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ORIGINAL ARTICLE

Linking seed dispersal and genetic structure of trees: a biogeographical approach

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ABSTRACT

Aim Natural and human-induced differences in frugivore assemblages can influence the seed dispersal distances of trees. An important issue in seed dispersal systems is to understand whether differences in seed dispersal distances also affect the genetic structure of mature trees. One possible approach to test for a relationship between seed dispersal and the genetic structure of mature trees is to compare the genetic structure of two closely related tree species between two biogeographical regions that differ in frugivore assemblages and seed dispersal distances. Previous studies on two *Commiphora* species revealed that *Commiphora guillauminii* in Madagascar has a much lower seed dispersal distance than *Commiphora harveyi* in South Africa. We tested whether the lower seed dispersal distance might have caused decreased gene flow, resulting in a stronger genetic structure in Madagascar than in South Africa.

Location Madagascar and South Africa.

Methods Using amplified fragment length polymorphism markers we investigated the genetic structure of 134 trees in Madagascar and 158 trees in South Africa at a local and a regional spatial scale.

Results In concordance with our hypothesis, kinship analysis suggests that gene flow was restricted mostly to 3 km in Madagascar and to 30 km in South Africa. At the local spatial scale, the genetic differentiation among groups of trees within sample sites was marginally significantly higher in Madagascar (F_{ST} = 0.069) than in South Africa (F_{ST} = 0.021). However, at a regional spatial scale genetic differentiation was lower in Madagascar (F_{ST} = 0.053) than in South Africa (F_{ST} = 0.163).

Main conclusions Our results show that lower seed dispersal distances of trees were linked to higher genetic differentiation of trees only at a local spatial scale. This suggests that seed dispersal affects the genetic population structure of trees at a local, but not at a regional, spatial scale.

Keywords

AFLP, *Commiphora*, frugivore assemblage, gene flow, Madagascar, pollination, South Africa, spatial structure.

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INTRODUCTION

An important issue in seed dispersal systems is to understand the impact of seed dispersal on the genetic structure of trees (Hardy *et al.*, 2006; Garcia *et al.*, 2007). Seed dispersal is expected to influence the genetic structure of trees at various spatial scales. At the local spatial scale (i.e. within a population in one forest), long seed dispersal distances are expected to lead to high gene flow and little genetic structure within a given population. Similarly, at the regional spatial scale (i.e. among populations located in different forests) long seed dispersal distances should lead to high gene flow and little genetic structure among populations (Nathan, 2005; Hardy *et al.*, 2006; Garcia *et al.*, 2007). Most tropical trees rely on animal

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vectors as their seed dispersers so that seeds escape mortality under parent trees and reach favourable sites (Howe & Smallwood, 1982; Wenny, 2001). Variations in frugivore communities, natural and human-induced (by, for example, different evolutionary histories or hunting pressure), are known to influence the seed dispersal distances of trees (Bleher & Böhning-Gaese, 2001; Cordeiro & Howe, 2003; Wang *et al.*, 2007). However, few studies have investigated if such variations in frugivore assemblages and seed dispersal distances have consequences for the genetic structure of trees.

When testing the influence of seed dispersal on the genetic structure of trees it is necessary to take into account that genetic structure is affected by a multitude of factors, for example gene flow through pollen, population size, habitat distribution, and the biogeographical and evolutionary history of populations (Hartl, 1980; Sork *et al.*, 1999; Hamrick, 2004). Thus, it remains a challenge to prove clearly that different seed dispersal distances lead to different genetic structures.

One approach to measuring the influence of seed dispersal distances on the genetic structure of trees is to use genetic markers to identify the parents of seeds and seedlings (Ouborg *et al.*, 1999; Sork *et al.*, 1999; Vekemans & Hardy, 2004). This approach has recently been used with a number of wind- and animal-dispersed tree species (e.g. Godoy & Jordano, 2001; Bacles *et al.*, 2006; Hardesty *et al.*, 2006; Garcia *et al.*, 2007) and showed that seed dispersal distances can be surprisingly high. Wang *et al.* (2007) showed that high hunting pressure caused limited seed dispersal and had consequences for the genetic structure of juveniles. However, a limitation of this approach is that it is logistically difficult to monitor the long-term consequences of seed dispersal distances for the population genetic structure from juveniles to mature trees. Genetic structure established at the seedling stage is likely to persist through the adult stage if mortality with age is random (Pacheco & Simonetti, 2000), whereas it may become less evident if balancing selection favours heterozygote survival (Hamrick *et al.*, 1993; Alvarez-Buylla *et al.*, 1996; Hardesty *et al.*, 2005; Jones & Hubbell, 2006). Thus, it remains a challenge to describe the long-term consequences of variation in seed dispersal distances for the genetic structure of mature trees.

