A Review of A DNA Dataset of Mangrove Populations from Las Perlas Archipelago, Panama.

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**Abstract**

Gene flow among populations is one of the important factors maintaining species integrity and the genetic diversity within species. In addition to geographic isolation, natural barriers such as rivers, shoreline, mountain ranges or even man-made canals or highways may interrupt the habitat and thus affect the population genetic structure of a species (Brown, 1989). Ten microsatellite DNA markers were used to assess genetic diversity within and among the population of *Rhizophora mangle* in the Las Perlas Archipelago on the Pacific coast of Panama. These were used on a sample of 199 adult trees which were randomly sampled from 12 plots covering the entire area of the Archipelago. Each plot was georeferenced using GPS (Etrex Vista, Garmin), and the information obtained was used in genetic analysis of the population structure of *R. mangle* in the chosen area.

Several methods were used to detect the genetic structure among the individuals. Two Bayesian clustering analysis (STRUCTURE 2.01 and BAPS 4.14) both identified a probable number of populations (K), partitioning the Archipelago into different populations. The best structure using “clustering of individuals” option was K = 5 with a probability of 0.45. However, when the analysis was made by “clustering groups”, the best structure obtained was K = 4 with the probability of 0.85, suggesting that geographical coordinates are crucial in determining a more realistic genetic structure. Molecular variance (AMOVA, Michalakis & Excoffier, 1996) analysis demonstrated 85% of intra-population genetic variation, while only 15% was due to inter-population genetic variability. Overall, the study revealed that a substantial genetic diversity exist within and among populations of *R. mangle*. 
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CONTENTS

CHAPTER 1: Introduction .................................................................1
1.1. Introduction to the study
1.2. The area of study and it’s mangrove community
1.3. Aims of the study

CHAPTER 2: Literature review on mangrove biology and ecology..............7
2.1. Mangroves ecology and adaptations
2.1.1. Morphological specializations of mangroves
2.2. Mangrove distribution and zonation
2.3. Mangrove Species richness and genetic diversity
2.4. Fauna associated with mangrove swamps
2.5. Mangroves importance, threats and conservation

CHAPTER 3: The implication of genetic analysis on our understanding
Of mangrove biology and distribution ..................................................25
5.1. The genetic structure
5.2. The effect of natural selection
5.3. Isolation by distance
5.4. Implication for conservation

CHAPTER 4: Conclusion and Recommendations .....................................33

CHAPTER 5: References............................................................................35

APPENDICES .......................................................................................... 54

FIGURES

Figure 1.1: Map of Las Perlas Archipelago, Panama indicating sampled plots.....6
Figure 2.1: Distribution of Mangroves around the world..............................13
Figure 3.1: The Las Perlas Special Management Zone.................................26
Chapter 1: 
Introduction

1.1. Introduction to the study

Mangroves serve several important functions, including the maintenance of coastal water quality, reduction in severity of storm, wave and flood damage, and as nursery and feeding grounds for commercial and artisanal fishery species (see Chapter 2). They are highly productive and provide food for a variety of fauna including benthic and pelagic marine animals such as fish and shellfish (Baran and Hambrey, 1998). However, mangrove forests, along with other marine ecosystems are experiencing an era of destruction and over exploitation by humans for several reasons such as reclamation projects, urbanisation, tourism developments, pollution and so on. Although they are considered to be pristine, mangroves on the Las Perlas islands are no exception and they are rapidly becoming threatened as many of these currently uninhabited islands become potential sites of development, in particular due to the flourishing tourism industry.

The Archipelago of Las Perlas forms a very complex and valuable ecosystem, not only hosting mangroves but it is also the destination for many whales, especially for the Humpback whale that migrates to mate and breed in the shelter of these islands. For these reasons, the Panamanian government decided to designate the islands as a protected management zone (http://striweb.si.edu/darwin-initiative/). This will be very helpful in monitoring and general management of the Archipelago, and it will indeed protect a large percentage of the total area of the land and marine ecosystems from overexploitation of the resources of the area. Through the Darwin Initiative Program, varied studies have been undertaken in the Archipelago in order to support the designation of Marine Protected Area zoning throughout this important Panamanian marine ecosystem, which also form an essential part of the Marine Biological Corridor of the Tropical Eastern Pacific (http://www.conservation.org/xp/frontlines/protectedareas/partners23-1.xml).
The Las Perlas Darwin Initiative project, which began in 2003 under the lead of Dr. Hamish Mair of Heriot-Watt University, undertook studies which included environmental data gathering as well as socio-ecological surveys of the fishery communities in order to begin a process of participation among local inhabitants of the islands and government for the implementation of a marine protected area. To date, these series of studies have played a crucial part in the success of the designation of a Marine Protected Area within the Gulf of Panama. Equally important, STRI staff-members are giving short seminars to the local people aimed at educating local communities regarding proper and effective management of resources through the Smithsonian’s Marine Education Program (http://www.conservation.org/xp/frontlines/protectedareas/partners23-1.xml).

Given the increased pressure on mangrove forests, there is an intense need to assess and monitor these resources using effecting management tools. The analysis of the genetic structure of mangrove population on the Las Perlas islands is aimed at understanding the genetic diversity and interactions between different geographic populations found on the islands. The practical work of this study was carried out by the STRI staff, and they generated the genetic data. The main focus of this dissertation is to analyse the data and describe the genetic structure and ecological diversity of these populations, as well as to establish any kind of interaction such as interbreeding.

Genetic variation, also referred to as “polymorphism” among organisms is an outcome of mutation, which is usually a result of both normal cellular processes and the interaction with the environment. In conjunction with selection and genetic drift, there arises genetic variation within and among individuals, species, and higher order taxonomic groups (Cordes and Liua, 2004). At the DNA level, types of genetic variation include: base substitutions, commonly referred to as single nucleotide polymorphisms (SNPs), insertions or deletions of nucleotide sequences (indels) within a locus, inversion of a segment of DNA within a locus, and rearrangement of DNA segments around a locus of interest (Cordes and Liua, 2004). Through long evolutionary accumulation, many different instances of each type of mutation should exist in any given species, and the
number and degree of the various types of mutations define the genetic variation within a species.

With the discovery of DNA marker technology, these variations have become valuable to geneticist in studies of such genetic variations within a genome of any particular individual, and thus of any given population. Furthermore, molecular marker technologies are developing with new ways of rapidly assessing diversity across many loci. Diversity Arrays Technology (DarT) has recently been used for genetic diversity assessment. DarT assesses polymorphism at thousands of genomic loci, providing binary scores in a single hybridisation-based assay and was developed originally using the rice genome (Jaccoud et al, 2001).

DNA Microsatellite markers are type II (i.e. are associated with anonymous genomic segments (O’Brien, 1991; Table 1)) unless they are associated with genes of known function, and their application has contributed to a rapid progress in population genetic structure studies. Other genetic markers that are used in genetic structure studies include mitochondrial DNA (mtDNA), Restriction fragment length polymorphism (RFLP), Random amplified polymorphic DNA (RAPD), Expressed sequence tags (EST), Single nucleotide polymorphism (SNP), Insertions/deletions (Indels) and so on (Cordes and Liua, 2004). Yang et al (2006) identified that present technologies (RFLPs, RAPDs, and SSRs) are limited by one of, or a combination of the following factors; low throughput and high cost of development and / or routine analysis. SNPs, even though they are highly abundant are costly in discovery and development. Based on their study, Yang and others argue that the use of DArT can alleviate such difficulties as it is low cost, high throughput and sequence independent.

Despite Yang et al’s arguments, DNA microsatellite technique is rapidly becoming the preferred method for studying population genetic structures both for plants and animals alike because microsatellites are useful genetic markers as they are usually highly
polymorphic, codominant and multi-allelic (Francisco-Candeira et al, 2007). For the same reasons, this technique was chosen to map and study the population structure of mangrove species *Rizophora Mangle* on several islands of the Las Perlas Archipelago in the Panamanian Gulf. Understanding the distribution of genetic diversity among and within populations is necessary for the efficient management and *in situ* conservation of any plant population. The importance of *in situ* conservation of plant genetic resources has gained greater recognition in the past 10 years (O’Brien, 1991). This emphasis was initiated with the ‘Convention on Biological Diversity’ (CBD), the treaty signed by 150 government leaders at the 1992 Rio Earth Summit (www.biodiv.org/convention) and gained momentum with the Global Action Plan of the Food and Agriculture Organisation of the United Nations (FAO) (Rome, 1996) which emphasised the priority needed for *in situ* methods of diversity maintenance.

### 1.2. The area of study and its mangrove community

The Las Perlas Archipelago lies within the Gulf of Panama on the Pacific coast of the country, approximately in the centre of the Gulf. It covers a large piece of land, ranging from coordinates 08°14’N and 79°07’W (see figure 2). There are more than a hundred small islands ranging from a few to hundreds of hectares in size. Among the major islands are Rey and San Jose which lie to the east and south west of the archipelago respectively (see figure 2). Generally, there are four species of mangroves occurring along the pacific coast of Panama, *Rhizophora mangle*, *Laguncularia racemosa*, *Avecinea germinans* and *Pelliciera rhizophorae*. However, according to surveys which were carried out on the Archipelago, the islands are mainly colonised by three species namely; *R. mangle*, *L. racemosa*, and *P. rhizophorae*. Each species is different, both in individual characteristics as well as in general distribution, however they all do share major characteristics of mangroves described in Chapter 2.

