the use of this technique is not as widespread as in earlier years, mainly because of its low polymorphism and its susceptibility to environmental factors. RFLP was the first method capable of detecting polymorphisms at the sequence level. RFLP analysis involves digesting DNA with restriction enzymes and separating the resulting fragments by gel electrophoresis. A number of short universal sequences can then be used as probes (usually radioactively labeled) and, in the course of an hybridisation reaction, recognise and attach to complementary DNA fragments. Mutation at restriction sites will result in differential banding patterns which are highly reproducible, co-dominant (enabling the distinction between heterozygotes and homozygotes) and fairly polymorphic.

This technique is informative but fairly complex and lengthy.

Targeted PCR relies on the design of primers targeting specific regions of the genome. Amplified products from different individuals can be compared on a gel, where differences in size represent various mutational events. An increasingly popular PCR-based technique is microsatellite analysis. Microsatellites, or simple sequence repeats (SSRs), are highly variable sites randomly dispersed across the DNA. Being co-dominantly inherited, highly reproducible and simple to use, SSRs are considered as one of the most useful population genetic tools. The main drawback of this technology is that it is not universal, and the identification of useful priming sites within the species of interest can be a complex matter. Nevertheless, the development of advanced enrichment techniques and transferability of primers across closely related species, make SSRs increasingly applicable to a variety of studies. Also fitting within the category of targeted PCR is DNA sequencing. When sequencing, PCR primers are selected to amplify a specific area of interest within the genome and each single nucleotide within the fragment obtained can be identified by way of a conventional sequencing reaction. Within plants there are three genomes and therefore three potential sources of sequence information: nuclear DNA (nDNA), chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA). These three genomes are inherited in different ways, have dissimilar mutation rates and thus can provide complementary evolutionary data. Finally, arbitrary priming PCR describes techniques such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP).

These procedures are based on the use of single, 'arbitrary' (ie. not specific to a specific region of the genome) primers, they require no prior genomic knowledge and can therefore be applied to any organism. RAPD relies on the use of a single primers nine to 20 bp long. The multiple products obtained by a low stringency PCR reaction can be visualised on a conventional gel. RAPD is simple, cheap and produces highly polymorphic markers; however its robustness can be questionable if consistent protocols are not followed. AFLP is an intermediate technique between RAPD and RFLP and relies on restriction digestion followed by two selective PCR amplifications. Despite being more complex and expensive than RAPD, AFLP is highly polymorphic and more reproducible.

Unfortunately, both systems produce dominant markers and therefore are less informative for certain applications.

3. Investigating Phylogenies

3.1 *Theory and Practice*

The highest hierarchical level of molecular investigation is the unraveling of evolutionary relationships between species, genera and even families. Traditionally this type of phylogenetic surveys rely on conserved DNA regions that have diverged in a quantifiable manner across species, but for which no within-species diversity can be expected. As a result, sampling can be limited to a single accession per taxon being investigated. The optimal DNA region to be sequenced in phylogenetic studies must be conserved enough to enable the development of universal primers (ie. primers that will work across all species), yet variable enough to discriminate between different taxa. When these conventional sequencing regions cannot distinguish among species, then molecular tools more appropriate for phylogeographic and population studies are likely to provide the necessary information. Conversely if these same regions differentiate between populations, then one might be dealing with cryptic species.

Ideally and when possible, the most informative phylogenetic approach is to combine sequence data from different genomes that evolve differently and follow distinct inheritance models. For instance, unlike nuclear DNA, chloroplast DNA (cpDNA) is uniparentally inherited (maternally inherited in most Angiosperms) and does not undergo recombination. Furthermore, evolutionary rates can vary within single genomes, so for instance genes (ie. regions coding for a specific function) are more conserved than non-coding region. The former regions might be less informative as they have fewer variable sites, especially at the lower hierarchical levels, yet it might be impossible to design primers for the latter regions because of the lack of universally conserved sites. It is therefore essential to carefully select the most appropriate sequencing region through a preliminary investigation before launching into a phylogenetic study. Sampling strategies will depend on the questions being asked and outgroups need to be selected carefully in order to positively contribute to the final findings. Some of the most commonly used regions for DNA sequencing studies are cpDNA genes such as *rbcL*, *atpB* and *matK* and nuclear ribosomal DNA genes such as 18S to name a few. Frequently used non-coding regions include the *trn*L intron and the intergenic spacer between *trn*L-F from cpDNA and the internal transcribed spacers (ITS1 and ITS 2) from nuclear ribosomal DNA.