A second approach to linking seed dispersal and the genetic structure of trees is a comparative analysis of many species, testing for the effects of life-history traits such as seed dispersal mode on genetic structure. Earlier comparative studies showed that genetic structure is affected by seed dispersal mode (e.g. Loveless & Hamrick, 1984; Hamrick *et al.*, 1993; Hamrick & Godt, 1996; Nybom & Bartish, 2000). However, these studies did not account for the phylogenetic relatedness of the species (Duminil *et al.*, 2007). The most recent analyses, controlling for phylogenetic effects, showed that seed dispersal mode was only weakly linked to genetic structure (Aguinagalde *et al.*, 2005; Duminil *et al.*, 2007). Duminil *et al.* (2007) conclude that 77–79% of the variation in genetic structure is accounted for by phylogenetic relatedness and that an effect of life-history traits on genetic structure might only be detectable if the particular, especially taxonomic, context of the species is taken into account.

A third approach is a biogeographical approach, comparing the same or at least closely related tree species in two biogeographical regions that differ in frugivore assemblages and seed dispersal distances; this approach thus has no or at least minimal phylogenetic effects. In the present study we took this biogeographical approach for two closely related, congeneric tree species and compared frugivore diversity, seed dispersal rates, seed dispersal distances and the genetic structure of mature trees of two *Commiphora* species (Burseraceae) between continental Africa and Madagascar. Originally we intended to compare the same species between Madagascar and South Africa; however, this is not possible because all Malagasy *Commiphora* species are endemics. In general, 96% of the tree and shrub species in Madagascar are endemics (Schatz, 2000).

The great advantage of a comparison between continental Africa and Madagascar is that these two regions differ markedly in frugivore assemblages, with a high diversity in continental Africa and a depauperate frugivore community in Madagascar (Fleming *et al.*, 1987; Goodman & Ganzhorn, 1997). Thus, *Comiphora guillauminii* H. Perrier in Madagascar is dispersed almost entirely by one frugivorous bird species, resulting in low seed dispersal rates and short seed dispersal distances within the studied forest (Böhning-Gaese *et al.*, 1999), and *Commiphora harveyi* Engl. in South Africa is dispersed by many frugivorous bird species, resulting in high dispersal rates and large seed dispersal distances within the studied forest (Bleher & Böhning-Gaese, 2001). Therefore, in the two study systems, the diversity of dispersers is linked to seed dispersal rates and to seed dispersal distances. Furthermore, the difference in frugivore assemblages between South Africa and Madagascar appears to have persisted over historical if not evolutionary time periods. Madagascar appears never to have had a high diversity of frugivorous birds; there is no evidence of frugivorous birds going extinct, either in historical or in earlier times (Goodman & Ganzhorn, 1997). A comparison between Madagascar and South Africa thus poses the possibility of linking local observations on seed dispersal (for more details see the section on Study Species) with an indirect historical approach to understanding gene flow, by studying the genetic structure of mature trees (Sork *et al.*, 1999).

Because of the profound differences in seed dispersal distances between the two species we use *Commiphora* as a model system to test whether restricted seed dispersal distances result in limited gene flow and higher genetic structure. As our data on seed dispersal distances came from two studies that were conducted at a local spatial scale (i.e. each within a population in one forest) we expected that gene flow and genetic structure would also differ between the tree species at the local spatial scale of analysis. We hypothesized that the shorter seed dispersal distances in Madagascar would result in lower gene flow, causing, in general, higher genetic differentiation among groups of trees within a forest (i.e. at a local

spatial scale) than in South Africa. We also tested for a spatial genetic structure between forests (i.e. at a regional spatial scale) to evaluate whether the expected relationship also held at larger spatial scales. Thus, if the shorter seed dispersal distances in Madagascar also resulted in lower gene flow between populations, we might also expect higher genetic differentiation between different patches of forest. We used amplified fragment length polymorphism (AFLP) (Vos *et al.*, 1995) to assess the spatial genetic structure in Madagascar and South Africa within and between forests. A limiting factor with this approach is that other factors besides seed dispersal, especially pollen transport distances, might differ between Madagascar and South Africa and influence the genetic structure of the trees. In the discussion, we identify such factors and evaluate whether they are likely to affect the results.