*L. racemosa* is distributed throughout the tropical latitudes of the Americas, however it tends to be restricted to the landward fringe of the mangrove community but it also well-
capable of pioneering into disturbed sites where it can form pure stands (Tomlinson, 1999). On the Pacific coast of Central America, it is generally found behind *R. mangle* stands. *L. racemosa* has a wide salt tolerance and is shade intolerant, with small but numerous propagules, which germinate while they disperse and can reach very high densities on the forest floor (Tomlinson, 1999), which are typical characteristics associated with a pioneer species. Dispersion within Panama occurs during mid-August till late November (Rabinowitz, 1978b).

*P. rhizophorae* is a characteristic species of the Central American region and has a limited distribution, which is restricted only to this area (see figure 3.3c). Significant stands of this species are now only found on the Pacific side of Costa Rica in areas of high rainfall (Jimenez, 1984). The limited distribution of this species is thought to be due to its low tolerance of salinity, which further justify why it is restricted only to areas with high run-off or rainfall (Jimenez, 1984). *P. rhizophorae* is characterised by small trees, usually 5 to 10m high but a maximum height of approximately 18m can be reached. Flowering occurs over an extended season during the months of November to February, with a peak occurring during October (Tomlinson, 1999).

*R. mangle* was found to be the most dominant species on the Las Perlas Archipelago, and it is because of this reason that it was used for this study. *R. mangle* trees can achieve a maximum height of around 30m, allowing the trees to form large and complex root systems. The propagules are rod-shaped with elongated enabling them to float and they are relatively log-lived. Dispersion within Panama occurs during September and October, although off-season production may also occur (Rabinowitz, 1978b). Although *R. mangle* is cold- and shade-intolerant, it is well-known for its wide range of salinity tolerance (Benfield, 2002).
Figure 1.1. Map of Las Perlas Archipelago, Panama indicating sampled plots.

1.3. Aim

The aim of this dissertation is to analyse the genetic data which were gathered by the Smithsonian Tropical Research Institute staff and establish the genetic structure of the *R. mangle* population of the Las Perlas Archipelago in the Gulf of Panama.
Chapter 2:
Literature review on mangrove biology and ecology

2.1. Mangroves ecology and adaptations

Mangroves can be defined as halophytic, mainly tree species that grow in tidal saline wetlands along tropical and subtropical coasts (Duke, 1992). Approximately 83 species from 24 plant families have been recognized, mainly consisting of trees and shrubs that normally grow above mean sea level in the intertidal zone of marine coastal environments or estuarine margins (Duke, 1992). Generally, mangrove forests are tough ecosystems to invade because few species can tolerate the hydrological and edaphic conditions that prevail in mangrove habitats. Such habitats are characterized by anoxia, salinity and frequent tidal inundation, therefore, the few species that occur there all exhibit special adaptation to enable them to survive.

These adaptations include pneumatophores (exposed breathing roots) to allow gas exchange in what are frequently anaerobic sediments, supporting prop roots for support in what may be shallow and unstable sediment, buoyant and viviparous propagules that allows dispersion of the seed in an aquatic environment and salt excretion glands to manage salt balance within the plant in what is frequently a saline environment (Tomlinson, 1999). Because of these universal traits, all mangrove species are potential colonizers, and usually can survive and grow provided that local conditions remain within their ranges of tolerance (Lal, 1990).

Two key factors control forest community structure at specific mangrove localities within the (mainly) tropical regions. Important environmental factors potentially controlling distribution and growth are tidal inundation, soil pore water salinity, sediment stability and type and fresh water input as discussed by several authors (Rabinowitz, 1978a,b,c; Jimenez et al., 1985; Smith, 1987a,b; Cardona & Botero, 1998; Jimenez and Sauter, 1991; Smith, 1992; Ellison and Farnsworth, 1993; Duke et al., 1998;). Soil porewater
salinities and soil wetness are considered the key physiological constraints on mangrove species growth and competitive potentials (Ball 1988, Smith 1992).

2.1.1. Morphological specializations of mangroves

The structure and anatomy of mangroves have received considerable attention over the past decades as plant ecologists strive to understand the functional processes, particularly those that are specialised and adapt plants to the mangal environments. That such physiological investigations of mangroves are relatively few, as Tomlinson (1999) puts it, is remarkable considering that it is the suite of functional characteristics that allows mangroves to survive tidal environments. The most remarkable morphological specializations of mangroves are vivipary, salt secretion, and aerial roots (Tomlinson, 1999). Although these characters are widely thought to have facilitated the adaptation of mangroves to harsh coastal environments, no single structural feature uniquely characterizes mangroves (Tomlinson, 1999).

(a) Salt secretion

High salinity makes it more difficult for mangroves to extract water from the soil, even though the soils on which mangroves grow are usually waterlogged. Consequently, many mangrove species have morphological characteristics and high water-use efficiencies characteristic of terrestrial xerophytes (Saenger, 1982; Clough et al., 1982; Clough and Sim, 1989; Ball, 1988; Ball et al., 1988). According to the mechanism of salt elimination, mangroves can be divided into two groups: salt-secreters and non-secreters. Secreters control their salt balance by excreting the absorbed salt metabolically via salt glands (Fahn, 1979; Fahn and Shimony, 1977; Tomlinson, 1999). In comparison, non-secreters selectively absorb only certain ions from the solution they come into contact with by the process of ultrafiltration (Morgany et al., 1999; Tomlinson, 1999). The structure of salt glands in salt-secreting mangroves is surprisingly similar in view of the fairly remote systematic affinity of the several families involved (Tomlinson, 1999). Whether salt secretion among mangroves evolved once or multiple times is not well understood.
Scholander and coworkers, have shown that the roots of some mangrove species exclude 80–95% of the salt in the soil solution, and they concluded that species without salt-secreting glands are more effective in excluding NaCl than those with salt-secreting glands (Scholander et al., 1962, 1964; Scholander, 1968). However, a more recent study found that *Avicennia marina*, a species with foliar salt-secreting glands, excluded salt with about the same efficiency as that reported by Scholander and coworkers for species without salt-secreting glands (Moon et al., 1986). If correct, this, according to Paliyavuth et al (2004), could challenge the above-stated, widely held theory in mangrove ecology that mangroves fall into two distinct groups of salt excluders and salt secretors.

Studies of the equilibrium water relations of mangroves also suggest that their ability to grow in a ‘physiologically’ dry environment without apparent adverse effects of severe water stress is associated with their ability to experience large negative shootwater potentials for only a small change in intracellular water content (Scholander et al., 1964; Scholander, 1968; Suárez et al., 1998). If this is the case, differences in shoot water relationships between species may help to explain observed zonation patterns in relation soil salinity and water availability. The study undertaken by Paliyavuth and colleagues was to investigate relationships between salt uptake and shoot water balance in four species of mangrove (*Avicennia alba* Bl., *Bruguiera gymnorrhiza* Lamk., *Xylocarpus granatum* Koen. and *Heritiera littoralis* Dry.) with widely different site preferences, in order to test three hypotheses, namely;

1. There is no difference in the efficiency with which mangrove species exclude NaCl, defined as the ratio of the concentration in the xylem divided by the external concentration around the roots, irrespective of their habitat preference, or whether or not they possess foliar salt-secreting glands.

2. The efficiency with which mangrove species exclude NaCl, as defined above, is independent of the soil salinity.

3. The slope of shoot water potential–relative water content isotherm increases with increasing salinity, thereby enabling mangroves to acquire water at high salinities without a significant loss of turgor, (Paliyavuth et al, 2004).
According to their observations, Paliyavuth’s team support the hypothesis that species without salt-secreting glands (e.g. *B. gymnorrhiza*) are more efficient in excluding salt than those with salt-secreting glands, such as *A. alba* (Scholander et al., 1962).

Though xylem salt concentrations are lower in non-salt-secreting species than in those with salt glands, salt nevertheless accumulates quite rapidly in the leaves of the former. Clough et al. (1982) estimated that for a non-salt-secreting species the average measured leaf chloride concentration could be reached within 10 days at soil salinity close to seawater. Assuming that all the salt being transported in the xylem ends up in the leaves, results of their study suggested that it could take 40–45 days for leaf sodium concentrations to reach their measured concentrations, which is still a relatively short time frame in relation to leaf longevity (which is estimated by Tomlinson (1999) to be about 6 to 12 months, with a maximum of 17 months). Hence there still remains the question of how mangroves without salt-secreting glands manage to regulate leaf salt concentrations (Clough et al., 1982).

