“Influence of pollination and seed dispersal on the genetic population structure of two *Commiphora* species in Madagascar and South Africa”

Dissertation
zur Erlangung des Grades
Doktor der Naturwissenschaften

Am Fachbereich Biologie
der Johannes Gutenberg-Universität
in Mainz

Friederike A. Voigt
geb. am 03.05.1974 in Karlsruhe

Mainz, den 01.03.2005

Kapitel 1 dieser Arbeit wurde veröffentlicht bei Cambridge University Press unter:

Voigt, F.A., S. Jung, N. Farwig and K. Böhning-Gaese (2005) Low fruit set in a dioecious tree: pollination ecology of *Commiphora harveyi* in South Africa. *Journal of Tropical Ecology* 21.

…dedicated to the arofys and the paperbarks and the mysterious beauty of tropical forests.

CONTENTS

1 Abstract of the thesis

Pollination and seed dispersal are important ecological processes for the establishment and the regeneration of plant populations. Besides their ecological importance, gene exchange occurs via both processes in and between plant populations. To assess how pollination and seed dispersal are influencing the genetic structure of plant populations, good field data about these processes are needed as well as genetic data to describe the population structure. For my thesis, I studied the pollination ecology of the South African tree species *Commiphora harveyi* (Burseraceae) and compared my results with the results of an earlier study about *C. guillauminii*, a tree species from the same genus from Madagascar. Both species have low visitation rates and a low number of pollinating insect species, resulting in a low fruit set. While their pollination ecology is very similar, they differ fundamentally in their seed dispersal rates. Previous studies showed that the seeds of the Malagasy species get dispersed by a lower number of frugivorous bird species than the seeds of the South African species. Based on these field data, I investigated the genetic population structure of both species, using AFLP marker. I expected that the lower seed dispersal rate may lead to a more reduced gene flow in the Malagasy than in the South African species. This should be reflected in a stronger genetic differentiation among populations in the Malagasy than in the South African species. My results contradict these expectations, the overall differentiation was lower in the Malagasy (F_{ST} = 0.05) than in the South African species (F_{ST} = 0.16). However, at a smaller spatial scale (below 3 km), the Malagasy species was genetically more strongly differentiated than the South African species, which was reflected by the high inter-population variance within the sample site (*C. guillauminii*: 72.2 - 85.5 %; *C. harveyi*: 8.4 - 14.5 %). This strong differentiation at a small spatial scale could arise from limited gene flow. Spatial autocorrelation analyses confirmed this pattern. The shape of the autocorrelogram suggested that gene exchange between individuals occurred only up to 3 km in the Malagasy species, whereas up to 30 km in the South African species. These results on the genetic structure correspond to the expectations based on the field data on seed dispersal. Thus, seed dispersal seems to be a key factor for the genetic structure in plant populations on a local scale.

2 General introduction

To understand the importance of plant-animal interactions is one of the challenges in community ecology. Two of the most important mutualistic plant-animal interactions are seed dispersal and pollination. Both of them are ecologically relevant for the regeneration of plant populations. Pollination is necessary for pollen transfer and for sufficient fruit set of plants (Boucher *et al*. 1982, Bronstein 1994). Seed dispersal is fundamental for seeds to escape the high mortality under a parent tree and to reach new sites (Howe & Smallwood 1982). Nevertheless, it is very difficult to assess long distance dispersal of pollen and seeds and their long-term consequences for plant populations (Cain *et al*. 2000). Besides their ecological importance, pollination and seed dispersal are also vectors for gene exchange in and between plant populations. Therefore, one possibility to understand the long-term consequences of pollination and seed dispersal is to study the genetic structure of plant populations.

2.1 Pollination

Pollination is an important ecosystem service and the primary step in plant reproduction. Many studies showed that outcrossing is necessary for successful pollination (Quesada *et al.* 2001, Barrett 2002). Even in some self-compatible plant species, pollen from conspecifics increased fruit set and decreased fruit abortion (Gaudeul & Till-Bottraud 2003, Herlihy & Eckert 2004). To enforce outcrossing, self-incompatibility and sexes on different individuals (dioecy) evolved as breeding system. However, since the stigma is only receptive for a short time, successful pollination cannot be guaranteed. Recent studies showed that most plant species suffer, at least at times, from pollen or pollinator limitation (either no or not sufficient pollen deposit on the stigma), or as Wilcock and Neiland (2002) stated: “too little, too much, too late, too mixed in composition or too poor in quality” (Wilcock & Neiland 2002, and citations within). To increase pollination success, plant-pollinator-interactions evolved in two different ways. On the one side, tight specialized pollinator-flower interrelations originated. The orchid *Angraecum sesquipedale* with its 30 cm long corolla and the by Darwin predicted hawk moth *Xanthopan morgani praedicta* (Darwin 1862, Nilsson 1992) is a typical example. On the other side, broad pollination

syndromes with mass flowering evolved and are often found in tropical trees species, attracting a wide variety of flower visitors (Bawa and Opler 1975). For example, some dioecious tree species get visited by up to 200 insect species (Bawa 1990). The longevity of trees, combined with high pollinator diversity and an oversupply of flowers increases the chances for successful pollination, particularly, since only one of their thousand flowers needs to be pollinated effectively.

Additionally, pollination success often depends on attractiveness of a tree individual (amount of pollen and nectar provided), distance to neighbouring conspecifics, pollinator diversity and simultaneously flowering species. Nevertheless, especially tropical dioecious tree species that are dependent on outcrossing have high fruit sets compared to monoecious and hermaphroditic plant species (Sutherland & Delph 1984). However, first studies showed that pollen limitation occurs also in dioecious tree species (Farwig *et al.* 2004). In the long-term, pollen limitation can result in a reduced fruit set, leading to a reduced regeneration potential of the species. Our knowledge of the long-term consequences of reduced fruit set in tropical tree species is sparse, mainly because of the long generation time of trees and the difficulty to work experimentally and to manipulate pollination on a large scale. One approach to handle this difficulty is to study pollination ecology in different ecosystems or environments and to compare pollination success. One example may be to investigate the pollination ecology of island populations with impoverished pollinator communities and populations on the mainland with a diverse pollinator spectrum.

2.2 Pollination and seed dispersal and their consequences for the genetic structure of plant populations

Mutation, genetic drift and gene flow are the contrasting forces acting on the genetic structure in populations (Hartl 1980). Whereas mutation and drift can result in a differentiation between populations, gene flow should lead to an exchange of the gene pools (Hamrick *et al.* 1993). The result of mutation, genetic drift and gene flow on the genetic structure depends on the frequency of gene flow which occurs via pollination and seed dispersal in plant populations (Shapcott 1999). Species with limited pollination and seed dispersal rate are likely to have reduced gene flow, resulting in considerable genetic

heterogeneity among populations while species with more extensive pollination or seed dispersal should have less spatial genetic structure (Fleming & Heithaus 1981, Howe 1990). Additionally, ecological factors, such as seed deposition pattern, adult densities and breeding system act in a genetic context and influence the genetic structure in plant populations (Loveless & Hamrick 1984, Vekemans & Hardy 2004).

The different vectors of pollination and seed dispersal such as wind, gravity or animals differ in their impact on the genetic structure of plant populations. In most studies on tropical plant species, gene flow through pollination acts over longer distances than through seed dispersal, with most seeds getting dispersed only over a short distance or just dropping under the parent plant (Dick 2001, Trapnell & Hamrick 2004). Thus, pollination should promote gene flow while seed dispersal might restrict it (Loiselle 1995a, b). Nevertheless, tropical tree species with effective seed dispersal through birds exhibit no genetic structure, suggesting that seed dispersal can promote gene flow over long distances as well (Chung *et al.* 2000). Thus, general assumptions about pollination and seed dispersal effectiveness are not sufficient to evaluate to which extend and spatial scale both processes are influencing gene flow in plant populations. Instead, detailed field data on pollination success, pollination and seed dispersal vectors, seed dispersal rates and seed dispersal distances are necessary.

2.3 Background of the thesis

Background for this thesis was a biogeographic comparison of the seed dispersal system of two dioecious *Commiphora* species, *C. guillauminii* from Madagascar and *C. harveyi* from South Africa. Both species have bird-dispersed fruits, but long-term studies showed that the frugivore community had a prominent influence on seed dispersal rates, seed dispersal distances, seedling establishment and the spatial pattern of seedlings and adults (Böhning-Gaese *et al.* 1995, 1999, Bleher & Böhning-Gaese 2000, 2001). Böhning-Gaese *et al.* (1995) stated that the depauperate frugivore community in Madagascar resulted in mainly one frugivorous bird species dispersing the seeds occasionally, and only 7.9 % of the seeds were carried away from the crown. The seedlings were clumped under female trees with low seedling survival (15 %), and the median seedling – female tree distance revealed a short distance of 0.9 m. Furthermore, the clumped spatial pattern of the seedlings was

reflected in the grouped pattern of the adult trees (Böhning-Gaese *et al.* 1999). In contrast, the high number of bird species in the South African frugivore community led to a large number of bird species visiting the tree. Bleher and Böhning-Gaese (2000) showed that 70.8 % of the seeds were dispersed away from the crown. One of the main seed dispersers were hornbills that are known for long distance dispersal (Holbrook & Smith 2000). The median seedling – female tree distance was 21 m. The seedling survival was high (36 %) and seedlings and adults were uniform distributed (Bleher & Böhning-Gaese 2001). These studies showed that differences in seed disperser diversity and effectiveness have ecological short-term consequences for the tree species studied. Since seed dispersal is also one possibility for gene exchange between plant populations, long-term consequences of differences in seed dispersal can be investigated with genetic studies on plant populations. Gene flow occurs also via pollination. Therefore, to investigate the genetic population structure of plant populations, the pollination ecology of the studied species should also be considered.

2.4 Aims of the thesis

In this thesis I investigated the pollination ecology of *Commiphora harveyi* and the genetic population structure of several *C. guillauminii*- and *C. harveyi*-populations using amplified fragment length polymorphism (AFLP). One aim of the thesis was to study the pollination system of *C. harveyi* in South Africa and to compare it with the established pollination system of *C. guillauminii* from Madagascar (Farwig *et al.* 2004). A second aim was to understand how differences in seed dispersal influenced the genetic population structure of the two species.

This thesis consists of two major chapters which can be read independently. Each chapter is organized like a journal publication containing an introduction, followed by a methods, results and discussion section and by a brief summary. The thesis closes with general conclusions.

In the first chapter, I focused on the pollination ecology of *C. harveyi*, a subtropical dioecious tree species with unspecialised small flowers. First, I quantified the floral display, studied pollination rate and pollinator diversity and performed pollination experiments. The study was conducted over one flowering season in co-operation with

Stefanie Jung, who collected half of the field data in the context of her Staatsexamensarbeit. Second, I compared my results with those found for *C. guillauminii,* a species from the same genus from Madagascar. A basic understanding of the pollination ecology of the two species was needed to evaluate the influence of pollination as vector for gene flow on the genetic population structure of the two species. In case the two species differ in their pollination ecology, e.g. one species would be visited by larger-bodied insects that may carry pollen over longer distances than weak fliers; I would expect gene flow via pollination to occur over longer distances in this species.

In the second chapter, I investigated the genetic population structure of 136 individuals of *C. guillauminii* and 158 individuals of *C. harveyi* from 12 and 15 sample sites in Madagascar and South Africa, respectively. Considering differences in seed dispersal rates of the two species, leaf material was explicitly sampled at different spatial scales. To consider the spatial scale at which pollination could be relevant for the genetic structure, I sampled groups of trees in a circle with a diameter of 300 m (small scale). To assess the spatial scale at which seed dispersal could be relevant for the genetic structure, sampled trees were grouped in a circle of 3 km (medium scale). For the largest spatial scale, sampled trees were grouped as sample sites. With this sampling design, it was possible to assess genetic differentiation among and within sample sites at different spatial scales. Additionally, I performed spatial autocorrelation analyses to test for isolation by distance and to get an indication until which distances individuals are still related to each other, and therefore, regular gene flow occurred.

3 Low fruit set in a dioecious tree: pollination ecology of *Commiphora harveyi* in South Africa

3.1 Introduction

Pollination by animals is an important plant-animal interaction (Boucher *et al*. 1982, Bronstein 1994), having particular significance in the tropics, where most trees are self-incompatible and up to 90 % depend on animals as pollinators (Bawa 1990, Buchmann & Nabham 1996, Dick *et al.* 2003). A decline in diversity and abundance of pollinators, for example caused through habitat fragmentation, can lead to a decrease in pollination rate (Cascante *et al*. 2002, Cunningham 2000a, b; Wilcock & Neiland 2002, Johnson *et al.* 2004).

Dioecious tree species which have the sexes on different individuals are common in the tropics (Bawa 1974, 1980a; Bawa & Opler 1975). While dioecy reduces selfing almost completely, pollinator (or wind) movements between individuals of both sexes are needed for successful reproduction (Bawa & Opler 1975, Bawa 1980a, Renner & Ricklefs 1995, Osunkoya 1999). In temperate regions, dioecious trees are mostly wind-pollinated, whereas in the tropics and subtropics they often depend on insects as pollination vectors (Bawa & Opler 1975, Bawa 1980a). Male and female trees can differ in their attractiveness to pollinators; staminate flowers provide pollen and mostly nectar, whereas pistillate flowers often have only nectar. Additionally, flower size and number of flowers per tree can differ between the sexes (Bawa & Opler 1975, Bawa 1980b, Ågren *et al.* 1986, Delph *et al*. 1996, Osunkoya 1999). These differences in flower morphology and floral rewards could possibly influence visitation rates and visitor diversity between the sexes (Bawa 1980b, Thomson *et al*. 1982, Bierzychudek 1987, Vamosi & Otto 2002, Farwig *et al*. 2004).

The dependency on pollinators and the difference in rewards offered between male and female trees could have consequences for the reproductive success of dioecious species. Fruit set could be restricted through pollinator or pollen limitation (Burd 1994). Nevertheless, dioecious plants have been recorded to have a higher fruit set than monoecious and hermaphroditic plants (73.8 % *versus* 42.1 %; Sutherland & Delph 1984). However, a pollination study conducted on the dioecious entomophilous tropical tree

Commiphora guillauminii in Madagascar revealed very different results: low pollinator diversity, low mean visitation rates and a fruit set of only 2.9 % (Farwig *et al*. 2004). The authors explained these results by the special island situation combined with the high percentage of endemic plants and animals on Madagascar (Farwig *et al*. 2004). Even though mutualistic plant-animal interactions on Madagascar are poorly investigated, existing studies suggest that plants on Madagascar interact with surprisingly few animal pollinators (Jenkins 1987, Nilsson 1992, Ratsirarson & Silander 1996).

In the present study, I investigated the pollination ecology of *Commiphora harveyi* in South Africa, a tree species in the same genus as *C. guillauminii*, but within a subtropical, continental situation. The comparison of the pollination ecology of the two species allows me to evaluate whether the pollination ecology of *C. guillauminii* is unique to tropical Madagascar or more common in tropical and subtropical dioecious trees than previously reported. The objectives of the study were, first to quantify the attractiveness of the different sexes: flower size, number of flowers per inflorescence, total number of flowers per tree, as well as the amount of nectar provided. Second, I determined the visitation rates for male and female trees and whether both sexes have a similar daily and seasonal visitation pattern. Third, I identified the natural fruit set and conducted hand-pollination experiments. Additionally, I excluded pollinators to test for non-pseudogamous apomixis. The last objective was the comparison of the pollination systems of the two *Commiphora* species.