3.2 *Mangrove Studies*

The fact that most mangrove taxa exist within larger, otherwise terrestrial clades supports the premise that taxa occupying the mangrove habitat arose independently from terrestrial clades in at least 15 plant families (Ricklefs and Latham, 1993). The fossil distributions of species occupying mangrove habitat support the generalisation that present distributions include their area of origin. When sister taxa (in this case species belonging to the same mangrove clade) are restricted to the same geographical region, one can surmise either that the ancestral taxon had similar distribution or that the ranges of the sister taxa have changed in parallel. The latter scenario is unlikely for mangroves whose sister taxa now occupy a substantially different terrestrial habitat. Parsimony would lead us to conclude that the common geographical distribution of sister mangrove taxa includes, in a very general sense, the place of the origin of the derived clade. Based on such cladistic analyses of mangroves and close, non-mangrove relatives, Ricklefs and Latham (1993) concluded that the distribution of Indo-West Pacific endemic mangroves originated in the Indo-West Pacific.

A similar conclusion was reached concerning the cladistic analysis of the Rhizophoraceae based on the morphological data of Juncosa and Tomlinson (1988).

Molecular data can provide a better understanding of the phylogeny of mangroves, as their characteristic features are phenotypically stable and not prone to environmental change. For example, on the basis of morphological and anatomical features, Rhizophoraceae has been included in the order Myrtales (Takhtajan 1980) or assigned to its own separate order Rhizophorales (Thorne 1992). However, a parsimony analysis of the DNA sequence data from the chloroplast gene *rbcL* (Conti *et al.* 1996), strongly suggests that Rhizophoraceae does not belong to the Myrtales. Rather, the analysis places Rhizophoraceae as part of a rosid clade including Euphorbiaceae, Humiriaceae and Malpighiaceae. A more recent compilation of the phylogeny of the eudicots by Savolainen *et al.* (2000) reviewed the phylogeny of 589 *rbcL* sequences from 308 families including some of those containing mangrove species.

The study revealed that Rhizophoraceae and Erythroxylaceae form a distinct subclade within the Malpighiales (this order also includes Humiaceae, Euphor-

biaceae and Salicaceae to name a few). The Malpighiales are a sister group to the Oxalidales which includes families such as Elaeocarpaceae and Cunoniaceae (Savolainen *et al.* 2000). This *rbcL* based study also contained information on most of the other mangrove-containing families. For instance, Avicenniaceae was found to form a distinct subclade with Acanthaceae, Pedaliaceae and Gesneriaceae within the Lamiales and Myrsinaceae was included within the Ericales (Savolainen *et al.* 2000). This and other large studies, such as that of Soltis *et al.* (2000) based on three genes and 560 angiosperms, have proved extremely useful in depicting interfamilial relationships.

More detailed studies of Rhizophoraceae based on a combination of data from cpDNA, nuclear ribosome DNA and morphology, have shown Rhizophoraceae to consist of three well supported monophyletic tribes: Macarisieae, Gynotrocheae and Rhizophoreae (Schwarzbach and Ricklefs 2000). The latter two tribes form a strongly supported subclade and the overall molecular data is compatible with morphological observations (for example fruit characters circumscribe the three tribes). Interestingly, within the tribe Rhizophoreae the genus *Bruguiera* occupies a basal position in comparison to the other genera (*Kandelia, Ceriops* and *Rhizophora*) in this mangrove group. This suggests a possible ancestral origin for this genus, and supports morphological observations on dispersal mechanisms. In *Bruguiera* the seedling disperses initially with the fruit, whereas in the other more advanced genera only the seedling disperses.