**Genes responsible for salt tolerance:**
Salinity stress was the focus of the work carried out by Miyama and Hanagata (2007). Part of their findings was that it can lead to changes in development, growth and productivity, and severe stress may threaten survival. Many studies have also been conducted to elucidate salt stress responses and the elements that might confer tolerance to sensitive plants (Cushman and Bohnert (2000), Hasegawa et al, (2000), Kreps at al, (2002) and Zhu (2001)). At the physiological level, the multitude of effects of salt stress indicates the importance of protecting the organism from the damage by reactive oxygen species (ROS) that inevitably increase as water deficit and increased ion uptake impair photosynthesis (Noctore and Foyer, 1998). Also implicated are the protective roles played by accumulation of metabolites that seem to act in more than one function, preventing radical formation, acting as low-molecular-weight chaperones, contributing to
redox control, and functioning as compatible solutes by decreasing osmotic potential (Sakamoto and Murata, 2000).

Genetic studies have shown that an over-expression of the transcription factor dehydration-responsive element binding-1A (DREB-1A) resulted in improved tolerance to several stress conditions, including drought, salt and cold (Kasuga et al, 1999). In some cases, a stress response can be improved by changing the expression of a single down stream gene. Most of these studies, however, have been on typical non-halophytic plants, such as Arabidopsis (Seki et al, 2002 and Takahashi et al, 2004), rice (Kawasaki et al, 2001), and barley (*Hordeum vulgare*) (Oztur et al, 2002), lacking of genetic potential to survive in high salinity environment.

Miyama and Haganata identified many potentially important stress-induced genes in Burma mangrove by microarray analysis. The major response observed by microarray analysis was the differential response to salt shock between leaf and roots. cDNA microarray analysis confirmed the stress responsive expression of a number of previously reported stress-inducible genes, such as CDPK Berberich and Kusano (1997), peroxidase (GO:0016491), vacuolar ATPase, and several PR-protein family genes (Hoffmann-Sommergruber, 2002). Their results indicated that there are similar molecular mechanisms of stress tolerance and responses between burma mangrove and model plants, but also some significant up-regulation of unknown genes or burma mangrove specific genes (Bg70), (Miyama and Hanagata 2007).

(b) Vivipary

Vivipary is a condition found in some seed plants in which the sexually produced embryo of the seeds, while still attached to the parent plant, continues its development without dormancy. Vivipary can be divided into two major different forms, known as “true vivipary” and “cryptovivipary,” representing the two situations in which the embryo grows to break through the fruit wall, and the seed coat, respectively (Tomlinson and Cox, 2000).
Among seed plants, vivipary is most well developed in mangroves (Tomlinson, 1999), however, true vivipary occurs occasionally in some seagrasses, such as *Amphibolis*. Since vivipary is such a remarkable character, the mechanism, including its evolutionary origin, is of great interest to many plant biologists. In particular, whether vivipary resulted from single or multiple origins has been debated and analyses based on the viviparous structure alone have not been conclusive (Farnsworth, 2000; Guppy, 1906; Van der Pijl, 1983).

(c) Root systems

A third feature of more highly specialized mangroves is that some parts of the root system become exposed to the atmosphere (Gill and Tomlinson, 1975; Tomlinson, 1999), thus forming what is referred to by ecologists as “aerial roots”. Tomlinson (1999) defined several types of aerial roots in mangroves, including stilt roots, pneumatophores, root knees, and plank roots. The mangrove root system plays three distinct roles; the aerating, anchoring/absorbing, and cable system. These morphological components are thought to have different origins in different species (Tomlinson, 1999).

Many paleobotanists have argued that the mangrove habitat is an ancient one and many seed plants share some primitive characters of mangroves. For example, vivipary was suggested to be the rule under uniform climatic conditions of early geological periods (Cridland, 1964; Raymond and Phillips, 1983). Retallack and Dilcher (1981) went even further, in light of fossil evidence, to suggest that angiosperms may have all radiated from coastal environments. The mainstream view, however, appears to be that most of those important adaptive attributes of mangroves were derived in specialized habitats rather than lost in general habitats. This view of independent and multiple evolutionary origins through convergent evolution is largely based on the occurrence of traits in different unrelated angiosperm families (Cox and Humphries, 1993; Ellison and Farnsworth, 2001; Farnsworth, 2000). While the scattered distribution of any adaptive trait in a phylogenetic
framework of mangroves may seem to be the apparent evidence for its multiple origins, the statistical support for such intuitions can often be unconvincing (Mooers and Schluter, 1999; Oakley and Cunningham, 2002; Pagel, 1999). This is especially true when the loss of a character is more likely than the gain which is believed by some plant ecologists to be the case for vivipary and salt secretion.

2.2. Mangrove distribution and zonation

Mangroves are distributed throughout the tropics, occurring in some 122 countries, covering about 18 million ha, and occupying almost one quarter of the world’s coastline (Kathirestan and Qasim, 2005). Forty percent of global mangrove cover is in Asia (see fig. 1), but the region has also experienced the highest loss of mangroves over the past decade, which has been primarily attributed to the development of aquaculture and tourism infrastructure.


Fig 2.1. World distribution of mangroves (www.flmnh.ufl.edu)

Mangrove forests are commonly seen with clear, monospecific stands which are parallel to the shore. Several theories have been proposed by botanists and plant ecologists to explain zonation patterns within mangroves, which, by far have included physico-
chemical gradients as well as competition between individuals. Rabinowitz (1978c) proposed that mangrove zonation pattern is controlled by tidal sorting of propagules according to size and buoyancy capacities. However, Delgado and other’s study challenged this hypothesis. They conducted a study on mangrove zonation, and they concluded that propagule size may not be the more important characteristic, based on their observation of *Avicennia* propagules which were over 30% larger than *Laguncularia* but also greater buoyancy which may restrict them to the middle and upper intertidal zones. But they agreed that the distribution of mangrove species with small propagules in the lower intertidal zone and species with large propagules in the middle to upper intertidal zones is a common observation for many mangrove forests, such as the Australian mangrove forests (Smith, 1987a). *Laguncularia* is usually considered as a pioneer of low intertidal zones, but it is not, however, typical for other environmental settings. In the downstream region of estuaries or under more marine influence, *R. mangle* is generally found colonizing mud bank deposits and lower intertidal zones, where higher salinities and more vigorous water movement exist. Under such conditions *Laguncularia* is found in higher elevations forming mixed stands with *Avicennia* and/or other species (Jiménez, 1994; Cantera and Arnaud, 1997). Throughout the Caribbean, *Laguncularia* has been observed as a dominant species in basin type mangrove forests (Jiménez, personal communication).

Mangrove establishment and colonization of new environments is initially dependent on the availability and successful dispersal of propagules to the sites of colonization (Ball, 1980; Clarke, 1993; Duke et al., 1998; Panapitukkul et al., 1998). High tree density increases the number of propagules that can be dispersed, and the closer the parent trees are to sites of colonization the higher the chances of successful dispersal. Both *Laguncularia* and *Avicennia* (the two species studied by Delgado and co-workers) produce large quantities of small, water-dispersed propagules (Jiménez, 1994), however, the dominance of *Laguncularia* adjacent to mud banks suggests that this species has an advantage for continued dominance of this zone.
Propagule buoyancy is thought to be an important factor affecting propagule dispersal and establishment in frequently flooded environments (Johansson et al., 1996). Propagules that can remain buoyant and viable for longer periods of time increase their effective range of dispersal (Rabinowitz, 1978a; Johansson et al., 1996; Duke et al., 1998). According to Delgado and others, *Laguncularia* propagules showed a floating period between 2 and 7 days, and the ability to establish and grow under water. *Avicennia*, however, floated during the 25-day experiment. This contrasts with the findings of Rabinowitz (1978a) who reported that for *Laguncularia* in Panama an average floating period of 23 days in freshwater and 31 days in salt water, while *Avicennia* floated during an 82-day period. *Avicennia* has an increased chance of long distance dispersal and deposition in the upper intertidal (spring high tide), but also of being carried out of the system by tidal action (Delgado et al., 2001). Propagules that sink rapidly, such as *Laguncularia*, may result in restricted dispersal away from parent trees.

Being a “sinker”, appears also to be an advantageous characteristic for a pioneer species colonizing frequently flooded environments such as mud banks. Similar to *Laguncularia*, propagules of *A. marina*, sometimes found in the seaward edge of mangroves (Semeniuk, 1985; Fujimoto and Miyagi, 1993; Osunkoya and Creese, 1997), sink within 1–3-day period (Steinke, 1975; Clarke and Myerscough, 1991; Clarke, 1993). This process of propagule dispersal, which relies on water level and buoyancy capacities, seems to agree with the hypothesis of “directed dispersal” proposed by Howe and Smallwood (1982), which assumes that adaptations for dispersal increase the probability of seeds to reach suitable sites for germination. During the study, establishment of *Avicennia* appeared to be more influenced by tidal water movements than *Laguncularia*. Under the action of relatively strong water currents buoyant *Avicennia* propagules tended to remain above the soil surface, making stranding more difficult. Those propagules that were able to stay on the bottom had to spend more energy and time reorienting their anchoring roots each time they were moved from their original position (personal observation of Delgado).