METHODS

Study species

Our study species are *Commiphora guillauminii* in Madagascar and *Commiphora harveyi* in South Africa. Both species are deciduous trees that grow up to 20 m in height. The Malagasy species is endemic to the west coast of Madagascar (Fig. 1); the South African species is found in subtropical forests, often in gorges (so-called scarp forests) along the east coast of southern Africa (Fig. 1; Palgrave, 1977). Both species are dioecious, have similar floral presentations, and similar small flowers (calyx 2–4 mm; de la Bathie, 1946; van Wyk & van Wyk, 1997; Voigt *et al.*, 2005). The flowers of both species are pollinated by small unspecialized insect species, have a low visitation rate in both sexes and do not display 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 Madagascar and the syrphid *Asarkina africana* in South Africa; both species were also pollinated by the honeybee *Apis mellifera* (Farwig *et al.*, 2004; Voigt *et al.*, 2005).

Female trees of both species have similar fruit presentations; they produce roundish fruits with outer coverings that split open when mature and expose a black seed partly enveloped by a fleshy red aril. Each fruit contains only one seed. The seed dispersal system of the species in Madagascar is unusually simple; seeds are basically dispersed by one bird species, the Lesser Vasa Parrot (*Coracopsis nigra*) (Böhning-Gaese *et al.*, 1999). *Coracopsis nigra* handles on average 93.2% of the fruits in a tree (Böhning-Gaese *et al.*, 1999). However, *C. nigra* usually nibbles off the arils and drops the seeds directly under the crown. Occasionally, *C. nigra* takes off with a seed still in its beak, thereby dispersing on average 7.9% of the seeds of a tree away from the crown (Böhning-Gaese *et al.*, 1999). In other regions in Madagascar and for other tree species *C. nigra* acts mostly as a pre-dispersal seed predator, destroying the seed and the embryo (Bollen & van Elsacker, 2004). Even though none of the hundreds of seeds that were shown to be handled by the parrot in a study by Böhning-Gaese *et al.* (1999) were destroyed, we cannot exclude the possibility that *C. nigra* might occasionally act as a seed predator. In addition, the seeds of *C. guillauminii* are occasionally dispersed by one diurnal lemur species (2.7% of the dispersed seeds, Böhning-Gaese *et al.*, 1999), but not by any nocturnal species (nocturnal lemurs, fruit bats). Seeds are secondarily dispersed by ants, but seed dispersal distances are small and the seedling establishment success of seeds dispersed by ants is much lower than that of bird-dispersed seeds (Böhning-Gaese *et al.*, 1999).

In contrast, fruits of the South African species attract a high diversity of seed dispersers, and most seeds (70.8%) are carried away from the crown (Bleher & Böhning-Gaese, 2001). In