3.2 Methods

3.2.1 Study site

The study took place during November and December 2002 in Oribi Gorge Nature Reserve (OGNR) on the South African East Coast. This 1850-ha nature reserve, located 110 km south of Durban and 22 km inland from Port Shepstone, is classified as coastal scarp forest (Cooper 1985). Average annual rainfall in the area is 1176 mm with the main rainfall season between October and March (Glen 1996). The monthly mean temperature is 19.2 ºC (www.worldclimate.com). For further details on Oribi Gorge Nature Reserve and coastal forests see Glen (1996) and Acocks (1988).

3.2.2 Study species

Commiphora harveyi Engl. (Burseraceae) (van der Walt 1973) is a deciduous tree found on the east coast of Southern Africa (Palgrave 1977, Pooley 1994) and grows up to 20 m in height. Its economic use is limited to the production of small goods such as spoons (van Wyk & van Wyk 1997). The species is dioecious, flowering from October to December (Pooley 1994). The flowers of female and male trees are small, whitish and are born in short axillary inflorescences (van Wyk & van Wyk 1997). Pistillate flowers are cryptically dioecious (Mayer & Charlesworth 1991) with staminodes. The fruiting season is from March to June (Bleher & Böhning-Gaese 2000, Pooley 1994). The fruits have an outer covering that splits in half when mature, exposing a single black seed, enveloped by a fleshy red aril that is dispersed by birds (Bleher & Böhning-Gaese 2000, 2001).

3.2.3 Floral display

To quantify floral display, I randomly chose trees of each sex (eight male and 12 female trees) in a 1-ha plot. I measured the width and the height of 2-15 flowers per tree with an electronic caliper. All flowers were taken from the lower third of the tree crown. To collect the data I either cut branches with a tree cutter or stood on an aluminium ladder. Average flower height and width was calculated for each tree. The differences in the means between male and female trees were assessed with a t-test (JMP 1995). To determine the attractiveness of each sex to potential pollinators, I counted flowers on 8-10 inflorescence per tree and inflorescences per tree on a representative part of the tree crown and then extrapolated the numbers to the whole crown. The total number of flowers per tree was calculated by multiplying the mean number of flowers per inflorescence with the number of inflorescences per tree. I tested for differences between the sexes in number of flowers per inflorescences, number of inflorescences per tree and total number of flowers per tree, using non-parametric Mann-Whitney U-tests.

I tried to measure nectar production of flowers with standardized microcapillaries, but the amount was low and variable in both sexes, preventing rigorous statistical analysis.

3.2.4 Flower visitors

Pollinators and their visitation rate were recorded on 16 randomly chosen trees (eight of each sex). Because the flowering period for a number of trees was shorter than the study period, I exchanged five female trees in the second and third observation block for five other female trees (see below). The study covered the complete flowering season of *C. harveyi* in Oribi Gorge NR (5 November - 10 December 2002) and was split into three observation blocks. The first two blocks lasted 12 d (5 - 16 November; 18 - 29 November). At the end of the flowering season only 9 d were left for the third block (2 - 10 December). The total observation time was 33 d. I divided a day into three time periods: morning (6h00 - 10h00), midday (10h00 - 14h00) and afternoon (14h00 - 18h00). Additionally, I made nocturnal observations on one female and one male tree (19h00 - 24h00), using night vision glasses (moonlight, nv 100, times 4.3).

In each of the seasonal observation blocks, I observed each tree in 30-min periods over the whole day, starting at the full hour (i.e. 6h00 - 6h30, 7h00 - 7h30, etc.). The observation units were randomized over the trees and during the course of the day. The total number of observation units was 528. For statistical analyses I excluded 54 units because the trees had no open flowers and 67 units due to heavy rain (during rain no insects visited the flowers). Therefore, the statistical analysis was based on 407 observation units (203.5 h).

In each observation unit, I observed several inflorescences simultaneously in the lower third of the crown. I recorded the number of open flowers observed and the identity and number of visiting insects - classified into visible distinguishable morphospecies. To do this, I either stood on an aluminium ladder or sat on the ground using binoculars (Zeiss, 6 x 18). To determine the morphospecies, I captured specimens of the most common visiting insects, using sweep nets. To detect possible pollinators, I looked for pollen on the insect body using lenses (10 x). Specimens were identified by specialists of the Plant Protection Research Institute in Pretoria and are now housed in the Ecology Department of the University of Mainz, Germany. For statistical analyses I transformed observation units into visits per flower h^{-1}. To compare visitation rates between the sexes, I calculated the mean visitation rate for each tree (4 - 33 observation units per tree). I used a non-parametric Mann-Whitney U-test to test for differences in visitation rates between male

and female trees; first combining visitation rates of all visiting species and, then, testing each species separately.

To test for a change in visitation rate during the course of the day, I calculated the mean visitation rate for each time period and each tree (1 - 12 observation units per period and tree). I used a Wilcoxon Matched-Pair Signed-Rank Test (JMP 1995) to test for differences in visitation rates between the time periods. I used the same approach to test for a change in visitation rate in the course of the flowering season. The mean visitation rate for each observation block and each tree (2 - 12 observation units per block and tree) was calculated and tested with a Wilcoxon Matched-Pair Signed-Rank Test (JMP 1995) for differences in visitation rate. The visitation rates were also analyzed with generalized linear models, using Poisson-distribution and log-link function. However, this was only possible for the combined visitation rate of all species and the results corresponded to those of the non-parametric Mann-Whitney U-test. Visitation rates of single species were too low for using generalized linear models. Thus, for comparison I present all results using non-parametric tests (JMP 1995).

3.2.5 Pollination experiments and fruit set

To calculate the natural fruit set, I estimated the total numbers of flowers and fruits on 19 female trees (observation trees included). Natural fruit set was defined as number of fruits divided by number of flowers.

On the same 19 trees I conducted pollination experiments. To exclude insect visitors, I completely covered 3 - 5 unopened inflorescences with mosquito gauze (mesh size: 1 mm). Every second or third day, I checked the status of the stigma of the single flowers. If a pistillate flower appeared receptive (slight change of the stigma), I pollinated it, using anthers from male trees. As flowers could not be covered or marked individually, I had to cover the whole inflorescence, potentially excluding seed predators and reducing mechanical damage. As a control, I covered other unopened inflorescences on the same tree without hand-pollination and tested for apomixis. For each mosquito gauze exclosure I recorded the number of pollinated flowers. To calculate the experimental fruit set I counted the fruits under each gauze at the end of the study period and divided them by the number of pollinated flowers. For analyses, six out of the 19 trees could not be considered, because the gauze or the whole branch had broken off in windy conditions. For the statistical

analysis, I determined natural and experimental fruit set for each tree. I tested for a difference between the two fruit sets, using the non-parametric Wilcoxon Matched-Pair Signed-Rank Test (JMP 1995) with the arcsine-transformed values. Moreover, I correlated fruit set with floral display (flowers per tree) using Spearman's Rho correlation.

3.2.6 Comparison with *C. guillauminii*

The methods and results of a study on the pollination ecology of *C. guillauminii* in Madagascar have already been published (Farwig *et al*. 2004). However, to make the comparison between *C. harveyi* and *C. guillauminii* easier, I give some basic information on the study conducted. The study took place between October and December 2001 in Kirindy forest, a dry deciduous forest in western Madagascar, with an average annual temperature of 24.7 °C and an average precipitation of 779 mm (Sorg & Rohner 1996). It is an entomophilous tree species with the flowers of female and male trees being small, reddish and born in inflorescences (de la Bathie 1946, Farwig *et al.* 2004). The pollination study was conducted as previously described for *C. harveyi* with the exception of hand-pollination that was not performed. To see whether the pollination ecology of the two species are comparable, I tested for differences in the attractiveness of the species to potential pollinators (number of flowers per inflorescence, number of inflorescences per tree, total number of flowers), visitor diversity, visitation rates and fruit set, using non-parametric Mann-Whitney U-tests. In each test, I compared among the male trees and among the female trees.

3.3 Results

The start of the flowering season for *C. harveyi* was difficult to determine, since it varied with tree size and habitat. Small trees in open habitat started to flower earlier than the larger trees in the closed forest. On the first observation date 5 November 2002 all trees in the study areas had open flowers, and after the last observation date (10 December 2002) there were only a few trees with some open flowers left. Male and female flowers opened at dawn and stayed open for 2 - 3 d, before they wilted and dropped off the tree.

3.3.1 Floral display

Male trees had significantly more flowers per inflorescence than female trees (male: median = 15, range = 9 - 22, N = 8; female: median = 5, range = 3 - 10, N = 19; Mann-Whitney U-test: Z = 3.86, P = 0.0001). Number of inflorescences differed marginally between the sexes (male: median = 1750, range = 800 - 5000, N = 8; female: median = 700, range = 10 - 3200, N = 19; Mann-Whitney U-test: Z = 1.89, P = 0.0588). The total number of flowers was significantly higher on male than on female trees (male: median = 25500, range = 8100 - 100000, N = 8; female: median = 2800, range = 30 - 32000, N = 19; Mann-Whitney U-test: Z = 2.97, P = 0.0029). Staminate flowers were significant longer than pistillate flowers (male: $\bar{x}$ = 3.43 ± 0.41 mm [$\bar{x}$ ± 1 SD, unless otherwise stated], N = 8; female: $\bar{x}$ = 2.17 ± 0.23 mm, N = 12; t-test: t_{18} = 8.82, P < 0.0001), but did not differ in flower width (male $\bar{x}$ = 1.46 ± 0.12 mm, N = 8; female: $\bar{x}$ = 1.54 ± 0.15 mm, N = 12; t-test: t_{18} = -1.27, P = 0.22). Both sexes produced nectar, but data were not sufficient to test for differences in nectar amount or sugar concentration between the sexes. Pollen of staminate flowers was moist and sticky.

3.3.2 Flower visitors

During 203.5 h of observations, I recorded a total of 28 visiting insect species (Table 1). I found no difference in the number of insect species visiting per tree between the sexes (male: $\bar{x}$ = 7.12 ± 2.78, N = 8; female: $\bar{x}$ = 7.0 ± 2.62, N = 8; t-test: t = 0.09; df = 14; P = 0.92). The most common visitors were *Asarkina africana* (Syrphidae) and *Apis mellifera* (Apidae). The most common visitors on male trees were *Asarkina africana, Apis mellifera* and a species from the family Calliphoridae (Diptera) and on female trees another Diptera species, a Formicidae and *Asarkina africana.* I found pollen on only three species (*Apis mellifera* (Hymenoptera), *Allodape peillix* and *Eristallinus modestus* (Diptera)). No flower visitors were recorded during night observations.

3.3.3 Visitation rates

The mean visitation rate for both sexes combined was 0.198 visits per flower h^{-1}, for male trees 0.243 visits per flower h^{-1} and for female trees 0.170 visits per flower h^{-1}. Total visitation rates did not differ significantly between the sexes (Table 1). Considering the

insect species separately, visitation rates differed significantly for *Asarkina africana*; they were 2.8 times higher on male than on female trees (0.0663 versus 0.0235 visits per flower h^{-1}; Table 1). The visitation rate for *Ischiodon aegypticus* differed marginally between the sexes. For all other insects, no difference between the sexes could be found. When controlling for multiple tests using table-wide sequential Bonferroni adjustment (Rice 1989), all differences in visitation rate between the sexes lost their significance.

Table 1: Visitation rates per flower h^{-1} for all visitor species together and for each species separately (grouped by orders). Data were analyzed for all trees sexes pooled (♂♀, N = 21), and for male (♂♂, N = 8) and female (♀♀, N = 13) trees separately. Given are mean values, because the median was in many cases zero. Additionally, the Z- and P-values from the Mann-Whitney U-tests are presented. Significant values are in bold. No P-value remained significant, after table-wide sequential Bonferroni correction (Rice 1989).

Species	Mean ♂♀ [Visits per flower h^{-1}]	Mean ♂♂ [Visits per flower h^{-1}]	Mean ♀♀ [Visits per flower h^{-1}]	Z	P
N	21	8	13		
All species	0.198	0.243	0.17	1.19	0.23
Diptera					
Asarkina africana	0.0398	0.0663	0.0235	2.57	**0.01**
Calliphoridae	0.0197	0.0209	0.0190	1.37	0.17
Dipt. IV	0.0183	0.0090	0.0240	-0.335	0.74
Allobacha sp.	0.0102	0.0130	0.0086	1.08	0.28
Eristallinus modestus	0.0049	0.0065	0.0039	0.681	0.50
Ischiodon aegypticus	0.0040	0.0049	0.0035	1.89	0.06
Dipt. VI	0.0025	0.0030	0.0023	0.213	0.83
Dipt. IX	0.0025	0.0015	0.0030	-0.119	0.90
Dipt. VII	0.0018	0.0047	0	1.18	0.24
Dipt. VIII	0.0007	0.0019	0	1.18	0.24
Hymenoptera					
Apis mellifera	0.0382	0.0636	0.0226	1.57	0.12
Formicide I	0.0160	0.0036	0.0236	-0.733	0.46
Belonogaster sp.	0.0080	0.0059	0.0093	0.318	0.75
Casioglossum sp.	0.0066	0.0010	0.0099	0.264	0.79
Formicide II	0.0060	0	0.0097	-1.07	0.29
Allodape peillix	0.0044	0.0093	0.0014	1.50	0.13
Hym VI	0.0012	0.0032	0	1.78	0.08
Hym V	0.0005	0.0012	0	1.18	0.24
Coleoptera					
Col. IV	0.0039	0.0103	0	1.18	0.24
Col. VII	0.0021	0.0012	0.0027	-0.581	0.56
Col. I	0.0016	0.0041	0	1.18	0.24
Col. VI	0.0013	0.0026	0.0005	0.355	0.72
Col. III	0.0012	0.0031	0	1.18	0.24
Col. VIII	0.0010	0	0.0016	-1.07	0.29
Col. II	0.0005	0.0014	0	1.18	0.24
Col. V	0.0004	0	0.0007	-0.686	0.50
Lepidoptera					
Lep. II	0.0005	0.0013	0	1.18	0.24
Lep. I	<0.0001	<0.0001	0	1.18	0.24

3.3.4 Daily pattern

Visitation rates were highest around midday with the same daily pattern for male and female trees (Figure 1; Table 2). Pooling both sexes, I found a significant difference between morning and midday and a marginal difference between midday and afternoon (Table 2). Considering the sexes separately, only the visitation rates on female trees differed significantly between morning and midday. When controlling for multiple tests using table-wide sequential Bonferroni adjustment, only the pooled values for all trees remained significant (Table 2)

Table 2: Test for changes in visitation rates in the course of the day and in the flowering season using Wilcoxon Matched-Pair Signed-Rank tests (JMP 1995). Presented are median differences between the periods, S- and P-values for all trees, sexes pooled (♀♂), for male (♂♂) and for female (♀♀) trees. Values that remained significant after table-wide, sequential Bonferroni correction (Rice 1989) are in bold.

		N	Median	S	P
Daily pattern					
morning versus midday	♀♂	20	-0.1273	-62.5	**0.005**
	♂♂	8	-0.2692	-12.0	0.109
	♀♀	12	-0.0628	-21.5	0.027
midday versus afternoon	♀♂	21	0.0500	51.0	0.058
	♂♂	8	0.3346	12.0	0.109
	♀♀	13	0.0260	14.0	0.301
Seasonal pattern					
first versus second period	♀♂	14	0.0185	-1.5	0.952
	♂♂	8	-0.0081	-2.0	0.844
	♀♀	6	0.0948	0.5	1.000
second versus third period	♀♂	12	0.1192	30.0	**0.005**
	♂♂	6	0.1151	7.5	0.063
	♀♀	6	0.1255	8.5	0.094

3.3.5 Seasonal pattern

The visitation rate declined in the course of the flowering season for male as well as for female trees (Figure 1). Pooling both sexes, the visitation rate was significantly lower in the third compared to the second observation block. This decrease in visitation rate was marginally significant for both sexes (male: P = 0.063; female: P = 0.094; Table 2). There

was no significant difference between the first and the second observation block for male trees, female trees, or for both sexes together (Table 2). After controlling for multiple tests using table-wide sequential Bonferroni adjustment the values for all trees remained significant (Table 2).