Parani *et al.* (1998) used RAPD and RFLP to investigate the genomic relationships between various genera considered to be 'true' mangroves and 'associate' mangroves.

Not surprisingly, they found association between related mangrove species and distinction between unrelated groups. However this type of analysis is inadequate for such distantly related taxa and therefore the amount of information that can be obtained from it is limited.

4. Phylogeographic Studies

4.1 *Theory and Practice*

Just as there are substantial differences among species, there may be large differences between particular groups within species. Genetic structuring of plant populations is strongly influenced by common ancestry and current patterns of interpopulation genetic exchange. Phylogeography is concerned with the processes governing the geographic distribution of genealogical lineages among and within closely related taxa, thus fitting between other micro and macro-evolutionary disciplines. This is an area of growing interest especially in the animal kingdom, with the majority of studies conducted to date being based on mitochondrial DNA (mtDNA). MtDNA is the preferred genome for animal phylogeographic studies, because its genes evolve at a faster rate than nuclear genes. Such mutational rates are sufficient to discern between different intraspecific evolutionary groups but not between single individuals. This approach has been particularly useful in defining Evolutionary Significant Units (ESU) for conservation. Some controversy exists in relation to the exact criteria defining ESUs, but in general these units should represent populations that have been historically isolated thus having discrete adaptational potential.

Understanding such distinctions at the population level is particularly important, as optimal management strategies should insure that the full array of differentially adapted groups are maintained as distinct units.

Unfortunately the same high evolutionary rates are not shared by plant mtDNA thus limiting its application. In plants, phylogeographic studies are frequently based on selected regions of cpDNA which, despite being less variable than animal mtDNA, is also known to be clearly structured geographically and to possess most of its evolutionary properties. Because of the lower informativeness of cpDNA, plant phylogeographic studies have been uncommon despite the fact that circumstances such as reticulated speciation (ie. speciation through hybridisation events) are more prevalent in plants than animals. In species where sufficient cpDNA variation has been detected to permit phylogeographic analysis, variation has been mainly revealed by restriction enzyme digests of whole cpDNA (RFLP), or of selected chloroplast loci (PCR-RFLP). Ultimately, direct sequencing of the variable cpDNA regions, as done for animal mtDNA, would be more desirable for studying relationships, however insufficient variation still restricts the more widespread use of this options. Other studies on plant population structure have relied on population genetic tools and nuclear DNA. In such studies, evolutionary divergence is measured by assessing differential allelic frequency or differential allelic fixation among populations. The majority of the examples below belong to this group.

4.2 *Mangrove Studies*

For speciation to occur reproductive isolation between two populations must be sufficient to cause genetic differentiation.

Such differentiation may eventually result in reproductive incompatibility.

Genetic differentiation can be brought about by a number of processes such as differing environmental stresses on the two populations, geographical separation, episodes of great extinction (bottleneck effects) or significant expansion from a small initial genetic pool (founder effects). Molecular techniques allow us to identify which of these processes are or have been active and to what extent they have resulted in genetic differentiation.

McMillan (1986) first used allozyme analysis to determine genetic differences amongst populations of *Avicennia germinans*. He found that allelic distribution from a number of loci differed between the plants from the western side and those on the Caribbean side of the Gulf of Mexico. Clearly, geographic separation and differing environmental conditions were limiting gene flow between the two populations thus resulting in genetic differentiation.

Genetic distinction based on allozyme analysis has also been described in other mangrove genera including *Bruguiera, Kandelia, Rhizophora* and *Sonneratia* (Baba *et al.* 1989, Goodall and Stoddart 1989, Huang 1993, Sun *et al.* 1998). Ballment *et al.* (1988) were able to differentiate three sibling taxa of *Ceriops* using allozymes, by detecting sufficiently high genetic divergence among taxa to justify the establishment of separate species. Their study showed that sympatric populations of *Ceriops tagal* and *C. australis* maintained fixed allelic differences at a number of loci, thus suggesting reproductive isolation and no interbreeding between two species indistinguishable on morphological grounds alone.