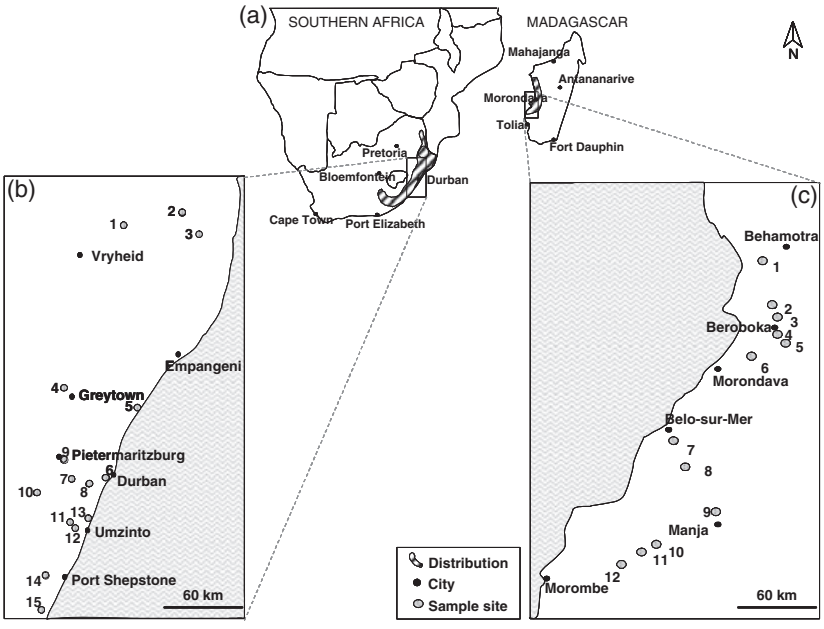

Figure 1 Range and sample sites of *Commiphora guillauminii* H. Perrier populations in Madagascar (range data taken from de la Bathie, 1946, complemented by herbarium records from Antananarivo) and of *Commiphora harveyi* Engl. populations in South Africa (range data taken from van Wyk & van Wyk, 1997). Numbers refer to sample sites (Table 1).

South Africa, 11 out of 15 bird species swallow the seeds, and some of the main dispersers are hornbills, which are known for long-distance dispersal (Holbrook & Smith, 2000).

We do not have measured data on seed dispersal distances. However, we can estimate seed dispersal distances from measured distances between seedlings and trees. In Madagascar, the median distance of a seedling to the *closest mature female* tree was measured in the field as 0.9 m. From the spatial distribution of trees and seedlings, using an individual-based, spatially explicit simulation model, the median distance of a seedling to its *mother* tree was calculated to be the same (i.e. 0.9 m; for more details on the simulation model see Bleher *et al.*, 2002). In South Africa, the median distance of a seedling to the *closest mature female* tree was measured in the field as 21.1 m; using the simulation model (Bleher *et al.*, 2002) we calculated a distance of 64.0 m from a seedling to its *mother* tree (Bleher & Böhning-Gaese, 2001). Thus, according to simulated seedling-mother tree distributions, distances between seedlings and their mother tree were 70 times greater in South Africa than in Madagascar (Bleher & Böhning-Gaese, 2001). This suggests that seed dispersal distances were also much greater in South Africa than in Madagascar. However, the spatial pattern of the seed shadow and the seedlings is also influenced by density- and distance-dependent seed and seedling mortality, which might differ between Madagascar and South Africa. We do not have data on seed mortality, but seedling mortality is much higher closer to adult trees in Madagascar than it is in South Africa (Bleher & Böhning-Gaese, 2001). This suggests that taking seedling-mother tree distances as a measure for seed dispersal distances overestimates seed dispersal distances in Madagascar and makes our approach conservative (see also Jansen *et al.*, 2008).

Finally, in spite of the fact that the overall population densities of mature trees are similar, the two species have a distinctly different spatial distribution on a local scale; the spatial distribution of the Malagasy species is significantly clumped, whereas that of the South African species is uniform (Bleher & Böhning-Gaese, 2001).

Plant materials and sample sites

We collected leaf material from 134 tree individuals of *C. guillauminii* in Madagascar and from 158 individuals of *C. harveyi* in South Africa. Trees were sampled at a local spatial scale (groups of trees within one forest) and at a regional spatial scale (in different forests, Fig. 1). In the following we term one forest a sample site. At the regional scale, trees were sampled in 12 sample sites in Madagascar and in 15 sample sites in South Africa (Fig. 1). The minimum distance between two sample sites was 8 km. Sample sites lay along a north–south gradient (Fig. 1). The greatest distance between the northern and southern sample site was 270 km in Madagascar (i.e. between sample sites Lonahena and Tsakobe) and 435 km in South Africa (i.e. between sample sites Jozini Dam and Umtavuna NR).

At the local spatial scale (i.e. within sample sites), trees were sampled in one to four groups (median = 2), each within a circle with a radius of 1.5 km. In the field, the position of each tree was recorded using a GPS 12 (Garmin Ltd, Olathe, KA, USA). Groups of trees were defined in ArcView GIS 3.2 (ESRI, Inc., Redlands, CA, USA) using the GPS position of each tree, laying circles with a radius of 1.5 km around each tree and defining all trees in the intersection as groups. The radius of 1.5 km was chosen *a posteriori* because it could be used as a consistent measure to subdivide the trees sampled within one sample site into smaller, non-overlapping groups. Within the groups trees were sampled randomly. In total, the sample size was 23 groups in Madagascar and 24 groups in South Africa. We made an effort to sample only mature trees of reproductive size. Leaf material was dried using silica in the field.