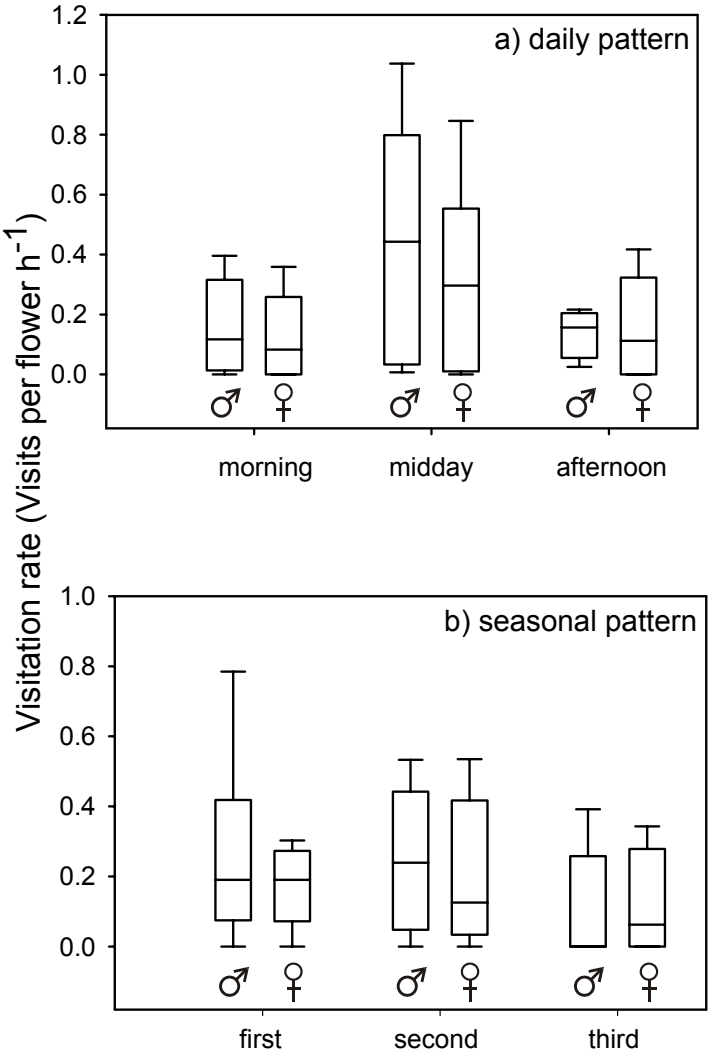

Figure 1: Daily (a) and seasonal (b) pattern of visitation rates (visits per flower h^{-1}) for male (m) and female (f) trees. Plotted are box-and-whisker plots with minimum value, 25 %-quartile, median, 75 %-quartile and maximum value. a) morning (6h00-10h00), midday (10h00-14h00) and afternoon (14h00-18h00). N = 8 for male trees and N = 12 (morning), N = 13 (midday, afternoon) for female trees. b) the first (05 - 16 November), the second (18 - 29 November) and the third (02 - 10 December) time period (N = 8 (first and second); N = 6 (third) for male trees and N = 9 (first); N = 10 (second); N = 6 (third) for female trees).

3.3.6 Fruit set

Median natural fruit set was 3.8 % (range = 2.7 - 7.1 %; N = 19) and the median fruit set in hand-pollinated flowers was 45.5 % (range = 5.6 - 75 %; N = 13). The experimental fruit set was significantly higher than the natural fruit set (Wilcoxon Matched-Pair Signed-Rank test: median of differences = -0.44, S = -36.0, P = 0.002, N = 13). There was a marginally significant negative correlation between floral display (flowers per tree) and natural fruit set (Spearman's Rho: R = -0.43, P = 0.065, N = 19). Inflorescences covered by mosquito-gauze did not develop fruits.

3.3.7 Comparison with *C. guillauminii*

The attractiveness to visitors of the two tree species differed only slightly. *Commiphora guillauminii* had significantly more flowers per inflorescence than *C. harveyi* for both sexes (Mann-Whitney U-test: $Z_{male} = -3.32$; $P < 0.001$; $N = 16$, $Z_{female} = 2.36$; $P < 0.05$; $N = 27$). The number of inflorescences per tree and the number of total flowers per tree did not differ between the species for neither sex. Considering the number of visiting insect species per tree, I found no difference between *C. guillauminii* and *C. harveyi* for male or for female trees (t-test: $t_{male} = 1.32$; $df = 14$; $P = 0.21$; $N = 16$; $t_{female} = -0.84$; $df = 14$; $P = 0.41$; $N = 16$). Despite a similar attractiveness and a similar visitor diversity, the visitation rate per flower h^{-1} was significantly higher on males of *C. guillauminii* than on males of *C. harveyi* (Mann-Whitney U-test: $Z = -5.81$; $P < 0.0001$; $N = 16$), the visitation rate between the females of the two species did not differ. Fruit set did not differ significantly between the two species (Mann-Whitney U-test: $Z = -0.39$: $P = 0.69$; $N = 27$). Important results on the comparison between the species are summarized in Table 3.

Table 3: Comparison of *Commiphora harveyi* (South Africa) and *Commiphora guillaumini* (Madagascar) in regards to flower width, flower length, flowers / inflorescence, inflorescences / tree, flowers / tree, # visiting species, visitation rate, natural and experimental fruit set, time of anthesis, daily and seasonal visitation peak, for male (♂♂) and for female (♀♀) trees. Data for *C. guillauminii* are taken from Farwig *et al*. (2004) and N. Farwig (unpublished data).

	Commiphora harveyi		***Commiphora guillauminii***	
	♂♂	♀♀	♂♂	♀♀
N	8	8 - 19	8	8
Mean flower width (mm)	1.46	1.54	2.33	2.74
Mean flower length (mm)	3.43	2.17	1.92	2.13
Flowers per inflorescence (median)	15	5	38.6	10.9
Inflorescences per tree (median)	1750	700	2070	215.5
Flowers per tree (median)	25500	2800	82800	2775
Total number of visiting species	25	18	18	16
Number of visiting species per tree (mean)	7.12	7.0	8.75	5.75
Mean visits per flower h^{-1}	0.2434	0.1697	1.07	0.18
Natural fruit set		3.8 %		2.9 %
Experimental fruit set		45.5 %		-
Anthesis	dawn		dusk	
Daily pattern	midday peak		morning peak	
Seasonal pattern	decline in course of the season		decline in course of the season	

3.4 Discussion

The flowers of *C. harveyi* were small and whitish and, thus, correspond to the general pattern described for tropical, dioecious tree species (Bawa & Opler 1975). Male trees of *C. harveyi* had significantly more and higher flowers than female trees, a common pattern found for animal-pollinated dioecious plants (Lloyd & Webb 1977, Ågren *et al.* 1986, Delph *et al*. 1996). This could be due to possible higher energetic cost of pistillate flowers (Cipollini & Wigham 1994, Humeau & Thompson 2001) or size differences of pistil and anthers (Delph *et al.* 1996). Alternatively, the more and higher staminate flowers could be the result of the intra-sexual competition as males in general need a higher visitation rate than pistillate flowers to reproduce successfully (Ågren *et al.* 1986, Osunkoya 1999). *Commiphora guillauminii* had generally more flowers per inflorescence, with female flowers being significantly larger than male ones. In both species, male trees had more flowers per inflorescence than female trees.

The flowers of *C. harveyi* were visited by 28 insect species. This is a relatively low number compared to other tropical and subtropical entomophilous tree species with similarly small 'generalized' flowers that are visited usually by up to 200 insect species (Bawa 1990, Ervik & Feil 1997, Soehartono & Newton 2001, Williams & Adam 2001). Most of the flower visitors on *C. harveyi* were small and unspecialized insects. This corresponds with other studies on dioecious tropical tree species with similar, inconspicuous flowers (Bawa & Opler 1975, Bawa 1980a, 1994; Farwig *et al*. 2004). I found on only three of the 28 insects pollen on their body and, thus, they appear to act as pollinators. Since I caught most insect species only once, it is possible that I underestimated the number of possible pollinators. The most important pollinator appears to be *Apis mellifera* (Table 1). This corresponds with bees being the most important pollinators for other tropical tree species of the family Burseraceae (Bawa 1990). The flowers of *C. guillauminii* were visited by an even lower total number of insect species. Since the pollen of *C. harveyi* was moist and sticky, I excluded the possibility of wind pollination in this species as observed in other primarily insect-pollinated systems (Anderson *et al.* 2000, Karrenberg *et al.* 2002).

Mean visitation rate was low with 0.2 visits per flower h^{-1} (Table 1). To my knowledge, this is the lowest visitation rate recorded in a dioecious tree species so far. In other plant species low visitation rates can be found as well (Motten 1986, Ashman & Stanton 1991, Ghazoul 1997), but they are still higher than recorded on *C. harveyi*. For example Liu *et al*. (2002) revealed a visitation rate of 2 - 13 visits per flower h^{-1} in the tropical monoecious genus *Musella* and McCall & Primack (1992) recorded 1.08 visits per flower h^{-1} in a South African Mediterranean plant community.

Visitation rates did not differ significantly between the sexes although male and female flowers differed in morphology and floral rewards. This result might be explained by the foraging behaviour of the visiting insect species. The insects might be nectar thieves on the nectar that is offered by both sexes. Nectar collection is known for bee-species that feed on nectar to satisfy their own energy demands and collect pollen only for their brood. For the other insect species visiting *C. harveyi*, no data on the foraging behaviour were available. Alternatively, insects may not be able to discriminate between males and females because of the staminodes in the pistillate flowers (cryptic dioecy). Anthers are important in attracting insects and are supposed to be the key to discriminating between staminate and pistillate flowers (Bawa 1980b, Charlesworth 1984, 1993; Anderson &

Symon 1989, Le Corff *et al*. 1998). In contrast to *C. harveyi*, *C. guillauminii* had a generally higher visitation rate with a higher visitation rate on male than on female trees. In the Malagasy species, pistillate flowers were not cryptically dioecious and, thus, the insects were probably able to discriminate between the sexes.

Daily and seasonal patterns in visitation rates were similar for both sexes (Figure 1). The daily pattern showed a peak around midday. While anthesis of *C. harveyi* took place at dawn, only a few insects were observed in the morning. This could lead to the accumulation of pollen and nectar by midday. Furthermore, the daily pattern of visitor activity matched the daily temperature pattern. High temperature around midday could result in high insect activity and this could lead to high visitation rates (Arroyo *et al.* 1985). An interrelation between temperature and insect activity is widely observed (Heinrich & Raven 1972, Heinrich 1974, Arroyo *et al*. 1985, McCall & Primack 1992, Wilcock & Neiland 2002). *Commiphora guillauminii* had its anthesis at dusk and a visitation peak in the morning. Since no nocturnal visitors were recorded, the authors explain the visitation peak with high nectar and pollen concentration in the morning (Farwig *et al.* 2004). Alternatively, the difference in the visitation peak of the two species could be explained by the difference between the subtropical and tropical climate at the two study sites. Oribi Gorge NR in South Africa has a monthly mean temperature of 19.2 °C (www.worldclimate.com) and Kirindy forest in Madagascar of 25 °C (Sorg & Rohner 1996). Thus, insect activity could be limited by cold morning temperatures in South Africa and high temperatures in the late morning and afternoon in Madagascar (McCall & Primack 1992). In both species, visitation rates declined in the course of the flowering season and coincided with a decline in open flowers on the trees. There was no evidence, that the study year had unusual climatic conditions.

Fruit set of *C. harveyi* was, at 3.8 %, very low. *Commiphora guillauminii* had an even lower fruit set of 2.9 %, much lower than the average fruit set of 73.8 % recorded for other dioecious plant species (Sutherland & Delph 1984). Focusing on dioecious tree species, Bawa & Opler (1975) recorded an average fruit set of 26 %. Low fruit sets have been reported for the monoecious palm *Neodypsis decaryi* (9.2 %) (Ratsirarson & Silander 1996) and the tropical dioecious palm *Chamaedorea alternans* (13 %) (Otero-Arnaiz & Oyama 2001). Thus, to my knowledge, the fruit sets of the two *Commiphora* species are the lowest so far reported in the literature for tropical and subtropical dioecious tree species.

The low visitation rates, the low visitor diversity and the low fruit set in both *Commiphora* species and the significant increase through hand-pollination in *C. harveyi* indicate that the low fruit set in these species could be caused by pollinator or pollen limitation. Pollen limitation is one of the main reasons for a low fruit set in dioecious plant species (Ratsirarson & Silander 1996, Liu *et al*. 2002, Otero-Arnaiz & Oyama 2001). For example, Burd (1994) compiled data on pollination experiments on 258 plant species and recorded pollen limitation in 62 % of them. However, the results of hand-pollination experiments have to be interpreted with caution. To compensate for resources invested, an unusually high fruit set can reduce growth, flower formation or seed production in the following year (Ackerman & Montalvo 1990, Fox & Stevens 1991, Ehrlen 1992, Calvo 1993, Ehrlen & Eriksson 1995). Additionally, it is unclear whether the fruit set could be increased on the basis of the whole tree (Johnston 1991) or whether increased fruit set would increase fruit abortion because of resource limitation (Schemske 1980, Stephenson 1981). The apparent oversupply of flowers could be a necessary adaptation to attract enough pollinators to ensure a sufficient visitation rate, resulting in an adequate number of fruits to sustain the population size. A number of studies indicate that fruit set is probably pollen limited within a season, but resource limited over a longer period of time (Fox 1992, Cunningham 1996, Wilcock & Neiland 2002).

To summarize, the two *Commiphora* species differed in the visitation rates of the two sexes. Although in both species male trees had more flowers per inflorescence, only in the Malagasy species did male trees have significantly higher visitation rates than female ones. In the South African species this could be caused by cryptic dioecy in the pistillate flowers, which might have made it difficult for the insects to distinguish the sexes. Therefore, despite the higher visitation rate in the Malagasy species, the fruit set was slightly higher in the South African one. Nevertheless, in comparison to other dioecious tree species the pollination systems of the two entomophilous *Commiphora* species are very similar with both having low visitation rates, low species diversity and low fruit sets. This indicates that the unusual pollination system of the Malagasy *C. guillauminii* is not caused only by the island situation of Madagascar, combined with its endemic flora and fauna, as assumed by Farwig *et al*. (2004). The present study suggests that low fruit set in dioecious subtropical and tropical tree species may be more common than previously reported. As I compared only two species at two sites, however, further studies on more species and mores sites are needed before I can make any generalizations.

3.5 Summary

Dioecious plant species differ in floral morphology and rewards between females and males. Pistillate flowers on female plants often lack pollen and can be less attractive to pollinators, which can have consequences for the visitation rates of the sexes. I studied the pollination ecology of the dioecious tree *C. harveyi* in a coastal scarp forest in eastern South Africa. Floral display, visiting insect species, visitation rate and natural fruit set were recorded. Additionally, I pollinated flowers by hand to determine experimental fruit set. I found that male trees had more and larger flowers per inflorescences than female trees. Both sexes produced nectar in low amounts. During 203.5 h of observation I recorded 28 insect species visiting the flowers. No difference in mean visitation rate (0.20 visits per flower h^{-1}) was recorded between the sexes. The daily and seasonal pattern was similar between the sexes. The natural fruit set was low (3.8 %) and increased significantly with hand-pollination (45.5 %), an indication of pollen limitation. I compared my results with the pollination system of *C. guillauminii* in Madagascar, a dioecious tree species on an island with a depauperate pollinator fauna. This comparison revealed a similar pattern with low visitation rates, low insect diversity and low fruit set, suggesting that this pattern may be more common in dioecious tree species than previously reported in the literature.