Similarly, *A. germinans* and *R. mangle* propagules had difficulty becoming established at a lower intertidal *Rhizophora* dominated zone, where tidal action regularly buoyed them away from the soil surface (McKee, 1995b). McMillan (1971) demonstrated a negative
relationship between water turbulence and *A. germinans* root development under laboratory experiments. Under field conditions mortality of *A. germinans* of clipped plots in a *Spartina* low marsh was attributed to the physical effects of the tides (Patterson et al., 1993, 1997). The effect of water movement has also been shown on the establishment of other mangrove and wetland species (Foote and Kadlec, 1988; Clarke and Allaway, 1993; Clarke and Myerscough, 1993).

The overall conclusion of Delgado et al (2001) was that both physical and biological factors affecting early stages of mangrove establishment are important in determining mangrove species’ distribution in different environmental settings. These factors include propagule abundance, dispersal, buoyancy and sensitivity to flooding and mechanical stress. For *Laguncularia*, high propagule production, effective dispersal, short propagule floating period, and low propagule sensitivity to moderate tidal action and hydrologic regimes, are important factors explaining its successful colonization of emergent mud banks and lower intertidal zones. *Laguncularia*’s limited distribution in upper intertidal zones, on the other hand, is closely related to restricted propagule dispersal and an intensive and selective crab propagule predation, which greatly increases its mortality in this zone. Restricted dispersal and stranding requirements (buoyancy) are important factors limiting *Avicennia* establishment in lower intertidal areas, giving an advantage to *Laguncularia* in this environment.

**Predation on seeds and its effect on distribution and zonation:**

Another factor that influences mangrove seedlings dispersal is predation by crabs. Several factors are related to differential crab-induced propagule predation. Crab preference among different mangrove species has been related to propagule characteristics such as size and chemical composition (Smith, 1987b; McKee, 1995a, c). Differential predation has also been related to depth and frequency of flooding, which can affect crab forage time and community structure, favouring some species over others or affecting size or age distributions (McKee, 1995a).
Based on the pattern of propagule predation observed across the intertidal gradient of point bars and islands, the dominance–predation model applies for *Laguncularia*. Predation was lowest where this species was dominant and highest where it was absent from the canopy. This model has been supported for some mangrove species (Smith, 1987b; Smith et al., 1989) but not for others (McKee, 1995a; McGuinness, 1997b; Sousa and Mitchell, 1999). In addition to predation, propagule desiccation and loss of viability were also causes of *Laguncularia* mortality in upper intertidal zones. In summary, limited propagule dispersal and selective crab predation of *Laguncularia* propagules are important factors limiting *Laguncularia* distribution in higher intertidal zones, favoring *Avicennia* development in this environment. Similarly, the effect of crab predation on the distribution and zonation of mangrove species has been shown for other mangrove ecosystems around the world (Smith, 1987b; Robertson, 1991; McKee, 1995a).

### 2.3. Mangrove Species richness and genetic diversity

Factors affecting species richness: landform, fresh water input/salinity regime. Species richness is higher in large estuaries with moderate rainfall and salinity regime. Lakshmi et al (2002) investigated the genetic diversity and species relationship among Rhizophoraceae species at both intra- and inter-population as well as species levels using molecular markers based analysis. DNA based molecular markers, unlike morphological markers are considered as stable and influenced very little by environmental fluctuations (Gottlieb, 1977; Hardrys et al., 1992). DNA based markers such as random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) have been widely used in a number of plant groups for variety of purposes such as cultivar identification, diversity studies, parentage determination, developing breeding programmes and conservation strategies. Similarly, markers specific to ribosomal DNA and chloroplast genes have provided reliable information for the analysis of genomic relationships above the level of species owing to their highly conserved nature (Lakshmi et al, 2002).
For a long time diversity in mangrove species has been studied based on ecology, morphology, floral physiology and structure of each vegetation types. All of these parameters vary extensively depending on the physical and environmental conditions of the habitat. Therefore, assessing the genetic diversity in these species using environmentally stable DNA markers helped in explaining the wide distribution of individual species and also the genetic potential of selection and evolution in mangroves (Spalding et al., 1997). It is generally believed that for long term survival and its adaptation a greater genetic diversity is a must for a species. Schaal et al. (1991) have stressed that species without adequate genetic diversity are at a greater risk of extinction.

Mating parameters (genes) and their effect on population genetic structure:
A study was carried out by Ge et al (2005) to investigate the mating system of a typical species of mangrove, *Bruguiera gymnorrhiza* (Rhizophoraceae) in the coastlines of south China. The relationships between the mating system and population structure or patterns of genetic variation among the populations were estimated. The information on the mating system parameters is helpful to explain the patterns of genetic diversity and enable to develop appropriate conservation strategies for mangrove species. The mixed mating model (Fyfe and Bailey, 1951) gives a robust and frequently used procedure for estimating mating system parameters. However, several new approaches have been developed (Brown, 1989), which enable further investigations of demographic and genetic causes of mating patterns. For example, the effective selfing model (Ritland, 1984, 1986) calculates mating system parameters in spatially structured populations. It should be noted however that, the mixed mating model is limited by certain assumptions, in which case the maternal plants are random samples from the population producing pollen. However, genetic diversity among populations could be caused by species’ biological characteristics and the life history.
2.4. Fauna associated with mangrove swamps

Mangrove vegetation contributes to habitat complexity and the diversity of the associated fauna of the mangrove ecosystem (Hutchings & Saenger, 1987; Lee, 1998). The dominant macrofauna in terms of numbers and species are the crustaceans and molluscs (Sasekumar, 1974; Jones, 1984). These macrofauna form an important link between mangrove detritus at the base of the mangrove food web and consumers at higher trophic levels, which include birds and commercial fish species (Macintosh, 1984). Macrofauna also modify the mangrove’s physical and vegetation structure through their burrowing activities (Smith et al., 1991) and by grazing on propagules, leaves and wood (Berry, 1972; Smith, 1987). Overall, crustaceans and molluscs play an important role in the ecological functioning of the mangrove ecosystem (Lee, 1998, 1999). Thus, their diversity and abundance may reflect the status and functioning of mangrove forest ecosystems and serve as potential biological/ecological indicators of habitat change in both natural and managed mangroves. Highly productive benthic bacterial populations are found in mangrove forests, ranging from 0.6-5.1 g of carbon release per m² per day (Alongi & Sasekumar 1992). Sediment bacteria are responsible for the bulk of detrital turnover in mangrove forests (Alongi 1990).

2.5. Mangroves importance, threats and conservation

Mangroves play a significant role in the global ecology. Part of their ecological significance lies in protecting the coast from erosion by wave action, hurricanes and floods. They also constitute a trap for sediments and nutrients and provide food, breeding grounds and nurseries for commercial species of fish and shellfish (Tomlinson, 1999). Furthermore, for human populations in coastal areas, they furnish important resources including firewood and timber (for furniture and construction) and many other harvestable benefits including a source for charcoal, tannin, paper, dyes and chemicals, thatch, honey and incense. The foliage of mangrove species is used to feed livestock, and several mangrove plants are used for traditional medicine.
Globally, nearly two thirds of all fish harvested depend on the health of wetlands, such as mangroves, seagrasses and coral reefs for various stages in their life cycle. An authoritative study carried out by an international group of scientists in the Caribbean, found that mangroves play a vital role in nurturing and protecting juvenile coral reef fish. Coral reefs were found to have more than twice as many snappers (\textit{Lutjanus apodus}) and grunts (\textit{Haemulon sciurus}) where healthy mangrove forests were found nearby. However, the destruction of mangroves may have caused local extinction of one of the largest herbivorous fish in the Atlantic – the rainbow parrotfish (\textit{Scarus guacamaia}). The researchers concluded that if the current rate of mangrove deforestation continues there are likely to be serious impacts on ecosystems and the productivity of fisheries, (EJF.2006. \textit{Mangroves: Nature’s defence against Tsunamis}).

Mangrove forests store and process huge amounts of organic matter, dissolved nutrients, pesticides and other pollutants that are dumped into them by human activities, and by absorbing excess nitrates and phosphates prevent the contamination of coastal waters. In so doing, they play a vital role in protecting coral reefs and seagrasses from siltation and eutrophication. Mangroves also function as a sink for atmospheric carbon dioxide, a major contributor to global warming (Lal, 1990).

However, over the last 50 years, nearly one third of the world’s mangrove forests have been lost due in particular to urbanisation, the demands of agriculture and aquaculture and the effects of pollution, which has resulted in loss of biological species (Kathirestan and Qasim, 2005). Globally, mangrove forests are among the most threatened habitats, with rates of loss exceeding those of rainforests and coral reefs (Yap, 2000). The development of shrimp aquaculture poses the gravest threat to the world’s remaining mangroves, and one estimate has attributed a large portion of recent mangrove loss to the industry. Other detrimental human activities include over-harvesting of wood for fuel and timber production; land clearance for agriculture and coastal development; mining; pollution; and damming of rivers, which alters water salinity.
A conservative estimate of the rate of mangrove destruction in the Asia and Pacific region is about 1% per year (Dorcey, 1986). Twenty to 50% of this may be attributed solely to the development in recent decades of culture ponds for shrimp and fish (Dover, 1999). Another significant contributor to degradation is the mangrove wood-chips industry based in Japan (Dorcey, 1986). Unfortunately, the conversion of mangrove forests for other uses is still fostered, directly or indirectly, by industries, governments and tourism operations (Dixon and Lal, 1994). Most of the damage to mangroves from shrimp farming is caused by direct conversion of mangrove land to shrimp ponds. Inorganic or organic pollution produced by shrimp farms can also lead to or exacerbate mangrove degradation. In India, mangroves were destroyed in order to control mosquito populations because they act as a shelter for mosquito larvae (http://www.ejfoundation.org/pdf/tsunami_report.pdf).