Also using allozyme analysis, Duke *et al.* (1998) confirmed the species status of *Avicennia alba, A. integra, A. marina* and *A. rumphiana* from the Indo-West Pacific and *A. germinans* from the Atlantic-East Pacific. As in the *Ceriops* study, the authors found fixed allelic differences at most loci even when sympatric populations were sampled. The authors were also able to confirm the distinction between three subspecific taxa: *A. marina* ssp. *marina, A. marina* ssp. *eucalyptifolia* and *A. marina* ssp. *australasica*. These subspecies differed in allelic frequencies which were revealed to be more reliable than the morphological characteristics previously adopted, especially at the geographical conjunction of varieties, where partial evidence of introgression was detected (Duke *et al.* 1998).

Overall, the allozyme data did not support an ancient origin of the *A. marina* varieties, with divergence time being estimated at about 2 my BP. Rather, Duke *et al.* (1998) suggested that such varietal differentiation was the result of geographical structuring caused by limited dispersal of propagules.

In another study on *Avicennia* based on the analysis of DNA markers, 109 RAPDs and 84 RFLPs, it was shown that the widely distributed *A. marina* was more closely related to *A. alba* (genetic distance 0.22) than to *A. officinalis* (genetic distance 0.37 Parani *et al.*, 1997). Lakshmi *et al.* (1997) used the same molecular system to investigate intraspecific variability in *Acanthus ilicifolius* to find that intrapopulation differentiation was much greater than interpopulation diversity.

Maguire and Saenger (2000) examined the tropical Indo-Pacific genus *Excoecaria* L. (Euphorbiaceae), which contains several poorly known components in Australia. In Australia, the most widespread species is the mangrove *E. agallocha* L. (type species) whose taxonomic and geographic limits are difficult to define from those of its closely related taxa. As a result, two additional taxa, *Excoecaria dallachyana* Baillon and *Excoecaria ovalis* Endl, have been described but could not be clearly differentiated from the type species. In order to determine the relationships between this closely related group of taxa, Maguire and Saenger (2000) investigated morphometric and DNA sequence data from the ITS1 region of nuclear ribosomal DNA. The authors showed that generally there were no within-species differences in either *E. agallocha* from eastern Australia or *E. ovalis* from Western Australia. However a sufficient number of between-species nucleotide substitutions were detected to justify their respective species status thus corroborating the morphometric data (Maguire and Saenger 2000). The only slight within-species difference was detected between the *E. ovalis* populations from the Gulf of Carpentaria and those from Western Australia, but such variation was not supported by morphometric differentiation. The ITS1 sequencing data also suggested that *E. dallachyana* is not closely related to either mangrove species (*E. agallocha* or *E. ovalis*) despite superficial morphological similarity.

5. Population Genetics and Breeding Biology

5.1 *Theory and Practice*

As it is important to recognise genetic distinction between groups of populations likely to represent local adaptation, it is important to understand the extent of overall genetic diversity representing evolutionary potential. Changes in gene diversity can be connected to environmental conditions as well as human impact, and generally are not distributed evenly throughout the range of habitats in which the species occurs.

The centres of diversity for single species, as well as the presence of rare genotypes within individual populations can be identified by using highly informative molecular tools.

Population geneticists study the changes in genetic structure, gene flow and overall diversity that occur within and among populations. Population genetic theory is used to quantify the amount, distribution and dynamics of genetic variation in populations and to elucidate their breeding and mating strategies. In the long term, the genetic pool available to a wild species must enable it to survive environmental pressures exceeding the limits of its developmental plasticity.

As a result, determining how much diversity exists within a species and understanding how it is maintained is essential for long term management and conservation. The mating system and breeding strategy of a species will influence the amount of gene flow within and across populations and as a result, the way genetic diversity is distributed. Being able to assess if a population is or is not mating randomly is particularly important.

For example selfing and inbreeding can cause loss of genetic diversity and evolutionary potential, especially in small populations. Similarly, isolation can reduce migration rates and gene flow, potentially causing genetic drift (ie. directional fluctuations in allelic frequencies).