4 Does seed dispersal matter? – Comparative population genetics of two congeneric tropical trees

4.1 Introduction

Pollination and seed dispersal are important processes to establish and sustain plant populations (Howe & Smallwood 1982, Bawa 1990, Wang & Smith 2002). Many field studies showed that both processes are crucial for the regeneration of plant species (Herrera 1998, Kearns *et al*. 1998, Wenny 2001). However, pollination and seed dispersal are not only ecologically important, but also relevant for gene flow in and between populations. Gene flow counteracts the effects of genetic divergence in populations caused by differences in selective forces and / or random genetic drift (Hartl 1980). Especially gene flow over long distances is ecologically important for gene exchange between populations. However, to assess pollination and seed dispersal over long distances is difficult based on field data (Cain *et al*. 2000, He *et al*. 2004, Trapnell & Hamrick 2004). Field data mainly document ecological processes such as pollination success as flower to fruit ratio (fruit set) or seed dispersal rates and seedling establishment. However, the consequences on the genetic population structure are hardly understood (Bohonak 1999, Ouborg *et al.* 1999).

Therefore, a new approach to answer these ecological questions was established in the last decade using molecular techniques (Aldrich & Hamrick 1998, Hamilton 1999, Sork *et al.* 1999, Vekemans & Hardy 2004). Nevertheless, most of the present studies are based either on field or on genetic data (Chung *et al*. 2000, Degen *et al*. 2001, Cascante *et al*. 2002, Gomes *et al.* 2004) and only few studies are combining both approaches (but see Dick 2001, Godoy & Jordano 2001). It is not possible to experimentally manipulate pollination and seed dispersal rates of tree species and to study the consequences on the genetic population structure. An alternative approach is to compare related tree species which are naturally differing in their pollination and seed dispersal system.

I chose such a biogeographic approach and compared two tropical tree species (genus *Commiphora)* from Madagascar (MAD) and South Africa (SA) using field data on pollination and seed dispersal (Bleher & Böhning-Gaese 2000, 2001, Farwig *et al*. 2004, Voigt *et al*. 2005). Both, the Malagasy *C. guillauminii* and the South African *C. harveyi* are dioecious, obligate outcrossing and pollinated by small unspecialised insects with a low pollination rate,

resulting in low fruit sets (Farwig *et al*. 2004, Voigt *et al*. 2005). Since in both species the pollinators are weak fliers, we expected gene flow via pollination to occur only at small spatial scales. Despite similar pollination ecology, seed dispersal is very different between the two species. Basically one frugivorous bird species disperses the seeds of the Malagasy species resulting in a dispersal rate of only 7.9 % with most seeds being dropped under the crown of the trees (Böhning-Gaese *et al*. 1995, 1999). In contrast, the seeds of the South African species are dispersed by a high number of frugivorous bird species resulting in most seeds being dispersed away from the crown (70.8 % dispersal rate) (Bleher & Böhning-Gaese 2000, 2001). Gene flow via seed dispersal should occur - in general - on a larger spatial scale. However, we expected gene flow via seed dispersal to act over larger distances in the South African than in the Malagasy species.

With these profound differences in seed dispersal rates between the two *Commiphora* species I have a good model system to test whether restricted seed dispersal results in limited gene flow. I expected that the low seed dispersal rates in the Malagasy species *C. guillauminii* resulted in low gene flow causing high genetic differentiation between populations. In contrast, high seed dispersal rates in the South African species *C. harveyi* should lead to high gene flow resulting in low genetic differentiation between populations.

I explicitly considered different spatial scales in my sampling design to take into account that pollination and seed dispersal might influence the genetic structure of the populations at different spatial scales. At the small spatial scale, i.e. the flight distance of a pollinator, I expected a similar genetic differentiation between populations of the two species. In contrast, on larger spatial scale, i.e. the flight distance of a seed disperser, I hypothesised a weaker genetic differentiation in the South African than in the Malagasy species.

I used amplified fragment length polymorphism (AFLP) (Vos *et al*. 1995) to study different populations of the Malagasy and South African *Commiphora* species.

4.2 Methods

4.2.1 Species studied

My study species are *Commiphora guillauminii* H. Perrier (Burseraceae) (de la Bathie 1946) from Madagascar and *Commiphora harveyi* Engl. (Burseraceae) (van der Walt 1973) from South Africa. Both species are deciduous trees that grow up to 20 m in height. *Commiphora guillauminii* is a narrow endemic to the west coast of Madagascar (Figure 1 A). *Commiphora*

harveyi is a tree species found in scarp forests along the east coast of Southern Africa (Figure 1 A; Palgrave 1977). Both species are dioecious and have small flowers (calyx 2 - 4 mm; Voigt *et al.* 2005) that are borne in inflorescences (de la Bathie 1946, van Wyk & van Wyk 1997). The flowers of both species are pollinated by small unspecialized insect species, have a low visitation rate in both sexes and are not able to perform apomixes (*C. guillauminii*: 0.62 visitors per flower h^{-1}, Farwig *et al*. 2004; *C. harveyi*: 0.20 visits per flower h^{-1}, Voigt *et al*. 2005). The most common pollinators were the stingless bee *Liotrigona mahafalya* in *C. guillauminii* and the syrphidae *Asarkina africana* in *C. harveyi*; both species were also pollinated by the honeybee *Apis mellifera*. Female trees of both species produce roundish fruits with outer coverings that split open when mature and expose a black seed partly enveloped by a fleshy red aril. The seed dispersal system of *C. guillauminii* is unusually simple and effectively carried out by only one species, the Lesser Vasa Parrot (*Coracopsis nigra*) (Böhning-Gaese *et al*. 1999). This results in a low percentage of dispersed seeds (7.9 %) with most seeds being dropped under the mother tree (Böhning-Gaese *et al*. 1999). In contrast, fruits of *C. harveyi* attract a high diversity of dispersers and most seeds (70.8 %) are carried away from the crown (Bleher & Böhning-Gaese 2000). Some of the main dispersers are hornbills, which are known for long distance dispersal (Holbrook & Smith 2000, 2002).

4.2.2 Plant materials and sample sites

Leaf material of tree populations of *C. guillauminii* and *C. harveyi* was collected from 12 sample sites in Madagascar and 15 sample sites in South Africa along a north-south gradient (Figure 1 B, C). Distance between the most southern and northern sample sites was 270 km in Madagascar (i.e. between sample sites Lonahena and Tsakobe) and 435 km in South Africa (i.e. between sample sites Umtavuna NR and Jozini Dam). Sample sites were separated by at least 8 km from one another. This is a distance which larger seed dispersers such as hornbills are still capable to cover (Holbrook & Smith 2002). In the following, this spatial scale is referred to as the largest spatial scale (Fig. 2 A). For descriptive genetic analyses, sample sites were grouped into geographic regions, based on UPGMA-tree (data not shown) and geographic proximity (Figure 1 B, C).

I collected leaf material of 10 - 20 trees per sample site. The position of each tree was recorded using a GPS (Garmin 12). Leaves collected were silica-dried in the field. To test for potential effects of pollination and seed dispersal at smaller spatial scales, I sampled the trees

within one sample site at two smaller, nested spatial scales. First, within the sample sites I collected samples from trees in 1 - 4 groups (median = 2). As groups I defined trees within a circle with a diameter of 3 km (in the following medium spatial scale, Figure 2 B). The distance of 3 km was assumed to be regularly covered by seed dispersers. Second, within each group the sampled trees were in 1 - 5 subgroups (median = 3). As subgroups I defined trees

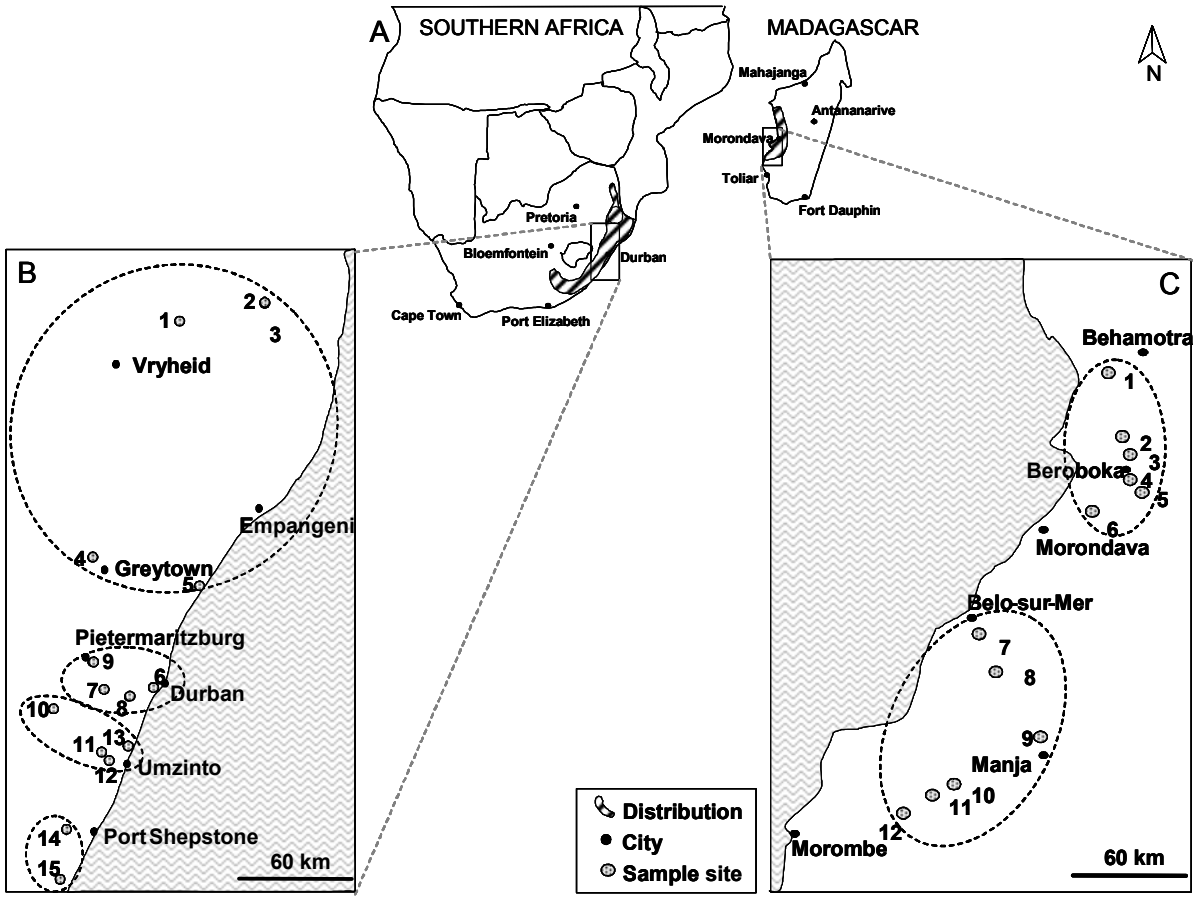

Figure 1: Distribution (A) and sample sites of *C. guillauminii* populations in Madagascar (C) and of *C. harveyi* populations in South Africa (B). Numbers refer to sample sites (Table 1), sample sites within dashed line represent regions for descriptive genetic analyses.

within a circle with a diameter of 300 m (in the following small spatial scale, Figure 2 C). The distance of 300 m was thought to represent a maximum distance a possible pollinator can fly regularly. Distances in the field were assessed based on GPS-distances between the trees. Circles of groups and subgroups were reconstructed in ARCVIEW 3.2 based on GPS-position of each tree, by laying circles with a diameter of 3 km and 300 m around each tree and defining all trees in the intersection as groups and subgroups, respectively.

4.2.3 DNA extraction and quantification

After grinding of leaf material in liquid nitrogen, the total genomic DNA was extracted using the DNeasyTM Extraction Kit (Quiagen). The standard protocol was slightly modified by using 500 µl buffer AP1 and 160 µl buffer AP2 which handled the amount of leave material better. DNA was stored at -20 ºC in AE elution buffer (Quiagen). DNA quantification was carried out spectrophotometrically for each AFLP sample with a GeneQuant RNA / DNA calculator Pharmacia (Uppsala Sweden). The genomic DNA concentration was standardized to 30 ng DNA/µl.

4.2.4 AFLP analyses

The AFLP procedure followed Vos *et al*. (1995), with the following modifications: for digestion 100 ng instead of 500 ng genomic DNA were used. To ensure complete digestion, the restriction-ligation step of the analyses was performed for 15 hrs at 23 ºC as the initial step of the analyses. Furthermore, instead of radioactive labelling, a multiplex analysis with fluorescent ´E´ primers (6-FAM, NED, HEX, Applied Biosystem) was used.

Genomic DNA was digested with EcoRI and MseI, and double-stranded EcoRI and MseI adapters were ligated to the sticky ends of the fragments (Vos *et al*. 1995). All 294 samples of both species were restricted - ligated in one reaction to avoid lab errors. In the following two-step amplification, preselective primers with one selective base (E + A, M + C) and selective primers with two additional selective bases (E + 3, M + 3) were used. The complete survey was done using three primer combinations: Eco-ACT (6-FAM) / Mse-CCT, Eco-ATG (HEX) / Mse-CGG and Eco-AGC (NED) / Mse-CTG. These were chosen for high variability of fragments after pilot screening with 16 primer combinations of DNA samples from five sample sites per species, covering the whole sampling area. All pipeting and PCRs were performed on a lab robot (RoboSeq 4204 SE; MWG). Selective amplification products were separated on 6 % polyacrylamid gels as a multiplex of three differently labelled products together with one internal size standard (GENESCAN ROX 500, ABI). Gels were run for approximately 4 hrs on an ABI 377 automated sequencer using GENESCAN analysis software (version 2.1 ABI). Since samples had to be run on five gels, 2 - 4 samples were put as internal standards on each gel to ensure comparability among the different gels. Fragments in the range 75-500 bp were scored automatically with GENOTYPER analysis software (version 3.1 ABI). When peak height exceeded the GENESCAN standard parameter-setting thresholds (blue, 60; green, 30; red, 40; yellow, 40) a peak (i.e. fragment) was scored as present (1),

otherwise as absent (0). An additional visual check of the electrophoretograms was made to correct possible misinterpretations of the automated GENOTYPER analysis. Ambiguous peaks were scored as missing data.

4.2.5 Data analysis

All analyses were performed similarly for both species. AFLP data analyses were based on 136 individuals of *C. guillauminii* and 158 individuals of *C. harveyi*. Descriptive statistic (polymorphic loci, Nei´s (1978) unbiased heterozygosity) was obtained using TFPGA, Version 1.3 (Tool For Population Genetic Analysis, Miller 1997). Since AFLPs generate dominant markers and heterozygotes cannot be distinguished directly, I used Lynch and Milligan´s Taylor expansion to estimate allele frequency and thus, determined indirect levels of heterozygosity (Lynch & Milligan 1994, Miller 1997). This assumes that populations are in Hardy-Weinberg-equilibrium and that AFLPs produce two alleles per locus (Lynch & Milligan 1994)

The percentage of private fragments per sample sites and region was derived from the binomial data matrix. Private fragments were defined as fragments that occur in more than one individual but are restricted to one sample site or region, respectively.

To estimate the partition of AFLP genotypic variation at the large spatial scale and within each sample site, analyses of molecular variance (AMOVA) were calculated with ARLEQUIN, Version 2.000 (Schneider *et al*. 2000). To calculate genetic differentiation for the entire data set, each sample site was defined as one population. In addition, pairwise F_{ST}-values were obtained for all combinations of sample sites. To test for isolation by distance (IBD) at the scale of the sample sites, I regressed the F_{ST} / 1 - F_{ST} against the log-transformed geographic distance separating the sample sites, based on these pairwise F_{ST}-values (Slatkin 1993, Rousset 1997). I tested for IBD with a Mantel-test using 1000 permutations, as implemented in TFPGA (Miller 1997).