Increasing awareness of the true value of mangrove ecosystems has led to renewed efforts to protect and restore them. According to Yap (2000), mangrove forests should be conserved and restored for several reasons, including; (1) conservation of the natural landscape; (2) to ensure the sustainable production of natural resources which usually serve multiple-use systems (e.g., timber, charcoal, shrimp harvest, and other uses mentioned above); and (3) to help protect coastal areas, such as from damage by cyclones and tidal bores. He further emphasizes that efforts at habitat restoration must be based on sound understanding of the ecological conditions prevailing at the affected area, on the biology of the species to be introduced as recruits or pioneers, and their ecological requirements. Ecological parameters vary considerably from site to site, so that it is highly likely that chances of success will be very site-specific as well. Success is of course also dependent on human communities surrounding the site to be restored (http://www.ejfoundation.org/pdf/tsunami_report.pdf)

Mangrove restoration or rehabilitation has been initiated successfully in various parts of the world, including Thailand (Khemnark, 1995; Field, 1996). Unfortunately, mangrove rehabilitation has, in many cases often been carried out simply by planting mangrove seedlings without adequate site assessment, or subsequent evaluation of the success of
planting at the ecosystem level (Field, 1996). Moreover, for economic reasons, mangrove reforestation efforts are often limited to only one or two tree species (e.g. Gan, 1995). This raises obvious questions regarding habitat change and reduced ecological function in mangrove plantations compared to natural, mixed species mangrove forests. Biodiversity is widely regarded to be important in maintaining genetic richness, ecological functioning and the resilience of the ecosystem (Schultze & Mooney, 1993; Heywood, 1995).

From an environmental management perspective, the results from Klong Ngao suggest that some groups of the macrofauna may be useful as biological indicators to assess the ecological impact of mangrove health and/or rehabilitation. A high dominance of one species of macrofauna may indicate a stressful environment, whereas a higher diversity of another species may be indicative of a more stable, mature area. For example, sesarmid crabs have been proposed as keystone species (Smith et al., 1991; Lee, 1998) because they have a significant impact on the bioturbation of the habitat and on nutrient recycling within the system. Indeed the concept of ecological indicator species has been used effectively in temperate regions and could be developed similarly for mangrove systems. The structure of mangrove crustacean and molluscan communities could be a useful tool for habitat assessment, but further research on the ecological role of nominated indicator/key species is needed (Macintosh et al, 2002). Overall, there is a paucity of ecological experiments on rehabilitated mangrove ecosystems and applied research is needed to test the options for enhancing mangrove ecosystem management (Field, 1999).

Economic values role and mangrove conservation:
Economic information is valuable to decision-makers trying to encourage efficient and sustainable use of mangroves. The type of economic value information needed will depend on what it will be used for. Mangrove economic value information is generally used in three broad ways: for advocacy purpose; when choosing between alternative uses; and when wanting developers to pay for externality costs of their activities on mangrove ecosystems (Dixon and Lal, 1994). Recognising that economic paradigm dominates government decisions, and economic development is often their primary goal, economic
valuation of natural resources is now commonly advocated. The Ramsar Bureau, under the Ramsar Convention, is encouraging countries to undertake economic valuations of wetlands (Rubec, 1999). In some parts of the world such as in the Pacific, the South Pacific Regional Environment Programme (SPREP) strongly recommends that island nations “conduct or sponsor research on economic values of wetland ecosystems and species of the Pacific Island region, and direct results to national land-use planning/management plans and conservation organizations” (Graduate Studies in Environmental Management and Development, National Centre for Development, Australia, 2003).

Another Nature Conservation Conference which was held in Rarotonga in the Cook Islands in the Pacific in July 2002 also identified economic valuation of natural resources as one of the key strategies needed in the region to encourage environmental conservation. By using economic value information it is assumed that one would be able to make stronger arguments and hopefully more readily convince key decision-makers, i.e. individuals, communities and governments, about the conservation of in situ values of wetlands than when only ecological information is used.

It should be understood that, for mangrove forests, any mangrove land-based activity produces some impact on the aquatic subsystems, affecting fisheries and other uses. Such externality costs, in the absence of property rights over the whole ecosystem, would then normally be borne by a third party or the society. However, the impact can be minimized and economic efficiency increased if the developer is made to pay. That is if the government adopts the principle of ‘impactor’/‘polluter’ pays (Graduate Studies in Environmental Management and Development, National Centre for Development, Australia, 2003). Thus, for mangrove land to be efficiently used, developers who wish to reclaim mangroves would, according to the ‘impactor pays principle’, pay for the full costs of forgone in situ goods and services. To make developers take into account their externality effects, governments would need to charge them a fee equivalent to the economic value of impacts in the form of a ‘tax’ or fee. Such payments would ideally
reflect the marginal value of the losses in forest products, fisheries output and other services. However, in practice, this may not always be feasible.
Chapter 3:
The implication of genetic analysis on our understanding of mangrove biology and distribution

As previously mentioned, the Smithsonian Tropical Research Institute of Panama, in partnership with the Darwin Initiative, and Heriot-Watt University teamed up in a three-year period (since April 2003), to develop a project that would give the Las Perlas Archipelago in Panama its recently-attained status of a “special management zone”. This status will provide the basis for a conservation strategy for the marine environment of the Archipelago. The main aim of the project was to map and assess the biodiversity of key marine habitats with the aim of providing comprehensive guidance to the appropriate Panamanian national authority (ANAM) to enable the establishment of this archipelago within the system of protected areas in Panama (http://striweb.si.edu/darwin-initiative/). Their objectives included producing school and community educational material, and to establish an “experience and information exchange” network to link related past and present Darwin Initiative project workers in the region and elsewhere. This was achieved through a series of surveys which included both biological as well as socio-economic studies. In order for proper management measures to be implemented, it is very important that the biology of the ecosystem in question is well understood. The process involved a lot of data gathering, which included genetic data which were used in writing up this thesis. Dr. Hector Guzman and his team from the Smithsonian Tropical Research Institute (STRI) collected the data and analysed it. The main purpose of this thesis was to interpret the results and relate it to the conservation of the Las Perlas Archipelago. For that reason, the materials and methods as well as the results obtained are included into the appendices of this report, instead of being part of the main body. Even though the Archipelago has been assigned the status of a “Special Management Zone” in May 2007, it is still crucial that the data is analysed and interpreted so that the information can be used for protecting mangrove populations in the Las Perlas Archipelago in a long run. Two other Heriot-Watt students are doing their dissertations on the Las Perlas Archipelago, with Katherine Nisbet who is examining zooplankton composition between a mangrove and a reef using night-
light lift net, and Anonin Guilbert Looking at the occurrence of a heavily exploited cockle (locally called *Concha negra* as well) within the same area.

**Fig. 3.1.** The Las Perlas Special Management Zone has a total area of 168,771 hectares, including two nautical miles around the satellite zones of Isla Galera and Roca Trollop (taken from [http://www.conservation.org/xp/frontlines/protectedareas/partners23-1.xml](http://www.conservation.org/xp/frontlines/protectedareas/partners23-1.xml))
3.1. The genetic structure

The most important finding of this study is that *R. mangle* of the Las Perlas Archipelago in the Gulf of Panama have substantial genetic diversity and structure. Analysis of microsatellite DNA variation, which was examined using several approaches detected significant genetic diversity among localities in the Las Perlas islands. Although the Las Perlas Archipelago is genetically structured, it however, at the same time maintain a significant level of gene flow among different genetic areas, as reflected by BAPS 4.04 which detected that 11.6% of collected individuals are significantly admixed, showing a commonality of some microsatellite alleles across all populations. This means the results can be interpreted as showing two levels of gene flow, with some alleles being retained in some areas creating unique alleles thus generating structure, and others present throughout the entire Archipelago.

The retention of genes may be explained by the fact that *R. mangle* have big, elongated propagules that are most likely to disperse very short distances at very local scale compared with ovoid and small seeds from other mangrove species such as black and white mangroves. In addition, *R. mangle* have pollen dispersed by wind exhibiting self-pollination. Short-distance dispersal of propagules and self-pollination are likely to generate "genetic patches" which explains the different populations detected by the analysis. However, some propagules can escape from the estuary system and other forms of barriers, and can thus disperse at long-distance, especially because of their rather relatively high longevity, i.e. one year, (Tomlinson, 1999). In other words, some alleles are private at short-scale inside estuaries while others are common at long-distance, resulting in a complex genetic structure. Interestingly, similar results were obtained in other areas from Panama, therefore *R. mangle* may be dispersing at long-distance independent of geomorphology and hydrology characteristics of the area, but more studies are necessary to validate this postulation.