The extent of gene flow is particularly consequential to the understanding of population dynamics. Generally there are two main methods to acquire such information: the indirect way (historical levels of gene flow are measured based on the distribution of diversity among populations) and the direct way (contemporary gene flow is estimated by direct observation and the identification of immigrant genotypes). Molecular techniques that can provide highly polymorphic, co-dominant markers are especially useful in population studies. A number of theoretical population genetics principles have been developed to facilitate the investigation of these and many other important mechanisms.

5.2 *Mangrove Studies*

Huang (1994) first studied the genetic variability between and within populations of the mangrove *Kandelia candel* in Taiwan using allozyme analysis. He concluded that a moderate level of genetic variation existed between the four mangrove populations studied as well as low within-population diversities. The author suggested that local environmental selection and restricted gene flow between the populations contributed to the limited genetic variability recorded for this species in Taiwan. Huang and Chen (1997) extended the study of *K. candel* to include the Ryukyu Archipelago. They found that the level of genetic variation in all six populations studied was lower than previously reported in other plant taxa. This low variation was largely due to high inbreeding rates, possibly a consequence of founder events in the recent past. As *K. candel* was previously shown to be predominantly outcrossing and as some genetic differentiation was recorded between populations, it is likely that the lack of diversity is also due to restricted gene flow between populations. The results suggest that on a macrogeographic scale, *Kandelia* populations consists of isolated and subdivided units with restricted gene flow. In a microgeographic study of 13 *K. candel* populations in Hong Kong, Sun et al. (1998) detected very low genetic diversity despite high outcrossing rates measured from the study of their mating system. As expected within such a small geographic scale, the authors found very low genetic differentiation between sites (with nearly four migrants per generation being estimated to move across populations). At that scale homogeneity is likely to be a result of recent coancestry (Sun *et al.* 1998).

Using allozymes, Duke *et al.* (1998) identified strong genetic structuring in *A. marina*, concluding that high levels of local diversity and outcrossing rates are not matched by high levels of gene flow between neighbouring populations. Gene flow was only recorded between geographically adjacent sites likely to be part of a single, large population. Overall there was strong evidence of isolation-by-distance, likely to be the consequence of reduced dispersal of propagules (Duke *et al.* 1998). Similar findings were reported in a study Maguire *et al.* (2000).

Table 1 : Intra- and interpopulation polymorphism in mangroves

Species	Method	Intra	Inter	Reference
Acanthus ilicifolius	RAPD	3.8-7.3	34.0	Lakshmi et al. 1997
Acanthus ilicifolius	RFLP	3.2-9.1	45.8	Lakshmi et al. 1997
Avicennia alba	Allozyme	0-9.1		Duke 1998
Avicennia alba	RAPD	37.8		Parani et al. 1997
Avicennia integra	Allozyme	0		Duke 1998
Avicennia germinans	Allozymes	0		Duke 1998
Avicennia marina	RAPD	17.8-38.9	76.7	Parani et al. 1997
Avicennia marina	RFLP		66.0	Parani et al. 1997
Avicennia m. marina	Allozyme	0-27.3		Duke 1998
Avicennia m. eucalyptifolia	Allozyme	18.2-45.5		Duke 1998
Avicennia m. australasica	Allozyme	9.1-54.5		Duke 1998
Avicennia officinalis	RAPD	32.3		Parani et al. 1997
Avicennia rumphiana	Allozyme	0		Duke 1998
Bruguiera cylindrica	RAPD	16.38-18.96	37.33	Lakshmi et al. in press
Bruguiera gymnorhiza	RAPD	12.68-16.98	31.08	Lakshmi et al. in press
Bruguiera parviflora	RAPD	14.16-19.36	32.67	Lakshmi et al. in press
Ceriops decandra	RAPD	16.23-19.62	27.77	Lakshmi et al. in press
Ceriops tagal	RAPD	14.68-16.28	39.33	Lakshmi et al. in press
Excoecaria agallocha	RAPD	21.4-31.0	65.0	Lakshmi et al. 1997
Kandelia candel	RAPD	14.45		Lakshmi et al. in press
Kandelia candel	Allozyme	11.1-16.7		Huang and Chen 1997
Kandelia candel	Allozyme	10.8		Sun et al. 1998
Rhizophora apiculata	RAPD	16.20-18.38	32.46	Lakshmi et al. in press
Rhizophora lamarckii	RAPD	17.10		Lakshmi et al. in press
Rhizophora mucronata	RAPD	18.02-19.68	33.33	Lakshmi et al. in press
Rhizophora stylosa	RAPD	16.43		Lakshmi et al. in press