In a second analysis, I studied the genetic variance at the two smaller spatial scales using Wright´s hierarchical F-statistic (Wright 1978, Weir & Cockerham 1984). Thereby, I defined all trees within a circle with a diameter of 3 km (groups, medium spatial scale, Figure 2 B) or of 300 m (subgroups, small spatial scale, Figure 2 C) as populations and nested them into sample sites. Introducing a hierarchical level allows to distinguish whether the genetic differentiation among populations is due to differentiation between the sample sites or due to high differentiation among populations within the sample sites (among circles of trees

with diameter 3 km or 300 m). In the following, genetic differentiation on the large spatial scale (sample sites) will be referred to as F_{ST}, on the **m**edium spatial scale (groups) as F_{SM}, and on the **s**mall spatial scale (subgroups) as F_{SS}.

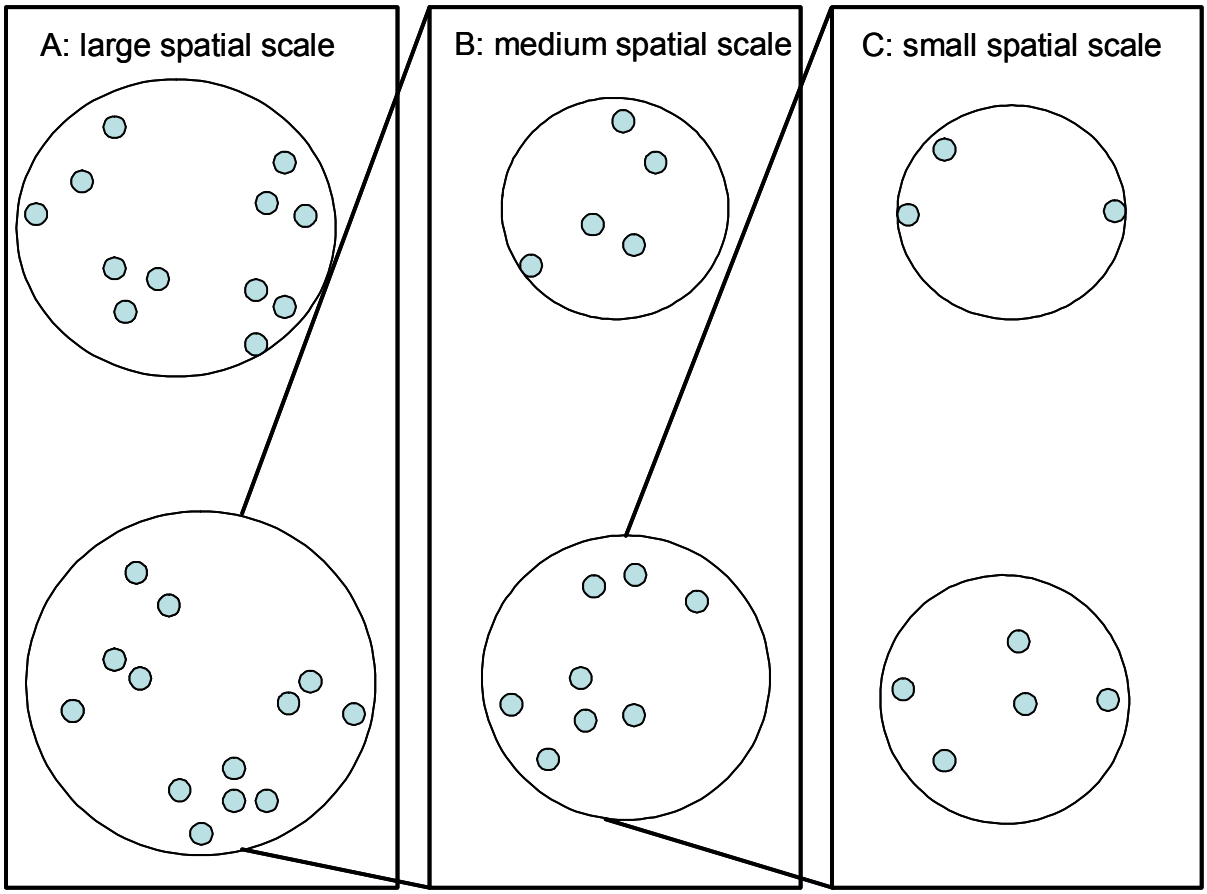

Figure 2: (A) For the overall genetic differentiation, sample sites are defined as populations; the maximum distance between trees within on sample site ranged from 5719 m - 9983 m in the Malagasy (MAD) and from 4200 m - 7651 m in the South African (SA) species. For the hierarchical F-statistic trees within one sample site are grouped on two smaller spatial scales. (B) For the analyses at the medium spatial scale, trees within a circle with a diameter of 3 km were defined as one group; distances between trees within groups ranged from 301 m - 2896 m (MAD) and from 302 m - 7651 m (SA). (C) For the analyses at the small spatial scale, trees within a circle with a diameter of 300 m were defined as one subgroup; distances between trees within subgroups ranged from 3 m - 300 m (MAD) and from 1 m - 299 m (SA). Circle mark sampled trees.

Additionally, I assessed the spatial genetic structure at different geographic scales using spatial autocorrelation analyses. I calculated kinship coefficients for each pair of individuals (Loiselle *et al*. 1995a) using the program SPAGEDI (Hardy & Vekemans 2002). A kinship coefficient is often defined as the probability of identity by descent of the genes, but Hardy and Vekemans (2002) use an estimator based on genetic markers that estimates

"relative kinship" as a ratio of differences of probabilities of identity in state (Rousset 2002). With this definition, the relative kinship coefficients between two individuals can also obtain negative values, if these individuals are less related to each other than randomly selected individuals (Hardy & Vekemans 2002). I plotted the kinship coefficients for each pair of individuals against the geographic distance separating the individuals. For the graphical representation of kinship, average multilocus kinship coefficients per geographic distance class were computed for the following distance classes: < 30 m, ≤ 300 m, ≤ 3 km, ≤ 30 km, ≤ 300 km and > 300 km.

4.3 Results

4.3.1 Descriptive population genetics

A total of 225 distinct loci were scored for the 136 Malagasy individuals and 184 loci for the 158 South African individuals, with 94.7 % and 96.2 % of them being polymorphic, respectively (Table 1 a, b). The mean number of polymorphic loci was 25.3 % ± 7.9 (mean ± SD in the following) in the Malagasy species and 27.9 % ± 5.9 in the South African species. Of 124 polymorphic loci in Madagascar 4.8 % were polymorphic within single sample sites and 21.8 % polymorphic regionally, of which 19.4 % were restricted to the northern region (north of Morondava) and only 2.4 % to the southern region (south of Morondava) (Table 1 a; Figure 1 C). Of 129 polymorphic loci in South Africa 7.0 % were restricted to single sample sites and 9.3 % occurred regionally (Table 1 b; Figure 1 B). The mean heterozygosity was 0.059 ± 0.009 in the Malagasy species and 0.073 ± 0.010 in the South African species (for heterozygosity of single sample sites, see Table 1 a, b). Heterozygosity was significantly lower in the Malagasy species than in the South African species (Mann Whitney U-test: S = 106.5, Z = -2.97, P = 0.003), which could be due to more private alleles in this species. In both species, the percentages of polymorphic loci and heterozygosity were significantly correlated for each sample site (Spearman´s rank correlation coefficient $r_{MAD} = 0.82$, P = 0.001, N = 12; $r_{SA} = 0.77$, P < 0.001, N = 15). In Madagascar, heterozygosity correlated significantly with geographical latitude and decreased from the northern to the southern sample sites (Spearman´s rank correlation coefficient $r_{MAD} = 0.727$, P = 0.007, N = 12). In South Africa, I found no correlation between heterozygosity and latitude (Spearman´s rank correlation coefficient $r_{SA} = -0.232$, P = 0.41, N = 15).

4.3.2 Large spatial scale

In the Malagasy species there was a low but significant differentiation between the sample sites (F_{ST} = 0.053, Table 2). The mean F_{ST}-value among all sample sites was 0.06 ± 0.04 and ranged from 0.00 - 0.19 (N = 66; Table 3a). In the South African species, I found a significant and moderate differentiation between sample sites (F_{ST} = 0.16, Table 2). The mean F_{ST}-value among all sample sites was 0.17 ± 0.07 and ranged from 0.02 – 0.33 (N = 105; Table 3b).

In both species, I found a significant relationship between pairwise genetic distances of sample sites (measured as F_{ST} / 1 - F_{ST}) and log-transformed geographic distances between them (Pearson product-moment correlation: r_{MAD} = 0.36, Mantel-P = 0.003; r_{SA} = 0.46, Mantel-P < 0.001). However, when I tested the northern and southern populations in the Malagasy species separately, I found no IBD-pattern (Pearson product-moment correlation: northern population: r = 0.12; Mantel-P = 0.36; southern population: r = -0.045; Mantel-P = 0.6). All pairwise genetic (F_{ST}) and geographic distances are presented in Table 3 a, b.

Table 1a: Sample sites in Madagascar with number of *C. guillauminii* trees studied (N), number of fragments, private fragments of sample sites and regions, Nei´s (1978) unbiased heterozygosity, percentage of polymorphic loci (99 % criterium). Sample sites are ordered from North to South, for numbers and regions refer to Figure 1.

no.	sample sites	code	N	# fragments	# private fragments		heterozygosity	% polymorphic loci
					sample sites	regions		
1	Lonahena	LA	8	77	0	24	0.0664	25.33
2	South of Belo s. Tsiribihina	SBO	4	73	0		0.0622	20.44
3	North of Berobuka	NBA	18	91	1		0.0683	32.44
4	Berobuka	BA	13	92	1		0.0695	32.44
5	Kirindy	KY	16	108	2		0.0671	39.11
6	Andromena	AA	10	79	0		0.0562	24.44
7	Kirindy-Mitea	KM	15	69	0	3	0.0568	22.22
8	Mukabe	MKE	17	85	0		0.0652	29.77
9	Manja	MA	5	57	0		0.0518	13.77
10	Migamba	MIG	15	86	0		0.0558	28.00
11	Bevoy / Mangoky	MY	4	53	0		0.0386	11.55
12	Antsakoabe	TSK	11	80	2		0.0539	24.00
		total	**136**	**225**	**6**	**27**		

Table1b: Sample sites in South Africa with number of *C. harveyi* trees studied (N), number of fragments, private fragments of sample sites and regions, Nei´s (1978) unbiased heterozygosity, percentage of polymorphic loci (99 % criterium). Sample sites are ordered from North to South, for numbers and regions refer to Figure 1.

no.	Sample sites	code	N	# fragments	# private fragments		heterozygosity	% polymorphic loci
					sample sites	regions		
1	Ithala GR	IAGR	11	89	0		0.0753	35.87
2	Jozini Damm	JD	9	71	1		0.0661	25.00
3	Mkuze GR	MEGR	6	65	1		0.0622	20.65
4	Montella	MA	5	61	0		0.0627	19. 02
5	Harold Johnson NR	HJNR	13	86	0	5	0.0755	35.87
6	Burman Bush NR	BBNR	10	68	1		0.0565	23.37
7	Shongweni	SGI	13	80	0		0.0742	31.52
8	Kranzkloof NR	KFNR	15	85	0		0.0911	35.87
9	Pietermaritzburg	PMB	7	70	0	2	0.0691	21.74
10	Helas-Helas Richmond	HHR	15	75	1		0.0905	29.35
11	Montezuma	MZ	11	79	0		0.0815	31.52
12	Vernon Crookes NR	VCNR	10	69	0		0.0718	26.09
13	Emizini NR	EINR	11	70	0	3	0.0778	26.62
14	Oribi Gorge NR	OGNR	13	82	1		0.0814	33.15
15	Umatvuna NR	UANR	7	69	4	2	0.0614	22.28
		total	**158**	**184**	**9**	**12**		

4.3.3 Medium and small spatial scale

In the hierarchical analyses of the Malagasy species, at the medium scale (Figure 2 B), I found an increased and significant differentiation ($F_{SM} = 0.083$). In the hierarchical analyses at the smallest spatial scales (Figure 2 C), the genetic differentiation increased again ($F_{SS} = 0.11$). In the Malagasy species, at the medium and small scale, 85.5 % and 72.7 % of the genetic differentiation was due to differentiation among groups or subgroups within sample sites, respectively. Thus, at both spatial scales, most of the differentiation was due to high variation between the defined groups / subgroups within sample sites (Table 2).

Table 2: Results of AMOVA of AFLP data from 12 sample sites of the Malagasy species (*C. guillauminii*) and from 15 sample sites of the South African species (*C. harveyi*) at large (among sample sites), medium and small spatial scales (for medium and small scale see Figure 2); ** P = 0.01; *** P < 0.001.

	Commiphora guillauminii	***Commiphora harveyi***
source of variation		
large spatial scale: populations = sample sites		
among sample sites (F_{ST})	0.053	0.159
within sample sites	0.947	0.841
medium spatial scale: groups = circles Ø 3 km		
among groups (F_{SM})	0.083***	0.168***
among sample sites	0.012 (14.5 %)	0.154*** (91.6 %)
among groups within sample site	0.069***(85.5 %)	0.014 (8.4 %)
within groups	0.917	0.832
small spatial scale: subgroups = circles Ø 300 m		
among subgroups (F_{SS})	0.11**	0.179***
among sample sites	0.03*** (27.3 %)	0.153*** (85.5 %)
among subgroups within sample site	0.08*** (72.7 %)	0.26*** (14.5 %)
within subgroups	0.89	0.82

In the South African species using the hierarchical approach as described above, F_{SM}- and F_{SS}-values increased only slightly at the medium and small scale (groups: $F_{SM} = 0.17$; subgroups: $F_{SS} = 0.18$). However, in the South African species only 8.4 % and 14.5 % of the genetic differentiation was due to differentiation between groups / subgroups within the sample sites (Table 2). Thus, in the South African species, on both spatial levels, the main differentiation was due to high variation between sample sites, and only a low differentiation between defined groups or subgroups within sample sites, respectively.

In both species the average kinship coefficient decreased with geographical distance. In the Malagasy species the kinship coefficient dropped nearly to zero in the distance class of

up to 3 km and in the South African species in the distance class of up to 30 km. Thus, trees seem to form a related group up to 3 km in the Malagasy species and up to 30 km in the South African species.