Within population diversity was shown to be higher than the diversity between populations (85% of the variance). This finding may be related to the large geographical
distribution and isolation of the populations. How the pattern of diversity observed evolved and has been maintained will be due to the combined effects of several genetic, environmental and human influences. Such factors include: population size, gene flow, breeding system and natural selection (Lowe et al., 2004; Coates and Byrne, 2005). In addition, several factors on the genome level need to be considered. These are mutation, polyploidy and linkage (Lowe et al., 2004). The geographical isolation of populations causes differences in allele frequency and type due to: the founder effect, gene flow, microsatellite mutation and genetic drift. By considering the genetic and physical evidence obtained in this study a picture may be gained as to the evolutionary history of the R. mangle populations, which enables the relative contributions of the causes identified above to be assessed.

The level of gene flow between populations is crucial in the level of diversity detected between populations and can occur through either pollen dispersal or seed migration. Between populations that are isolated from one another, such as Rey and Pedro Gonzales, no gene flow is occurring, which means that mutations that occur from a specific event at a locus in one population will not be present in the other population. Therefore, such events will result in unique alleles in a population. This pattern was observed in the data where groups maintained unique alleles, (Appendix B). For example, if the inter-population data set is considered, unique alleles were observed across the 3 loci (RM7, RM36 and RM46) in all island groups (see Appendix B).

Genetic diversity within wild populations is important for maintaining the ability of organisms to adapt to environmental change (Wright, 1977), which, for Las Perlas mangroves, will become ever more important as human actions alter the natural processes and patterns of the forests. Low genetic diversity may affect populations by impeding fertility, productivity, disease resistance, and survival (Wright, 1977). Identification of the extent of genetic interchange among populations has the potential to alert managers to discrete demographic units within the Las Perlas Archipelago. This will also help confirm or deny the status of the Las Perlas mangroves population as a functional meta-population (i.e., a set of spatially disjunct populations among which there is some
immigration; Wells and Richmond, 1995). Microsatellite analyses including this study may also form the basis for sound decision-making surrounding any future supplementation (transplanting) programs that may become necessary, for example to maximize genetic diversity or maintain genetically similar populations within the Archipelago. Of particular interest will be to study and examine whether the recent anthropogenic fragmentation of mangrove forests in the study area has affected the genetic makeup of populations, and whether any loss of genetic diversity from the area has occurred so far, apart from what might be expected naturally. In many places both anthropological activities and overexploitation have threatened the continuing existence of many species of mangroves and this should be avoided in the Las Perlas Archipelago.

Genetic depletion that is characteristic of species with a history of fragmented populations and small population sizes is believed to have dramatic impact on the ability of a species to survive environmental changes (Wise et al., 2002), as could be associated with certain fitness traits (Paschke et al., 2002). Thus, investigations of levels and patterns of genetic diversity of endangered species is expected to provide insights into the evolutionary process and mechanism of the species as well as information useful for developing conservation plans to preserve genetic diversity (Falk and Holsinger, 1991) (see section 5.3).

3.2. The effect of natural selection

There is a possibility that the SSR diversity detected between the different populations is representative of local environmental adaptation and has been directed by the process of natural selection. Generally, SSRs are assumed neutral so that any observed diversity patterns are a result of random genetic drift or gene flow (Valdes et al, 1993). However, it is also understood that evolutionary mechanisms which either link SSRs to loci under selection or where the SSRs themselves have a functional role may deviate SSRs from neutral expectations (Li et al, 2002). Therefore selective pressure may have a contributory role to the hierarchical variance detected.
As explained by Slatkin, (1995), mechanisms by which SSRs could be influenced by natural selection include genetic hitchhiking in selective sweeps whereby an SSR has close linkage to a selective locus and thus other ‘neutral’ SSR alleles diminish. Other studies have revealed a regulatory functional role in selection at certain loci with substantial data indicating that SSR expansions and/or contractions in protein-coding regions can lead to a gain or loss of gene function via frameshift mutation or expanded toxic mRNA (Li et al, 2004).

A further possible reason which would explain the very high level of diversity found within *R. mangle* is the “niche-width” variation theory (Van Valen, 1965), which assumes that there is a higher level of diversity found within populations that are found within more stressful environments. Due to the increased pressure on the mangroves populations as well as on the environments due to such events of global warming, it is worth looking into whether the increase in diversity found within certain populations does indeed correlate with more stressful ecological conditions. But because the Las Perlas Archipelago is considered to be pristine, human activities may be exempted from this effect.

### 3.3. Isolation by distance

“Isolation by distance” refers to the fact that distance-dependent gene flow generally limits the genetic differences among natural populations (Slatkin, 1993). In the presence of isolation by distance, populations in geographic proximity to each other will be more similar at the genetic level than those that are far away from each other as dully displayed by the results of this study when clustering analysis was performed using spatial information. In natural populations, geographical barriers often limit pollen dispersal, while in populations of cultivated species, both seed and pollen dispersal are often a consequence of human activity and artificial (human) selection tends to reinforce existing groups. In this case, natural barriers that limit pollen dispersal are mainly estuaries.

Putative relict plant populations exhibit higher levels of genetic diversity relative to their descendant populations (Comes and Kadereit, 1998 and Hewitt, 2000). When populations
are small and isolated from one another, genetic drift influences genetic structure and increases differentiation among populations (Barrett and Kohn, 1991 and Ellstrand and Elam, 1993). The evident genetic differentiation among populations of *R. mangle* does not appear to be correlated with geographic distance among the populations, because despite higher diversity is observed within populations than among populations. The absence of such a correlation suggests an important role for genetic drift (Fischer and Matthies, 1998) which increases levels gene flow. Breeding system may also lead to a complex genetic structuring within populations, but this was not examined during this study. In future, loss of genetic variability may result from succession because of reduced seedling establishment combined with the elimination of genotypes via competition, poor adaptation, or stochastic events (McNeilly and Roose, 1984).

### 3.4. Implication for conservation

Generally, tropical forests necessary for the survival of mangrove species have largely been disturbed or destroyed by human activities at an alarming rate during the past century. However, the Las Perlas is one of the least affected areas of mangrove stands remaining. Therefore, in the long term, the most suitable strategy for the conservation of *R. mangle* and other mangrove species is the protection of its habitat, thus the designation of the Archipelago as a Special Area of Management will be the most appropriate tool. A further management measure may be aimed at increasing the number of plants in small populations. Since *R. mangle* can spread vegetatively via rhizomes in suitable habitats, a strategy seems feasible involving propagation via rhizome segments and tissue culture techniques, followed by cultivation in garden plots, and subsequent reintroduction into their original wild habitat.

The overall genetic diversity of a taxon has great implications for its long-term survival and evolution (Frankel et al., 1995 and Avise and Hamrick, 1996). Therefore, knowledge of the levels and patterns of genetic diversity is important for designing conservation strategies for threatened and endangered species (Hamrick, 1983, Hamrick and Godt, 1989 and Francisco-Ortega et al., 2000). The present study, together with the results of earlier studies carried out through the Darwin project, has conservation implications for
*R. mangle* and perhaps other mangrove species on the Las Perlas Archipelago. The fact that most of the diversity is found within populations suggests that preserving the maximum genetic diversity of *R. mangle* requires the protection of most of the known populations. Hamrick (1994) suggested that if more than 80% of the total genetic diversity resides within populations, five strategically placed populations should maintain 99% of the total genetic diversity for conservation purposes. However, if most of the genetic diversity is observed between populations, it would imply that more populations are needed to maintain genetic diversity of the species when implementing *ex situ* conservation strategy, but this is not the case with *R. mangle*.

Furthermore, low levels of heterozygosity and high genetic differentiation observed in *R. mangle* may be the consequence of low rate of natural recruitment, clonal growth, gene drift, and habitat fragmentation. Based on this, it can be suggested that *in situ* conservation be an important and practical measure for maintaining the genetic diversity of this species. *Ex situ* conservation should sample from different populations across the distribution range of the species to conserve the high genetic diversity (as reflected by intra-population data, 85%). Preserving the genetic diversity of threatened and endangered species is one of the primary goals in conservation planning because long-term survival and evolution of species depend on the maintenance of sufficient genetic variability within and among populations to accommodate new selection pressures brought about by environmental changes (Barrett and Kohn, 1991). Genetic data can be important additions to demographic data and information about reproductive biology for the adequate management of such populations and species.