In this study, microsatellite markers were used to assess genetic variation throughout the worldwide range of *A. marina*. Despite the greater informativeness of SSRs and evidence of the species being a preferential outerosser, the authors generally detected low within-population genetic diversity and some evidence of inbreeding (especially in the most isolated populations). High levels of genetic structure were also measured (Fst = 0.41) with number of migrants being estimated at less than 1 in most pairwise population comparisons. Overall, the findings from this SSR-based study indicated that genetic structure in *A. marina* is not purely the result of geographical distance, but is the consequence of the formation of independent subunits caused by inadequate dispersal mechanisms (Maguire *et al.* 2000). Such findings support previous observations that propagule dispersal in *A. marina* is limited by longevity, ocean currents and the presence of suitable environmental conditions (Clarke and Myerscough 1991, Clarke 1992). A further study by the same researchers but based on a smaller number of *A. marina* individuals, compared the data obtained using two different techniques : AFLPs and SSRs (Maguire *et al. in press*). The authors found that while the levels of polymorphism obtained were similar for the two techniques, SSRs were more adequate for population based investigations, whereas AFLPs were particularly useful for fingerprinting single individuals and monitoring propagation programs.

Finally, random amplified polymorphic RAPD and RFLP markers were used to estimate intra-and interspecific variation in three species from the mangrove genus *Avicennia* (Parani *et al.* 1997).

Intrapopulation polymorphism among the 10 populations of *A. marina* varied between 17.8 and 38.9%. The authors found that when sympatric, populations of *A. marina* were less genetically variable than populations of *A. officinalis* (Pichavaram population, 32.3%) and *A. alba* (Coringa population, 37.8%). Interpopulation variation in *A. marina* (76.7% for RAPDs and 66% for RFLPs) was greater than the variation estimated within any individual population of this same species, confirming the high degree of divergence between the populations found in other studies (Table 1).

6. Conclusion

As a considerable amount of genetic research as been conducted on mangrove species, it is now possible to draw preliminary conclusions on the evolutionary processes that operate in mangrove species.

At the phylogenetic level, gene sequencing corroborated the observation that mangroves have evolved in parallel within very different families. Nevertheless, within their families, mangrove species form distinct clades often incorporating unique genera (eg. the Rhizophoreae in the Rhizophoraceae). At a phylogeographic level, morphological distinction within taxa often corresponds to species or subspecies status (eg. *Avicennia*). In fact, all the molecular techniques used to date appear to agree in finding great genetic differentiation among populations. As geographic distance increases between populations, so does genetic identity. Most mangrove population genetic studies indicate that intrapopulation diversity is consistently smaller than interpopulation diversity. In other words, it appears that for most mangrove species, gene flow among population is limited even across relatively confined geographic distances. This suggests that the evolution of mangrove propagules is likely to have been directed more towards buoyancy than long-range dispersal.

Nevertheless, sporadic long-range dispersal events do occur. From a genetic point of view such rare episodes, known as founder events, establish populations with small genetic pools. Such processes explain the lack of intrapopulation diversity recorded in most studies despite the fact that most species are preferential outcrossers. Subsequent environmental stresses and lack of genetic flow ensure that geographically isolated populations remain genetically distinct.

From a conservation and management point of view, it is therefore important to reach a balance between the need to preserve local adaptation and the necessity to ensure optimal evolutionary potential. Overall the best strategy is to maintain local provenances distinct while ensuring that maximum genetic diversity is kept within them.

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