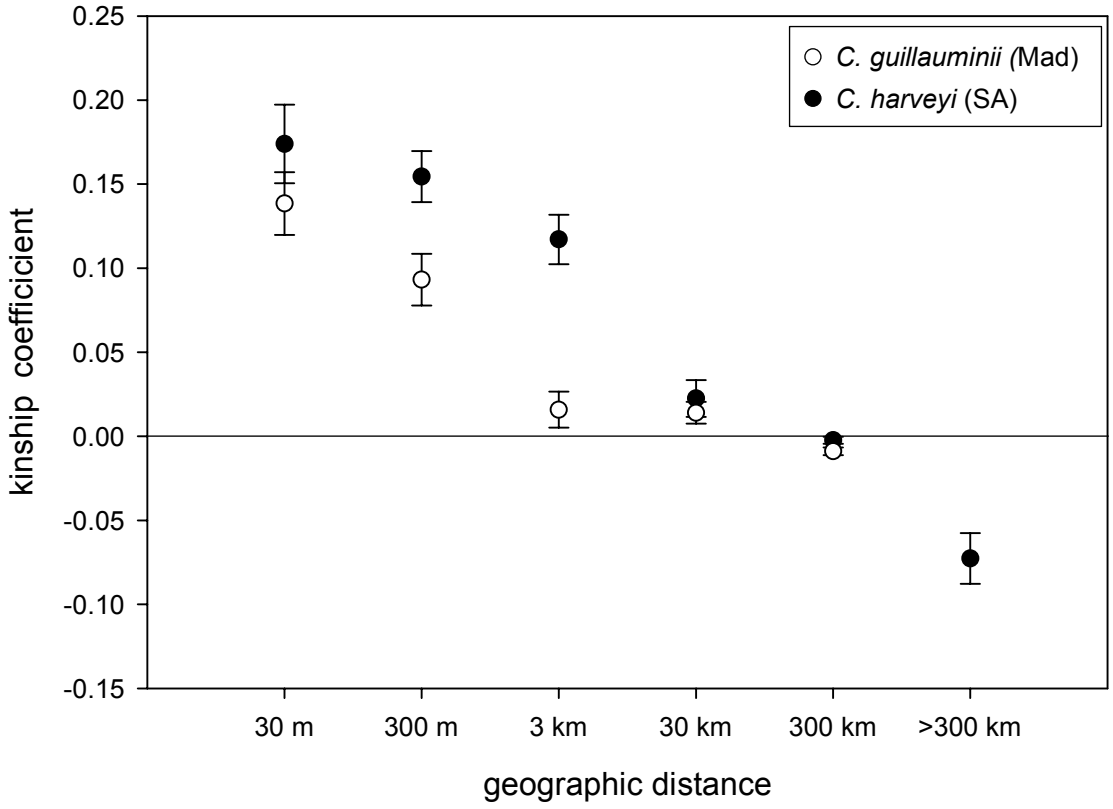

Figure 3: Kinship coefficient among 136 Malagasy and 158 South African *Commiphora* individuals per geographic distance class; displayed are multilocus jacknife values (mean ± SE).

Table 3a: Pairwise F_{ST}-values and geographic distances of sample sites; above diagonal: genetic differences (F_{ST}, AMOVA ARLEQUIN); below diagonal: geographic distances (km) between sample sites in Madagascar, sample sites are ordered from North to South.

	LA	**SBO**	**NBA**	**BA**	**KY**	**AA**	**KM**	**MKE**	**MA**	**MIG**	**MY**	**TSK**
LA	-	0.029	0.026	0.017	0.046	0.038	0.045	0.059	0.099	0.088	0.037	0.095
SBO	38.85	-	0.099	0.039	0.078	0.050	0.119	0.133	0.143	0.141	0.057	0.120
NBA	51.40	12.34	-	0.002	0.023	0.024	0.047	0.045	0.119	0.086	0.061	0.071
BA	63.66	25.06	13.12	-	0.00	0.009	0.035	0.017	0.079	0.044	0.033	0.062
KY	68.03	30.26	18.16	8.44	-	0.045	0.066	0.050	0.114	0.076	0.074	0.080
AA	80.61	41.78	31.56	22.02	21.79	-	0.040	0.060	0.140	0.088	0.074	0.070
KM	167.89	130.69	121.54	104.14	109.14	90.12	-	0.021	0.112	0.04	0.077	0.017
MKE	183.67	145.81	134.71	125.25	122.62	104.59	22.10	-	0.096	0.023	0.066	0.009
MA	212.96	174.26	163.29	150.72	147.98	132.79	60.59	44.50	-	0.071	0.196	0.077
MIG	247.30	174.39	201.40	187.21	185.40	168.88	81.70	64.31	45.46	-	0.100	0.030
MY	258.92	221.06	211.09	200.26	198.42	179.58	92.38	75.89	57.78	12.59	-	0.032
TSK	270.98	233.87	223.92	212.08	211.58	193.23	103.53	88.79	72.27	27.54	16.13	-

Table3b: Pairwise F_{ST}-values and geographic distances of sample sites; above diagonal: genetic differences (F_{ST}, AMOVA ARLEQUIN); below diagonal: geographic distances (km) between sample sites in South Africa, sample sites are ordered from North to South.

	IAGR	**JD**	**MEGR**	**MA**	**HJNR**	**BBNR**	**SGI**	**KFNR**	**PMB**	**HHR**	**MZ**	**VCNR**	**EINR**	**OGNR**	**UANR**
IAGR	-	0.053	0.050	0.144	0.069	0.206	0.148	0.180	0.215	0.219	0.172	0.202	0.267	0.209	0.267
JD	71.41	-	0.018	0.173	0.070	0.202	0.135	0.166	0.262	0.206	0.122	0.171	0.247	0.185	0.265
MEGR	87.49	24.17	-	0.207	0.049	0.173	0.138	0.199	0.250	0.230	0.195	0.236	0.276	0.209	0.285
MA	178.23	226.59	222.53	-	0.155	0.329	0.177	0.246	0.319	0.285	0.244	0.289	0.314	0.240	0.305
HJNR	186.70	202.23	188.97	96.12	-	0.117	0.067	0.149	0.218	0.193	0.157	0.154	0.209	0.133	0.175
BBNR	254.81	279.89	267.24	107.60	78.56	-	0.128	0.201	0.273	0.221	0.189	0.193	0.203	0.117	0.208
SGI	271.10	295.12	283.93	101.37	99.01	28.39	-	0.117	0.163	0.137	0.121	0.130	0.161	0.061	0.131
KFNR	258.55	280.46	268.83	95.42	82.97	17.13	16.18	-	0.107	0.065	0.067	0.085	0.132	0.133	0.163
PMB	255.05	287.69	279.55	73.55	107.55	60.77	38.33	42.82	-	0.075	0.161	0.151	0.131	0.156	0.227
HHR	289.20	332.14	324.42	111.55	151.40	90.16	61.79	74.33	45.33	-	0.090	0.076	0.135	0.132	0.195
MZ	308.91	338.25	329.12	151.58	142.27	65.02	45.74	59.31	61.10	58.15	-	0.022	0.114	0.133	0.167
VCNR	210.21	340.78	328.87	144.49	140.82	63.53	46.65	59.52	72.88	61.10	07. 07	-	0.109	0.127	0.172
EINR	308.24	328.59	315.93	140.29	126.99	49.02	39.10	49.12	72.37	73.55	23.00	16.86	-	0.139	0.210
OGNR	366.44	400.09	388.39	194.45	201.15	122.84	104.70	118.33	120.98	907.93	58.21	59.84	74.56	-	0.031
UANR	409.76	435.29	423.24	229.53	234.80	157.22	140.07	153.67	156.45	123.47	93.49	94.39	107.89	36.50	-

4.4 Discussion

4.4.1 Descriptive population genetics

With three AFLP primer pair combinations, I found 225 loci in 12 populations in the Malagasy *C. guillauminii* and 184 loci in 15 populations in the South African *C. harveyi*. These results are in range of other AFLP studies on trees (Muluvi *et al*. 1999, Gaudeul *et al*. 2000, Wang *et al*., 2003, He *et al*. 2004). In both species, heterozygosity was low compared to other AFLP studies on trees in which heterozygosity ranged from 0.17 - 0.35 (Rivera-Ocasio 2002, Wang *et al*. 2003, He *et al*. 2004). However, Muluvi *et al*. (1999) stated similar low values of 0.026 - 0.099 in *Moringa oleifera*. The significant lower heterozygosity in the Malagasy than in the South African species could be caused by its smaller distribution (Hamrick & Godt 1989, Wolf *et al*. 2000). The Malagasy species is a narrow endemic species that is restricted to the west coast of Madagascar (Figure 1 A), whereas the South African species has a much wider range along the east coast of southern Africa (Figure 1 A). This pattern fits to the expected positive correlation between genetic diversity and the size of the distribution area (Frankham 1997). Similar results were found by Dawson and Powell (1999). They compared gene diversity of *Prunus africana* between mainland Africa and Madagascar and found the lowest gene diversity in the Malagasy populations as well.

The decrease in heterozygosity and private fragments from north to south in the Malagasy populations coincided with approaching the edge of the distribution area of the species (Figure 1 A). I found no geographic pattern in the South African species; this could be due to the fact that I sampled in the middle of the distribution area of the species (Figure 1 A).

4.4.2 Large spatial scale

The genetic differentiation of the two species observed is in disagreement with my initial hypothesis. The South African populations are genetically more distinct than the Malagasy populations ($F_{ST} = 0.16$ in South Africa *vs* $F_{ST} = 0.05$ in Madagascar). Comparing my overall F_{ST}-values to Hamrick and Godt´s allozyme data (Hamrick & Godt 1989; 1996, 1997), I found that the values of the South African species are similar to the expectations based on its life-history traits (woody, long-lived, outcrossing, animal-dispersed). However, this comparison is only very coarse, since it is difficult to compare different

marker systems (Whitlock & McCauley 1999). The genetic differentiation between the sample sites in the South African species may be influenced also by its habitat distribution. The species is restricted to northerly slopes and can often be found at steep slopes of gorges (MacDevette *et al.* 1989). Thus, the species occurs in a naturally fragmented habitat, which might lead to limited gene flow and a high differentiation between populations. Furthermore, non-overlapping flowering or fruiting period is known to reduce gene flow between populations (Hall *et al.* 1994, Jordano & Godoy 2000). I observed a shift in flowering period between the northern (e.g. Ithala Game Reserve, No. 1 in Figure 1 B) and the southern populations (e.g. Oribi Gorge NR; No. 14 in Figure 1 B), with the northern populations starting to flower up to three weeks earlier.

Based on its life-history traits, the Malagasy species should have a similar differentiation, if not even a higher genetic differentiation between sample sites. Following Hamrick and Godt (1997), endemic species are genetically stronger differentiated than species with a wider geographical distribution area. However, the unexpectedly low F_{ST}-value in the Malagasy species could be explained by the higher number of alleles scored in this species. Theoretical studies showed that the more alleles are taken into account to calculate the F_{ST}-value, the smaller the value for the differentiation between sample sites becomes (Whitlock & McCauley 1999). Alternatively, the high variation within the local populations reduced the variance left to describe the differentiation between the sample sites. In total, on the largest spatial scale the genetic population structure of the two species seemed to be influenced more by biogeographic factors such as habitat distribution than ecological processes such as seed dispersal.

I stated an IBD-pattern in the Malagasy species, but it disappeared after analysing the northern and southern populations separately. The missing IBD-pattern within these two regions could indicate that there was no migration between the different populations at the sample sites and thus, they are genetically separated. These two regions are not only different in the number of private loci, but were separated by PCO and cluster analyses as well (F.A. Voigt, unpublished data). This separation could be caused by an arid area south of Morondava that might act as natural barrier. So even before human influence on Madagascar (ca 0 AD, Burney 1997) when there were still large tracts of coastal forest left, the northern and southern regions might not have been connected, resulting in reduced gene flow and the observed genetic divergence.

In the South African species, I detected an IBD-pattern. This pattern has often been observed when long distances are considered in relation to the natural distribution area of a species (Bekessy *et al*. 2002, Bouvet *et al*. 2004).

4.4.3 Medium and small spatial scale

The hierarchical $F_{SM/SS}$-analyses at the medium and small scale detected that in the Malagasy species most genetic differentiation was recorded between groups or subgroups within sample sites. This is an indication of low gene flow at the local scale (Dutech *et al.* 2002). The same pattern was also found in the spatial autocorrelation analyses. Already at a distance of 3 km, individuals were hardly related to each other (Figure 3). The negative slope of the autocorrelogram indicates that there was an IBD-pattern in the Malagasy species, but on a smaller scale (up to 3 km) than considered for the IBD-analyses, for which sample sites were separated by at least 8 km. Both results confirm the field observations which recorded very limited seed dispersal (Böhning-Gaese *et al.* 1999). Thus, there seems to be restricted gene flow between groups or subgroups within sample sites and only low pollen transport and seed dispersal over medium distances of 300 m - 3 km resulting in highly structured groups and subgroups on a small spatial scale (Ng *et al*. 2004). This pattern fits to the strongly clumped spatial distribution of the species. Bleher and Böhning-Gaese (2001) state a medium distance between conspecific trees within groups of only 11.9 m. These groups of trees could reflect related individuals on a small spatial scale in accordance to genetically structured plant populations. Furthermore, the kinship coefficient already decreased strongly between the distance classes of 30 m to 300 m. This may be explained by restricted pollen flow at short distances within the subgroups (van Rossum *et al.* 2004) and is concurrent with the field data on pollination (Farwig *et al.* 2004).

At the two smaller spatial scales, the $F_{SM/SS}$-values increased in the Malagasy species, indicating stronger structured groups and subgroups at a smaller spatial scale. A similar highly structured pattern at the local scale with a modestly structured pattern at the regional scale was found in a study on the herb *Silene alba* using cpDNA (McCauley *et al.* 2003). The authors suggested that forces shaping the local population structure may vary from one sample site to another. Since southern populations of the Malagasy species are at

the end of their distribution area, northern and southern populations may experience different selection pressures.

In the South African species, the hierarchical analyses at the two smaller spatial scales revealed that only a small percentage of the variance was found between groups and subgroups within sample sites. Such a low genetic variance within sample sites can be caused by high gene flow at the local scale, in this case at the scale of 300 m - 3 km. The spatial autocorrelation analyses confirmed this pattern. Even though relatedness was decreasing monotonously with distance, individuals were still related to each other up to a distance of 30 km (Figure 3). The decrease of the kinship-coefficient with geographic distance fits to the IBD-pattern on the scale of the sample sites. The IBD-pattern, combined with a spatially uniform distribution of the adult trees (Bleher & Böhning-Gaese 2001) reinforce that there is a regular migration between the sample sites and thus, gene exchange within and between the plant populations. This exchange may be caused by the high number of frugivorous birds that disperse the seeds of the South African *Commiphora* species, including long-distance seed dispersers such as hornbills. This nearly random genetic pattern at the medium and small spatial scale was also found in other forest tree species that are wind- or insect-pollinated and have efficient seed dispersal through birds (Hamrick *et al*. 1993, Gibson & Wheelwright 1995, Chung *et al*. 2000, Degen *et al*. 2001).

In *C. harveyi*, most of genetic differentiation revealed by the hierachical $F_{SM/SS}$-statistic was between the sample sites. Since fruits were dispersed by large-bodied seed dispersers such as hornbills, I could expect high gene flow also at larger distances than 30 km and a lower differentiation between the sample sites. However, compared to other studies on gene flow over long distances, gene flow above 3 km is already a very long distance (see Loiselle *et al.* 1995b, Heuertz *et al*. 2003, Dutech *et al*. 2002). Additionally, even with field data on long-distance seed dispersal, it is not possible to predict seedling establishment in the new population, which is crucial for successful genetic interchange of populations (Shapcott 1998). Furthermore, the fragmented habitat distribution and the large distances between the sample sites could contribute to the high differentiation between the sample sites (see above).

To conclude, the low seed dispersal rate in the Malagasy species was connected with a high local genetic differentiation and the high seed dispersal rate in the South African species was connected with a low local differentiation. Thus, seed dispersal seems to have a strong influence on the genetic population structure on a spatial scale of

300 m - 3 km, and even up to 30 km. This study showed that broad assumptions on the breeding system are not sufficient to interpret the genetic structure of plant populations. Instead detailed field data are necessary. Combining detailed field data on a small spatial scale with genetic data from a wide geographical range has the benefit that field data yield the required data base to interpret the genetic data and the genetic data make it possible to evaluate whether field data are representative for the studied system. Therefore, the complement of the two data sets can help to reveal the dynamic nature of plant-animal interactions.