The populations of Pedro Gonzales, Vivero sureste and Ensenada Pasa de Tierra (Rey Este) harbour relatively high amounts of the genetic diversity within *R. mangle*, and should therefore be the priority sites for *in situ* conservation. It must be noted, however, that microsatellite markers are not expressed phenotypically, therefore they may not have much direct relevance to natural selection and adaptation. Nevertheless, like other supposedly ‘neutral markers’, microsatellite may serve as an indicator of local adaptation, e.g., via linkage with other functional gene regions.
Chapter 4:
Conclusion and Recommendations

In conclusion, substantial genetic diversity was revealed by microsatellite DNA markers among populations of the Las Perlas Archipelago of Pacific Panama. Analysis of clustering of groups using spatial information identified the best structure of $K=4$ (where $K$ denotes populations) with a fairly high probability of 0.85. Furthermore, AMOVA analysis revealed that a higher level of genetic diversity lies within populations than among populations (0.85%). The remaining proportion of variation represents among populations variation. This implies that a small number of populations are required for \textit{in situ} conservation to preserve the diversity. When pairwise $F_{ST}$ values were compared to estimate the degree of differentiation between pairs of subpopulations, it can be concluded that most localities (93.5%) showed significant genetic differentiation ($P<0.05$), see Table 4.2. As previously stated, the Las Perlas mangrove stands are considered to be pristine relative to other Pacific mangroves forests. The fact that such biological diversity has been spared from massive habitat destruction, exploitation, and artificial propagation should be encouraging of efforts to protect these populations.

A number of researchers (such as Hedrick, 1999 and references therein) have shown that, for highly variable loci such as microsatellites, $F_{ST}$ is constrained by high within-population diversity. As gene diversities within subpopulations and the total population approach 1.0, the difference between them, which should represent the diversity among subpopulations, approaches zero. It should therefore be noted that a certain degree of bias may exist due to that reason. Further studies in the evolution and history of the \textit{R. mangle} community is recommended, which will further strengthen the results of this study. Whether the genetic structure of the community has already been altered by anthropogenic activities should also be examined. Further more, though the current study did not include phenotypic analysis, it would be interesting to determine whether there were any significant differences in phenotypic means for several traits among the identified subpopulations once the identity of each group or subpopulation had been
determined on the basis of genetic markers. It is thus suggested that future studies include this type of analysis and compare the data to genetic data obtained in this study.
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**Websites**

[www.biodiv.org/convention](http://www.biodiv.org/convention)
www.flmnh.ufl.edu
http://www.stri.org
http://striweb.si.edu/darwin-initiative/
APPENDIX A

Materials and Methods used to gather and analyse the data

Field Data collection and analysis

As mentioned before, the data were gathered by the STRI staff and the methods they used are briefly discussed below.

199 adult trees of *R. mangle* were randomly collected from 12 different plots covering the entire area of the Las Perlas Archipelago in the Gulf of Panama. Each plot was geo-referenced using a GPS (Etrex Vista, Garmin?) in order to verify the data for genetic analysis. One leaf was collected from each selected individual tree, and these leaves were then preserved and dried with DRIERITE™ and stored until they could be processed. In order to extract the DNA, dried leaves were mechanically disrupted using a FastPrep FP 120 machine (Bio101), followed by extraction of the genomic DNA using DNeasy plant extraction kit (Qiagen, Valencia, Calif.). The quality and quantity of the obtained DNA was tested using agarose gel electrophoresis at 1.5% and Low Mass Ladder (Invitrogen). For PCR process direct DNA was used for extraction because *R. mangle* is known to generate low DNA quantity (about 1-5 ng/µl).

Individuals of *R. mangle* were genotyped via PCR reaction of six microsatellites loci (RM7, RM11, RM19, RM21, RM36 and RM46) (Rosero *et al.* 2001). For the PCR process a final volume of 10 µL was used, with the following components, 0.1 µM labeled M13 universal forward primer (5’-CACGACGTTGTAAAACGAC-3’, Steffens *et al.* 1993), 0.1 µM forward primer and 0.03 µM reverse primer which contained a tail at the 5’end with the M13 universal forward primer sequence. In addition, we used 1X Taq Buffer (Quiagen), 0.2 mM of each dNTP, 0.25 U of Taq (Quiagen) and standardized Mg²⁺ concentration for each locus such as 2.0 mM (RM11), 2.5 mM (RM7), 3.0 mM (RM19, RM46) and 3.5 mM (RM21, RM36). For the thermocycler program the initial temperature of 94°C for 3 min was used, followed by 35 cycles with the following
temperatures, 94°C for 40s for DNA denaturation, 50°C (RM19, RM21 and RM36) or 55°C (RM7, RM11, RM46) for 40s for DNA annealing and 72°C for 30s for the final extension process. After the 35 cycles a final extension at 72°C for 4 min was carried out. Amplified fragments were electrophoretically separated on ABI 3130xl Sequencer. Fragment size was determined relative to the Liz500 molecular size standard using software Genemapper™ version 4.0.

Divergence among populations was assessed by an analysis of molecular variance (AMOVA, Michalakis & Excoffier 1996) using estimates of $F_{ST}$ (Weir & Cockerham 1984) with Arlequin 3.1 (Exoffier et al. 2005). To supplement $F_{ST}$ analyses, the Bayesian clustering method of Pritchard et al. (2000), and BAPS 4.14 were used to identify cryptic genetic structure using only genotypic data. Based on Hardy–Weinberg expectations, Pritchard et al.’s (2000) program STRUCTURE was also used. This method uses multilocus genotypes to infer the fraction of an accession's genetic ancestry that belongs to a population, for a given number of populations ($K$). Both programs identified the most probable number of populations ($K$) subdividing the sample into a number of different clusters with the minimum departures from HWE and Linkage equilibrium and simultaneously assign the individuals to different clusters. To perform the analysis with STRUCTURE 2.01, the following parameters were selected; admixed model, uniform prior probability of $K$ and an arbitrary threshold of 0.700 to assign populations or individuals to a specific cluster. The posterior probabilities were estimated using a Markov chain Monte Carlo method (MCMC). The results were based on 500,000 iterations of MCMC which were performed after a burn-in of length 50,000, with a model of correlated allele frequencies and probable number of populations $K$ from one to 15 and ten iterations for each $K$ calculated. The true $K$ was estimated using the method of Evanno et al. (2005) based on ten iterations. However, STRUCTURE may tend to identify more populations than are biologically relevant (Falush et al. 2003). While a value of $K = 4$ was chosen for the final analysis, other values of $K$ are possible and would not qualitatively affect our conclusions.
The Bayesian Analysis of Population Structure (BAPS 4.14) was run in two different ways during the population mixture analysis, 1) clustering of individuals, and 2) clustering of groups using spatial information (Corander et al. 2006c). For clustering analysis of individuals the program was run ten times for each K and the maximum number of clusters higher than total number of plots (K = 20) was fixed in order to avoid misleading results (Corander et al. 2006a). After population admixture analysis, the admixture likelihood of each individual was calculated using the following four parameters; minimum size of a population, 100 iterations to estimate admixture coefficients for the individuals, 200 reference individuals for each population and 10 iterations to estimate the admixture coefficients for the reference individuals.

APPENDIX B
Results of the Analysis done by the STRI staff

The three programs used to analyze the data (i.e. AMOVA, STRUCTURE 2.01, and BAPS 4.14) detected significant genetic structure among localities in Las Perlas Archipelago (Table 4.1, 4.2, 4.3 and 4.4). AMOVA analysis showed that 85% of genetic variability is attributed to within groups (Table 4.1). The Bayesian approach of using STRUCTURE 2.01 to determine the number of population subdivisions within these populations detected the uppermost genetic structure level, dividing the genetic diversity in two groups. According to this, the Archipelago could be divided into two parts with a vertical line, clustering west islands such as Gibraleon and Pedro Gonzalez into a single group separated from the other localities, most of them in the East side and South. However, in spite of this suggestion, Table 4.3 is showing that this group is not completely isolated because some individuals from other localities were assigned to this cluster. This may be suggesting a commonality of many microsatellite alleles across all populations due to gene flow, which makes it possible to obtain composite genotypes that are shared by both populations.
BAPS 4.14 proved to be more sensitive than STRUCTURE 2.01 in detecting cryptic population structure under two different options. The best structure using “clustering of individuals” option was K=5, with a probability of 0.45 (Table not shown). When the analysis of clustering of groups was done using spatial information, the best structure was K=4 with a probability of 0.85, suggesting that geographic coordinates are very useful in determining a more realistic genetic structure. Table 4 and Figure 1 show that Gibraleon Island retains unique alleles compared with other study localities, meaning that it is genetically different from others. North islands such as Mogo-Mogo and Mina are in another group, together with La Ensenada plot which is located to the south-east. The other group is consisted of Rey localities and southern islands such as Grillo and finally, there is another group mainly for Pedro Gonzalez individuals.

Pedro Gonzalez, Vivero sureste, Casaya sureste, and Ensenada Pasa de Tierra (Rey Este) are areas with high gene flow with other areas from Archipelago, as it is demonstrated by individuals collected from these four areas which were split into different clusters. AMOVA genetic structure comparing pairwise of sampled areas overestimated genetic structure and therefore resolution to detect areas of gene flow was lost. On the contrary, analysis by STRUCTURE 2.01 and BAPS 4.14 were more powerful in detecting a more realistic genetic structure.