4.5 Summary

The genetic structure of plant populations is influenced strongly by pollination and seed dispersal, the two vectors for gene flow. Previous studies on a Malagasy and a South African *Commiphora* species revealed that both tree species have a similar pollination ecology, but the Malagasy *C. guillauminii* has a much lower seed dispersal rate than the South African *C. harveyi*. I hypothesized that the lower seed dispersal rate may cause decreased gene flow, resulting in a stronger genetic structuring among the Malagasy than the South African populations. I used AFLP markers to investigate the population genetics of 136 Malagasy and 158 South African *Commiphora* trees. Unexpectedly, the overall genetic differentiation was lower in the Malagasy ($F_{ST} = 0.05$) than in the South African species ($F_{ST} = 0.16$). Nevertheless, the hierarchical F-statistics revealed that most of the inter-population variance in the Malagasy species was between populations within sample sites (72.7 - 85.5 %) whereas in the South African species only a low amount of the genetic differentiation between populations within sample sites (8.4 - 14.5 %) was revealed. This pattern could be caused by low gene flow in Madagascar and high gene flow in South Africa at the scale of populations within sample sites. Spatial autocorrelation analyses suggest that gene flow is restricted mostly to 3 km in the Malagasy species and to 30 km in the South African species as predicted from field data on seed dispersal. Thus, seed dispersal seems to be a key factor for the genetic population structure of trees on the local scale.

5 General conclusion

Pollination and seed dispersal are important ecosystem processes for the regeneration of plant populations. Both processes are also responsible for gene flow in and between plant populations. Limited gene flow can result in genetic drift and mutation and may lead to differentiation of plant populations. Pollination and seed dispersal can occur through different vectors such as wind, gravity or animals. Depending on the effectiveness of the vectors, both processes differ in their spatial impact on the genetic structure of plant populations. Thus, their influence on the genetic structure of plant populations can only be assessed based on good field data on pollination and seed dispersal. In this thesis, I studied the pollination ecology of a South African *Commiphora* species and based on differences in seed dispersal, compared its genetic structure with the genetic structure of a Malagasy *Commiphora* species.

In a first study, I investigated the pollination ecology of the South African *Commiphora harveyi* (Burseraceae), a typical insect-pollinated dioecious tree in a mainland context. Results were compared with those of the related *C. guillauiminii* in an island context with a sparse pollinator fauna in Madagascar. I addressed the question whether differences in the pollinator fauna would influence the pollination ecology and the fruit set of the two species. The pollination ecology of the South African species was assessed by recording floral display, visiting insect species, visitation rate, natural fruit set and by hand-pollination experiments. The results showed that male trees had more and larger flowers per inflorescences than female trees and both sexes produced little nectar. Irrespective of the diverse pollinator fauna, only 28 insect species were recorded visiting the flowers, with a low mean visitation rate (0.20 visits per flower h^{-1}) in both sexes. The natural fruit set was low (3.8 %) and increased significantly with hand-pollination (45.5 %), an indication of pollen limitation. The comparison of the two species revealed a similar pattern in the Malagasy species. *Commiphora guillauminii* had also low visitation rates, low insect diversity and low fruit sets. Thus, irrespective of the different pollinator diversity, the pollination systems of the two entomophilous *Commiphora* species were very similar and especially the fruit set in both species was low compared to other dioecious tropical tree species.

In a second approach, I studied the genetic structure of several *C. harveyi* and C. *guillauminii* populations, relating to the similar pollination ecology and differences in seed

dispersal. Considering, that the Malagasy *C. guillauminii* had a much lower seed dispersal rate than the South African *C. harveyi*, I assumed that the lower seed dispersal rate may lead to less gene flow, resulting in a stronger genetic differentiation among the Malagasy than the South African populations. I used AFLP markers to investigate the population genetics of 136 Malagasy and 158 South African *Commiphora* trees from several sample sites. Against my hypothesis, the overall genetic differentiation was lower in the Malagasy (F_{ST} = 0.05) than in the South African species (F_{ST} = 0.16).

The overall differentiation does not allow to distinguish whether this inter-population variance originated by differentiation between populations within sample sites or differentiation among the several sample sites. Therefore, I performed hierarchical F-statistics. This approach revealed that most of the inter-population variance in the Malagasy species was among populations within sample sites (72.7 - 85.5 %), whereas in the South African species only a low amount of genetic differentiation (8.4 - 14.5 %) was revealed between populations within sample sites. This pattern could derive from limited gene flow in Madagascar and high gene flow in South Africa within sample sites. The assumed gene flow pattern in the two species was confirmed by spatial autocorrelation analyses. The shape of the autocorrelogram suggested that gene flow was restricted mostly to 3 km in the Malagasy species and to 30 km in the South African species. These results reflect the genetic structure of the tree populations as predicted from the field data on seed dispersal. Thus, seed dispersal seems to be a key factor for the genetic population structure of tree species on the local scale.

This thesis has the methodical advantage of combining detailed field data on a small spatial scale with genetic data from a wide geographical range. This approach overcomes the restrictions of field data which are mostly reflecting the present situation and represent usually, only one or a few study areas. It differs from many genetic studies, which cover wide geographical areas, but rely in their interpretation which influence seed dispersal or pollination have on the genetic population structure on common assumptions. This thesis shows on the one hand exemplarily that common assumptions on the breeding system are not sufficient to interpret genetic data. Based on common assumptions both species have insect-pollinated flowers and bird-dispersed fruit and thus, should have a similar genetic differentiation. On the other hand these two species are good examples for the contradictorily data in literature which influence seed dispersal might have on the genetic structure of plant populations. In the tree species *Camellia japonica*, Ueno *et al.*

(2000) explained clumped pattern and weak genetic structure with limited seed dispersal and extensive gene flow through pollination, what is understandable since seeds are dispersed by gravity. However, even when bird dispersal is considered, the influence seed dispersal might have on the genetic structure, can be different. Some authors, such as Gibson and Wheelwright (1995) explained high genetic diversity in their tree species studied with effective seed dispersal by birds (as in *C. harveyi*). Whereas other studies (e.g. Loiselle *et al.* 1995a, b) explained genetic structure mainly with pollination, neglecting influence of seed dispersal by birds, since most seeds drop under the parent tree (as in *C. guillauminii*). Even in models on the influence of breeding system on the spatial genetic structure in plants, seed dispersal is considered only over short distances (Hamilton & Miller 2002, Heueretz *et al*. 2003). However, it is not possible to generalize based on the dispersal vector, to which spatial scale seed dispersal can influence the genetic structure in plant populations. Only detailed field data can give an adequate base for it. Few genetic studies combined detailed field data with genetic data, e.g. paternity analyses. Dick (2001) combined field data on the pollination of *Dinizia excelsa* with microsatellite data and showed that pollination occurred over a distance up to 3.2 km. In comparison to other pollination studies, this is one of the longest distances observed. However, they cover again only small geographical scales and it is difficult to generalize.

To conclude, the combined approach of field data with genetic data in this thesis has on the one hand the advantage that the detailed field data present the required data base to interpret the genetic data. On the other hand, the genetic data on a broad geographical scale give the possibility to evaluate whether field data are representative for the studied system. Thus, the two data sets can complement each other and can broaden our understanding of plant-animal interactions.

6 Literature cited

Ackerman J, Montalvo A (1990) Short- and long-term limitations to fruit production in a tropical orchid. **Ecology**, 71, 263-271.

Acocks J (1988) Veld types of South Africa. **Memoirs of the Botanical Survey South Africa**, 57, 1-146.

Ågren J, Elmqvist T, Tunlid A (1986) Pollination by deceit, floral sex ratios and seed set in dioecious *Rubus chamaemorus* L. **Oecologia**, 70, 332-338.

Aldrich PR, Hamrick JL (1998) Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. **Science**, 281, 103-105.

Anderson GJ, Symon DE (1989) Functional dioecy and andromonoecy in *Solanum*. **Evolution**, 43, 204-219.

Anderson GJ, Bernardello G, Lopez P, Stuessy TF, Crawford DJ (2000) Dioecy and wind pollination in *Pernettya rigida* (Ericaceae) of the Juan Fernandez Islands. **Botanical Journal of the Linnean Society**, 132, 121-141.

Ashman TL, Stanton M (1991) Seasonal variation in pollination dynamics of sexually dimorphic *Sidalcea oregana* ssp. *spicata* (Malvaceae). **Ecology,** 72, 993-1003.

Arroyo MTK, Armesto JJ, Primack R (1985) Community studies in pollination ecology in the high temperate Andes of central Chile. II. Effect of temperature on visitation rates and pollination possibilities. **Plant Systematics and Evolution**, 149, 187-203.

Barrett SCH (2002) Sexual interference of the floral kind. **Heredity**, **88**, 154-159.

Bawa KS (1974) Breedings systems of tree species of a lowland tropical community. **Evolution**, 28, 85-92.

Bawa KS (1980a) Evolution of dioecy in flowering plants. **Annual Review of Ecology and Systematics**, 11, 15-39.

Bawa KS (1980b) Mimicry of male by female flowers and intrasexual competition for pollinators in *Jacaratia dolichaula* (D. Smith) Woodson (Caricaceae). **Evolution**, 34, 467-474.

Bawa KS (1990) Plant-pollinator interactions in tropical rain forests. **Annual Review Ecological Systematics**, 21, 399-422.

Bawa KS (1994) Pollinators of Tropical Dioecious Angiosperms - a Reassessment - No, Not Yet. **American Journal of Botany**, 81, 456-460.

Bawa KS, Opler PA (1975) Dioecism in tropical forest trees. **Evolution**, 29, 167-179.

Bekessy SA, Allnutt TR, Premoli AC, Lara A, Ennos RA, Burgman MA, Cortes M, Newton AC (2002) Genetic variation in the vulnerable and endemic Monkey Puzzle tree, detected using RAPDs. **Heredity**, 88, 243-249.

Bierzychudek P (1987) Pollinators increase the cost of sex by avoiding female flowers. **Ecology**, 68, 444-447.

Bleher B, Böhning-Gaese K (2000) Seed dispersal by birds in a South African and a Malagasy *Commiphora* species. **Ecotropica**, 6, 378-392.

Bleher B, Böhning-Gaese K (2001) Consequences of frugivore diversity for seed dispersal, seedling establishment and the spatial pattern of seedlings and trees. **Oecologia**, 129, 385-394.

Böhning-Gaese K, Gaese BH, Rabemanantsoa SB (1995) Seed dispersal by frugivorous tree visitors in the Malagasy tree species *Commiphora guillaumini*. **Ecotropica**, 1, 41-50.

Böhning-Gaese K, Gaese BH, Rabemanantsoa SB (1999) Importance of primary and secondary seed dispersal in the Malagasy tree *Commiphora guillaumini*. **Ecology**, 80, 821-832.

Bohonak AJ (1999) Dispersal, gene flow, and population structure. **Quarterly Review of Biology**, 74, 21-45.

Boucher DH, James S, Keeler KH (1982) The ecology of mutualism. **Annual Review of Ecology and Systematics**, 13, 315-347.

Bouvet JM, Fontaine C, Sanou H, Cardi C (2004) An analysis of the pattern of genetic variation in *Vitellaria paradoxa* using RAPD markers. **Agroforestry Systems**, 60, 61-69.

Bronstein JL (1994) Our current understanding of mutualism. **Quarterly Review of Biology**, 69, 31-51.

Buchmann SL, Nabham GP (1996) **The forgotten pollinators**. Island Press, Washington DC.

Burd M (1994) Bateman principle and plant reproduction - the role of pollen limitation in fruit and seed set. **Botanical Review**, 60, 83-139.

Burney DA (1997) Theories and facts regarding Holocene environmental change before and after human colonizaion. In: Goodman SM, Patterson BD (eds) **Natural change and human impact in Madagscar**, pp. 75-89. Smithsonian Institution Press, Washington DC.

Cain ML, Milligan BG, Strand AE (2000) Long-distance seed dispersal in plant populations. **American Journal of Botany**, 87, 1217-1227.

Calvo RN (1993) Evolutionary demography of orchids: intensity and frequency of pollination and the cost of fruiting. **Ecology**, 74, 1033-1042.

Cascante A, Quesada M, Lobo JJ, Fuchs EA (2002) Effects of dry tropical forest fragmentation on the reproductive success and genetic structure of the tree *Samanea saman*. **Conservation Biology**, 16, 137-147.

Charlesworth D (1984) Androdioecy and the evolution of dioecy. **Biological Journal of the Linnean Society**, 23, 333-348.

Charlesworth D (1993) Why are unisexual flowers associated with wind pollination and unspecialized pollinators? **American Naturalist**, 141, 481-490.

Chung MG, Chung MY, Oh GS, Epperson BK (2000) Spatial genetic structure in a *Neolitsea sericea* population (Lauraceae). **Heredity**, 85, 490-497.

Cipollini ML, Whigham DF (1994) Sexual dimorphism and cost of reproduction in the dioecious Shrub *Lindera Benzoin* (Lauraceae). **American Journal of Botany**, 81, 65-75.

Cooper KH (1985) **The conservation status of indigenous forests in the Transvaal, Nataland O.F.S., South Africa**. Wildlife Society of Southern Africa, Durban.

Cunningham SA (1996) Pollen supply limits fruit initiation by a rain forest understorey palm. **Journal of Ecology**, 84, 185-194.

Cunningham SA (2000a) Depressed pollination in habitat fragments causes low fruit set. **Proceedings of the Royal Society of London Series B-Biological Sciences**, 267, 1149-1152.

Cunningham SA (2000b) Effects of habitat fragmentation on the reproductive ecology of four plant species in mallee woodland. **Conservation Biology**, 14, 758-768.

Darwin, C (1862) **On the various contrivances by which British and foreign orchids are fertilised by insects**. London: Murray.

Dawson IK, Powell W (1999) Genetic variation in the Afromontane tree *Prunus africana*, an endangered medicinal species. **Molecular Ecology**, 8, 151-156.

Degen B, Caron H, Bandou E, Maggia L, Chevallier MH, Leveau A, Kremer A (2001) Fine-scale spatial genetic structure of eight tropical tree species as analysed by RAPDs. **Heredity**, 87, 497-507.

de la Bathie HP (1946) **Flore de Madagascar et des Comores**. 106e famille. Burseraceae. Tanarive. Madagascar.

Delph LF, Galloway LF, Stanton ML (1996) Sexual dimorphism in flower size. **American Naturalist**, 148, 299-320.

Dick CW (2001) Genetic rescue of remnant tropical trees by an alien pollinator. **Proceedings of the Royal Society of London Series B-Biological Sciences**, 268, 2391-2396.

Dick CW, Etchelecu G, Austerlitz F (2003) Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. **Molecular Ecology**, 12, 753-764.

Dutech C, Seiter J, Petronelli P, Joly HI, Jarne P (2002) Evidence of low gene flow in a neotropical clustered tree species in two rainforest stands of French Guiana. **Molecular Ecology**, 11, 725-738.

Ehrlen J (1992) Proximate limits to seed production in a herbaceous perennial legume, *Lathyrus vernus*. **Ecology**, 73, 1820-1831.

Ehrlen J, Eriksson O (1995) Pollen limitation and population-growth in a herbaceous perennial legume. **Ecology**, 76, 652-656.

Ervik F, Feil JP (1997) Reproductive biology of the monoecious understory palm *Prestoea schultzeana* in Amazonian Ecuador. **Biotropica**, 29, 309-317.

Farwig N, Randrianirina EF, Voigt FA, Kraemer M, Böhning-Gaese K (2004) Pollination ecology of the dioecious tree *Commiphora guillauminii* in Madagascar. **Journal of Tropical Ecology**, 20, 307-316.

Fleming TH, Heithaus ER (1981) Frugivorous bats, seed shadows, and the structure of tropical forests. **Biotropica (supplement)**, 13, 45-53.

Fox JF (1992) Pollen limitation of reproductive effort in willows. **Oecologia**, 90, 283-287.

Fox JF, Stevens GC (1991) Costs of reproduction in a willow: experimental responses vs. natural variation. **Ecology**, 72, 1013-1023.

Frankham R (1997) Do island populations have less genetic variation than mainland populations? **Heredity**, 78, 311-327.

Gaudeul M, Taberlet P, Till-Bottraud I (2000) Genetic diversity in an endangered alpine plant, *Eryngium alpinum* L.. (Apiaceae), inferred from amplified fragment length polymorphism markers. **Molecular Ecology**, 9, 1625-1637.

Gaudeul M, Till-Bottraud I (2003) Low selfing in a mass-flowering, endangered perennial, *Eryngium alpinum* L. (Apiaceae). **American Journal of Botany**, 90, 716-723.

Ghazoul J (1997) The pollination and breeding system of *Dipterocarpus obtusifolius* (Dipterocarpaceae) in dry deciduous forests of Thailand. **Journal of Natural History**, 31, 901-916.

Gibson JP, Wheelwright NT (1995) Genetic-structure in a population of a tropical tree *Ocotea tenera* (Lauraceae) - influence of avian seed dispersal. **Oecologia**, 103, 49-54.

Glen HF (1996) A description of the vegetation of Oribi Gorge Nature Reserve, Natal, part I. **Trees in South Africa**, 46, 18-27.

Godoy JA, Jordano P (2001) Seed dispersal by animals: exact identification of source trees with endocarp DNA microsatellites. **Molecular Ecology**, 10, 2275-2283.

Gomes V, Collevatti RG, Silveira FAO, Fernandes GW (2004) The distribution of genetic variability in *Baccharis concinna* (Asteraceae), an endemic, dioecious and threatened shrub of rupestrian fields of Brazil. **Conservation Genetics**, 5, 157-165.

Hall P, Chase MR, Bawa KS (1994) Low genetic-variation but high population differentiation in a common tropical forest tree species. **Conservation Biology**, 8, 471-482.

Hamilton MB (1999) Tropical tree gene flow and seed dispersal. **Nature**, 401, 129-130.

Hamilton MB, Miller JR (2002) Comparing relative rates of pollen and seed gene flow in the island model using nuclear and organelle measures of population structure. **Genetics**, 162, 1897-1909.

Hamrick JL, Godt MJW (1989) Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) **Plant population genetics: Case histories from nature**, pp. 43-63. Sunderland, MA: Sinauer,

Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plant species. **Philosophical Transactions of the Royal Society of London Series B-Biological Sciences**, 351, 1291-1298.

Hamrick JL, Godt MJW (1997) Effects of life history traits on genetic diversity in plant species. In: Silverton J, Franco M, Harper JL (eds) **Plant life histories: Ecology, phylogeny and evolution**, pp. 102-118. Cambridge Royal Society, Cambridge.

Hamrick JL, Murawski DA, Nason JD (1993) Influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. **Vegetatio**, 107/108, 281-297.

Hardy OJ, Vekemans X (2002) SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. **Molecular Ecology Notes**, 2, 618-620.

Hartl D (1980) **Principles of Population Genetics**. In: Sinauer Associates, pp 488.

He TH, Krauss SL, Lamont BB, Miller BP, Enright NJ (2004) Long-distance seed dispersal in a metapopulation of *Banksia hookeriana* inferred from a population allocation analysis of amplified fragment length polymorphism data. **Molecular Ecology**, 13, 1099-1109

Heinrich B (1974) Thermoregulation in endothermic insects. **Science**, 178, 747-756.

Heinrich B, Raven PH (1972) Energetics and pollination ecology. **Science** 176, 597-602.

Herlihy CR, Eckert CG (2004) Experimental dissection of inbreeding and its adaptive significance in a flowering plant, *Aquilegia canadensis* (Ranunculaceae). **Evolution**, 58, 2693-2703.

Herrera CM (1998) Long-term dynamics of Mediterranean frugivorous birds and fleshy fruits: A 12-year study. **Ecological Monographs**, 68, 511-538.

Heuertz M, Vekemans X, Hausman JF, Palada M, Hardy OJ (2003) Estimating seed vs. pollen dispersal from spatial genetic structure in the common ash. **Molecular Ecology**, 12, 2483-2495.

Holbrook KM, Smith TB (2000) Seed dispersal and movement patterns in two species of *Ceratogymna* hornbills in a West African tropical lowland forest. **Oecologia**, 125, 249-257.

Holbrook KM, Smith TB, Hardesty BD (2002) Implications of long-distance movements of frugivorous rain forest hornbills. **Ecography**, 25, 745-749.

Howe HF, Smallwood J (1982) Ecology of seed dispersal. **Annual Review of Ecology and Systematics**, 13, 201-228.

Howe HF (1990) Seed dispersal by birds and mammals. Implications for seedling demography. In: Bawa KS, Hadley M (eds) **Reproductive ecology of tropical forest plants***,* pp. 191-218. Man and Biosphere Series, Volume 7. UNESCO and Parthenon Publishing Group, Paris.

Humeau L, Thompson JD (2001) The allometry of flower size dimorphism in dioecious *Dombeya* species on La Reunion. **Ecology Letters**, 4, 221-228.

Jenkins MD (1987) **Madagascar: an environment profile**. IUCN/UNEP/WWF, Gland.

JMP (1995) **Statistics and graphics guide**. Version 3.1 SAS Institute Inc., Cary, North Carolina.

Johnson SD, Neal PR, Peter CI, Edwards TJ (2004) Fruiting failure and limited recruitment in remnant populations of the hawkmoth-pollinated tree *Oxyanthus pyriformis* subsp *pyriformis* (Rubiaceae). **Biological Conservation**, 120, 31-39.

Johnston MO (1991) Pollen limitation of female reproduction in *Lobelia cardinalis* and *L. siphilitica*. **Ecology**, 72, 1500-1503.

Jordano P, Godoy JA (2000) RAPD variation and population genetic structure in *Prunus mahaleb* (Rosaceae), an animal-dispersed tree. **Molecular Ecology**, 9, 1293-1305.

Karrenberg S, Kollmann J, Edwards PJ (2002) Pollen vectors and inflorescence morphology in four species of *Salix*. **Plant Systematics and Evolution**, 235, 181-188.

Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: The conservation of plant-pollinator interactions. **Annual Review of Ecology and Systematics**, 29, 83-112.

Le Corff J, Agren J, Schemske DW (1998) Floral display, pollinator discrimination, and female reproductive success in two monoecious *Begonia* species. **Ecology**, 79, 1610-1619.

Liu AZ, Kress WJ, Wang H, Li DZ (2002) Insect pollination of *Musella* (Musaceae), a monotypic genus endemic to Yunnan, China. **Plant Systematics and Evolution**, 235, 135-146.

Lloyd, DG, Webb CJ (1977) Secondary sex characters in plants. **Botanical Review**, 43, 177-216.

Loiselle BA, Sork VL, Graham C (1995a) Comparison of genetic variation in bird-dispersed shrubs of a tropical wet forest. **Biotropica**, 27, 487-494.

Loiselle BA, Sork VL, Nason J, Graham C (1995b) Spatial genetic-structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). **American Journal of Botany**, 82, 1420-1425.

Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. **Annual Review of Ecology and Systematics**, 15, 65-95.

Lynch M, Milligan BG (1994) Analysis of population genetic-structure with Rapd markers. **Molecular Ecology**, 3, 91-99.

MacDevette DR, MacDevette DK, Gordon IG, Barhoolomew, RLC (1989) Floristic of the Natal indigenous forests. In: Geldenhuys CJ (ed). **Biogeography of the mixed evergreen forests of Southern Africa.** Occasional report No. 45. Foundation for research development, Pretoria, South Africa, (http://easd.org.za/ Publicat/natalfl.htm).

Mayer SS, Charlesworth D (1991) Cryptic dioecy in flowering plants. **Trends in Ecology and Evolution**, 6, 320-325.

McCauley DE, Smith RA, Lisenby JD, Hsieh C (2003) The hierarchical spatial distribution of chloroplast DNA polymorphism across the introduced range of *Silene vulgaris*. **Molecular Ecology**, 12, 3227-3235.

McCall C, Primack RB (1992) Influence of flower characteristics, weather, time of day and season on insect visitation rates in three plant communities. **American Journal of Botany** 79, 434-442.

Miller MP (1997) Tools for populations genetics analyses (TFPGA) 1.3. a Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author; http: //bioweb.usu.edu/ mpmbio/tfpga.htm.

Motten AF (1986) Pollination ecology of the spring wildflower community of a temperate deciduous forest. **Ecological Monographs**, 56, 21-42.

Muluvi GM, Sprent JI, Soranzo N, Provan J, Odee D, Folkard G, McNicol JW, Powell W (1999) Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam. **Molecular Ecology**, 8, 463-470.

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

Ng KKS, Lee SL, Koh CL (2004) Spatial structure and genetic diversity of two tropical tree species with contrasting breeding systems and different ploidy levels. **Molecular Ecology**, 13, 657-669.

Nilsson LA (1992) Long pollinia on eyes: hawk-moth pollination of *Cynorkis uniflora* Lindley (Orchidaceae) in Madagascar. **Botanical Journal of the Linnean Society**, 109, 145-160.

Osunkoya OO (1999) Population structure and breeding biology in relation to conservation in the dioecious *Gardenia actinocarpa* (Rubiaceae) - a rare shrub of North Queensland rainforest. **Biological Conservation**, 88, 347-359.

Otero-Arnaiz A, Oyama K (2001) Reproductive phenology, seed-set and pollination in *Chamaedorea alternans*, an understorey dioecious palm in a rain forest in Mexico. **Journal of Tropical Ecology**, 17, 745-754.

Ouborg NJ, Piquot Y, Van Groenendael JM (1999) Population genetics, molecular markers and the study of dispersal in plants. **Journal of Ecology**, 87, 551-568.

Palgrave KC (1977) **Trees of South Africa**. C. Struik Publishers. Cape Town, Johannesburg.

Pooley E (1994) **The complete field guide to trees of Natal Zululand & Transkei**. Natal Flora Publications Trust, Durban.

Quesada M, Fuchs EJ, Lobo JA (2001) Pollen load size, reproductive success, and progeny kinship of naturally pollinated flowers of the tropical dry forest tree *Pachira quinata* (Bombacaceae). **American Journal of Botany**, 88, 2113-2118.

Ratsirarson J, Silander JA (1996) Reproductive biology of the threatened Madagascar triangle palm: *Neodypsis decaryi* Jumelle. **Biotropica**, 28, 737-745.

Renner SS, Ricklefs RE (1995) Dioecy and its correlates in the flowering plants. **American Journal of Botany**, 82, 596-606.

Rice WR (1989) Analyzing tables of statistical tests. **Evolution,** 43, 223-225.

Rivera-Ocasio E, Aide TM, McMillan WO (2002) Patterns of genetic diversity and biogeographical history of the tropical wetland tree, *Pterocarpus officinalis* (Jacq.), in the Caribbean basin. **Molecular Ecology**, 11, 675-683.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F- statistics under isolation by distance. **Genetics**, 145, 1219-1228.

Rousset F (2002) Inbreeding and relatedness coefficients: what do they measure? **Heredity***,* 88, 371-380.

Schemske DW (1980) Evolution of floral display in the orchid *Brassavola nodosa*. **Evolution**, 34, 489-493.

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN Version 2.000: **A software for population genetics data analysis**. Genetics and Biometry Laboratory, University of Geneva, Geneva.

Shapcott A (1998). The patterns of genetic diversity in Carpentaria acuminata (Arecaceae), and rainforest history in northern Australia. **Molecular Ecology,** 7, 833-847.

Shapcott A (1999) Comparison of the population genetics and densities of five Pinanga palm species at Kuala Belalong, Brunei. **Molecular Ecology**, 8, 1641-1654.

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. **Evolution**, 47, 264-279.

Soehartono T, Newton AC (2001) Reproductive ecology of *Aquilaria* spp. in Indonesia. **Forest Ecology and Management**, 152, 59-71.

Sorg JP, Rohner, U (1996) Climate and tree phenology of the dry deciduous forest of the Kirindi Forest. In: Ganzhorn JU, Sorg JP (eds) **Ecology and economy of a tropical dry forest in Madagascar**, pp. 57-80. Primate Report special issue 46-1, Göttingen.

Sork VL, Nason J, Campbell DR, Fernandez JF (1999) Landscape approaches to historical and contemporary gene flow in plants. **Trends in Ecology and Evolution**, 14, 219-224.

Stephenson AG (1981) Flower and fruit abortion: proximate causes and ultimate functions. **Annual Review of Ecology and Systematics**, 12, 253-279.

Sutherland S, Delph LF (1984) On the importance of male fitness in plants: patterns of fruit set. **Ecology**, 65, 1093-1104.

Thomson JD, Maddison WP, Plowright RC (1982) Behaviour of bumblebee pollinators of *Aralia hispida* Vent. (Araliaceae). **Oecologia**, 54, 326-336.

Trapnell DW, Hamrick JL (2004) Partitioning nuclear and chloroplast variation at multiple spatial scales in the neotropical epiphytic orchid, *Laelia rubescens*. **Molecular Ecology**, 13, 2655-2666.

Ueno S, Tomaru N, Yoshimaru H *et al.* (2000) Genetic structure of Camellia japonica L. in an old-growth evergreen forest, Tsushima, Japan. **Molecular Ecology**, 9, 647-656.

van der Walt JJA (1973) The South African species of *Commiphora*. **Bothalia**, 11**,** 53-102.

Vamosi JC, Otto SP (2002) When looks can kill: the evolution of sexually dimorphic floral display and the extinction of dioecious plants. **Proceedings of the Royal Society of London Series B-Biological Sciences**, 269, 1187-1194.

van Rossum F, Bonnin I, Fenart S, Pauwels M, Petit D, Saumitou-Laprade P (2004) Spatial genetic structure within a metallicolous population of *Arabidopsis halleri*, a clonal, self-incompatible and heavy- metal-tolerant species. **Molecular Ecology**, 13, 2959-2967.

van Wyk B, van Wyk P (1997) **Field guide to trees of southern Africa**. Struik Publishers Ltd. Cape Town.

Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. **Molecular Ecology**, 13, 921-935.

Voigt FA, Jung S, Farwig N, Böhning-Gaese K (2005) Low fruit set in a dioecious tree: pollination ecology of *Commiphora harveyi* in South Africa. **Journal of Tropical Ecology**, 21, 1-10.

Vos P, Hogers R, Bleeker M, Reijans M, vn de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau,M. (1995) AFLP: a new technique for DNA fingerprinting. **Nucleic Acids Research**, 23, 4407-4414.

Wang BC, Smith TB (2002) Closing the seed dispersal loop. **Trends in Ecology and Evolution**, 17, 379-385.

Wang, X. R., V. E. Chhatre, Song W, Zackrisson O, Szmidt AE (2003) Island population structure of Norway spruce (Picea abies) in northern Sweden. **International Journal of Plant Sciences**, 164, 711-717.

Weir BS, Cockerham CC (1984) Estimation of F-statistics for the analysis of population structure. **Evolution**, 38, 1358-1370.

Wenny DG (2001) Advantages of seed dispersal: A re-evaluation of directed dispersal. **Evolutionary Ecology Research**, 3, 51-74.

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: F-ST not equal 1/(4Nm+1). **Heredity**, 82, 117-125.

Wilcock C, R. Neiland (2002). Pollination failure in plants: why it happens and when it matters. **Trends in Plant Science,** 7, 270-277.

Williams G, Adam P (2001) The insect assemblage visiting the flowers of the subtropical rainforest pioneer tree *Alphitonia excelsa* (Fenzl) Reiss. ex Benth. (Rhamnaceae). **Proceedings of the Linnean Society of New South Wales**, 123, 235-259.

Wolf AT, Howe RW, Hamrick JL (2000) Genetic diversity and population structure of the serpentine endemic *Calystegia collina* (Convolvulaceae) in northern California. **American Journal of Botany**, 87, 1138-1146.

Wright S (1978) **Evolution and the genetics of populations**, Vol. 4 Variability within and among natural populations. University of Chicago Press, Chicago.