**Tables and figures**

**Table 4.1.** AMOVA results comparing variance components among and within collected areas (populations).

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum of squares</th>
<th>variance components</th>
<th>percentage of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among populations</td>
<td>11</td>
<td>87.73</td>
<td>0.21 Va</td>
<td>15.13</td>
</tr>
<tr>
<td>Within populations</td>
<td>382</td>
<td>447.03</td>
<td>1.17 Vb</td>
<td>84.87</td>
</tr>
<tr>
<td>Total</td>
<td>393</td>
<td>534.8</td>
<td>1.38</td>
<td></td>
</tr>
</tbody>
</table>
**Table 4.2.** Pairwise values of $F_{ST}$ (Weir & Cockerham 1984 among localities of *Rhizophora mangle* from the Caribbean after 10,000 permutations using Arlequin 3.1 (Excoffier *et al.* 2005). All localities showed significant genetic differentiation ($P<0.05$) except in five pairwise comparisons (in bold).

<table>
<thead>
<tr>
<th></th>
<th>Mogo-mogo</th>
<th>Mina</th>
<th>La Ensenada</th>
<th>Gibraleon</th>
<th>Vivero Sureste</th>
<th>Pedro Gonzalez</th>
<th>Casaya Suroeste</th>
<th>Ensenada Pasa de Tierra (Rey Este)</th>
<th>Ensenada Rio Ostion (Rey Noreste)</th>
<th>Bodega</th>
<th>Grillo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mogo-mogo</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mina</td>
<td>0.074</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>La Ensenada</td>
<td>0.135</td>
<td>0.151</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gibraleon</td>
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<td>0.304</td>
<td>0.276</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vivero Sureste</td>
<td>0.079</td>
<td>0.174</td>
<td>0.143</td>
<td>0.190</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pedro Gonzalez</td>
<td>0.102</td>
<td>0.167</td>
<td>0.140</td>
<td>0.088</td>
<td>0.071</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casaya Suroeste</td>
<td>0.147</td>
<td>0.163</td>
<td>0.205</td>
<td>0.244</td>
<td>0.028</td>
<td>0.118</td>
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<tr>
<td>Ensenada Pasa de Tierra (Rey Este)</td>
<td>0.082</td>
<td>0.166</td>
<td>0.125</td>
<td>0.180</td>
<td><strong>-0.009</strong></td>
<td>0.072</td>
<td>0.025</td>
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<td></td>
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<td></td>
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<tr>
<td>Ensenada Rio Ostion (Rey Noreste)</td>
<td>0.169</td>
<td>0.207</td>
<td>0.157</td>
<td>0.249</td>
<td>0.032</td>
<td>0.143</td>
<td>0.037</td>
<td><strong>0.020</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bodega</td>
<td>0.381</td>
<td>0.357</td>
<td>0.312</td>
<td>0.447</td>
<td>0.136</td>
<td>0.342</td>
<td>0.152</td>
<td>0.132</td>
<td>0.054</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Grillo</td>
<td>0.175</td>
<td>0.263</td>
<td>0.212</td>
<td>0.280</td>
<td>0.047</td>
<td>0.143</td>
<td>0.087</td>
<td>0.048</td>
<td><strong>0.026</strong></td>
<td>0.081</td>
<td>0</td>
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<tr>
<td>Ensenada Rio Cacique (Rey Sureste)</td>
<td>0.205</td>
<td>0.277</td>
<td>0.242</td>
<td>0.326</td>
<td>0.030</td>
<td>0.174</td>
<td>0.043</td>
<td>0.038</td>
<td>0.033</td>
<td><strong>0.046</strong></td>
<td><strong>0.030</strong></td>
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</table>
Table 4.3. Bayesian population admixture analysis based on spatial clustering of *R. mangle* groups from the Las Perlas Archipelago, using STRUCTURE 2.01 (Pritchard *et al.* 2002). The posterior probability of the number of clusters was maximum at $K = 2$. The average admixed proportion of each predefined group in each one of the four inferred clusters is indicated in the table. Each group was assigned to a single cluster if its admixed proportion was equal or larger than 0.600 (in bold). The individuals assigned to any cluster with an admixed proportion threshold higher than 0.600 are indicated in parenthesis and in bold. In addition, individuals assigned to different clusters with an admixed proportion less than 0.600 are indicated.

<table>
<thead>
<tr>
<th>Sample locality</th>
<th>Clusters</th>
<th>Individuals not assigned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Mogo-mogo</td>
<td>0.943</td>
<td>0.057</td>
</tr>
<tr>
<td>(8)</td>
<td>(8)</td>
<td>(0)</td>
</tr>
<tr>
<td>Mina</td>
<td>0.958</td>
<td>0.042</td>
</tr>
<tr>
<td>(20)</td>
<td>(20)</td>
<td>(0)</td>
</tr>
<tr>
<td>La Ensenada</td>
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<td>0.105</td>
</tr>
<tr>
<td>(19)</td>
<td>(16)</td>
<td>(0)</td>
</tr>
<tr>
<td>Gibraleon</td>
<td>0.021</td>
<td>0.979</td>
</tr>
<tr>
<td>(20)</td>
<td>(0)</td>
<td>(20)</td>
</tr>
<tr>
<td>Casaya Suroeste</td>
<td>0.550</td>
<td>0.450</td>
</tr>
<tr>
<td>(18)</td>
<td>(10)</td>
<td>(8)</td>
</tr>
<tr>
<td>Pedro Gonzalez</td>
<td>0.394</td>
<td>0.606</td>
</tr>
<tr>
<td>(20)</td>
<td>(7)</td>
<td>(12)</td>
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<tr>
<td>Vivero Sureste</td>
<td>0.579</td>
<td>0.421</td>
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<tr>
<td>(20)</td>
<td>(11)</td>
<td>(8)</td>
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<tr>
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<td>(9)</td>
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<td>(1)</td>
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<td>Grillo</td>
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<td>0.482</td>
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<tr>
<td>(14)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
<tr>
<td>Ensenada Rio Cacique (Rey Sureste)</td>
<td>0.703</td>
<td>0.297</td>
</tr>
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</table>
Table 4.4. Bayesian population admixture analysis based on spatial clustering of *R. mangle* groups from the Las Perlas Archipelago, using BAPS 4.14 (Corander *et al.* 2006c). The posterior probability of the number of clusters was maximum at $K = 4$. The average admixed proportion of each predefined group in each one of the four inferred clusters is indicated in the table. Each group was assigned to a single cluster if its admixed proportion was equal or larger than 0.600 (in bold). The individuals assigned to any cluster with an admixed proportion threshold higher than 0.600 are indicated in parenthesis and in bold. In addition, individuals assigned to different clusters with an admixed proportion less than 0.600 are indicated. Percentages of significant admixed individuals ($P < 0.05$) for each sample group vary from cero to 35%.

<table>
<thead>
<tr>
<th>Sample locality</th>
<th>Clusters</th>
<th>Individuals not assigned</th>
<th>Percentage of significant admixed individuals ($P &lt; 0.05$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I  II III IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mogo-mogo</td>
<td>0.940 0.000 0.014 0.046</td>
<td>0</td>
<td>12.5</td>
</tr>
<tr>
<td>(8)</td>
<td>(8) (0) (0) (0)</td>
<td>(0)</td>
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<tr>
<td>Mina</td>
<td>0.913 0.000 0.064 0.023</td>
<td>1</td>
<td>15.0</td>
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<tr>
<td>(20)</td>
<td>(18) (0) (1) (0)</td>
<td>(1)</td>
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</tr>
<tr>
<td>La Ensenada</td>
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<td>0</td>
<td>12.5</td>
</tr>
<tr>
<td>(19)</td>
<td>(19) (0) (0) (0)</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td>Gibraleon</td>
<td>0.012 0.973 0.012 0.004</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>(20)</td>
<td>(0) (20) (0) (0)</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td>Vivero Sureste</td>
<td>0.317 0.178 0.074 0.432</td>
<td>3</td>
<td>35.0</td>
</tr>
<tr>
<td>(20)</td>
<td>(6) (2) (0) (9)</td>
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</tr>
<tr>
<td>Location</td>
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<td>0.202</td>
<td>0.570</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Casaya Suroeste</td>
<td>0.192</td>
<td>0.092</td>
<td>0.120</td>
</tr>
<tr>
<td>Ensenada Pasa de Tierra (Rey Este)</td>
<td>0.343</td>
<td>0.151</td>
<td>0.043</td>
</tr>
<tr>
<td>Ensenada Rio Ostion (Rey Noreste)</td>
<td>0.185</td>
<td>0.114</td>
<td>0.028</td>
</tr>
<tr>
<td>Bodega</td>
<td>0.153</td>
<td>0.000</td>
<td>0.008</td>
</tr>
<tr>
<td>Grillo</td>
<td>0.165</td>
<td>0.026</td>
<td>0.099</td>
</tr>
<tr>
<td>Ensenada Rio Cacique (Rey Sureste)</td>
<td>0.138</td>
<td>0.053</td>
<td>0.068</td>
</tr>
<tr>
<td>Total</td>
<td>(68)</td>
<td>(26)</td>
<td>(14)</td>
</tr>
<tr>
<td>(Percentage)</td>
<td>34.2</td>
<td>13.1</td>
<td>7.0</td>
</tr>
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</table>
Figure 4.1. Spatial genetic structure (K=4 as indicated by different colours) of *R. mangle* in Las Perlas Archipelago based on Voronoi tessellation representation and obtained using BAPS 4.14 (Corander *et al*. 2006c).