DNA extraction and quantification

After grinding of the leaf material in liquid nitrogen, the total genomic DNA was extracted using the DNeasyTM Extraction Kit (Qiagen, Venlo, Netherlands). The standard protocol was slightly modified by using 500 μL of buffer AP1 and 160 μL of buffer AP2, which handled the amount of leaf material better. DNA was stored at −20°C in AE elution buffer (Qiagen). DNA quantification was carried out spectrophotometrically for each AFLP sample with a GeneQuant RNA/DNA calculator (Amersham Pharmacia Biotech, Uppsala, Sweden). DNA concentration was reduced to 30 ng μL^{-1} with sterile distilled water for further reactions.

AFLP analyses

The AFLP procedure followed Vos *et al.* (1995), with the following modifications. In a simultaneous restriction–ligation reaction we used 100 ng instead of 500 ng genomic DNA. To ensure complete digestion, the restriction–ligation step of the analysis was performed for 15 h at 23°C as the initial step of the analysis. A multiplex analysis with fluorescent 'E' primers (6-FAM, NED, HEX, Applied Biosystems, Foster City, CA, USA) was used.

Simultaneous restriction (with *Eco*RI and *Mse*I) and ligation of the genomic DNA was carried out. Double-stranded *Eco*RI and *Mse*I adapters were ligated to the sticky ends of the fragments (Vos *et al.*, 1995). All 292 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 undertaken 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 pipetting and polymerase chain reactions (PCRs) were performed on a lab robot (RoboSeq 4204 SE; MWG-Biotech AG, Ebersberg,

Germany). Selective amplification products were separated on 6% polyacrylamide gels as a multiplex of three differently labelled products together with one internal size standard (GENESCAN ROX 500, Applied Biosystems). Gels were run for *c.* 4 h on an ABI377 automated sequencer using GENESCAN analysis software (version 2.1, ABI). Because samples had to be run on five gels, two to four samples were used 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), and otherwise as absent (0). An additional visual check of the electrophoretograms was made to correct possible misinterpretations of the automated GENOTYPER analysis. For consistency of data handling this visual check was performed by the same person (F. A. Voigt). Some of the peaks were difficult to interpret. These peaks were conservatively scored as missing data.

Data analyses

Data analyses were based on all 134 individuals in Madagascar and all 158 individuals in South Africa. Descriptive statistics (polymorphic loci, Nei, 1978; unbiased heterozygosity) were calculated using TFPGA, version 1.3 (Tools for population genetic analysis; Miller, 1997). Because AFLPs generate dominant markers, and heterozygotes cannot be determined directly, we used Lynch and Milligan's Taylor expansion to estimate allele frequency and levels of heterozygosity indirectly (Lynch & Milligan, 1994; Miller, 1997). This method 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 group and per sample site 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 group or sample site, respectively.

Unfortunately, in our study, sample sizes, especially within groups of trees, were small. To account for this we tested our hypotheses using four types of analyses. Some of the analyses were repeated analyses of different subsets of the sampled trees; others used all trees and partitioned the genetic variation among trees within and among sample sites and groups. If all analyses show the same results, this provides evidence for a consistent pattern.

We first assessed the spatial genetic structure based on pairwise kinship coefficients between individuals, F_{ij}, using the program SPAGEDI (Hardy & Vekemans, 2002). F_{ij} measures the extent of genetic similarity between individuals i and j relative to the mean genetic similarity between random individuals in the sample. 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). We 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: < 0.03, ≤ 0.3, ≤ 3, ≤ 30, ≤ 300 and > 300 km. Although average sample size within groups of trees was small, overall sample sizes across all spatial scales correspond to other studies that successfully used kinship analyses (e.g. Hardy *et al.*, 2006). Our nested spatial sampling design resulted in decent sample sizes in each distance class (> 78 pairs of individuals) except for the largest in Madagascar, and high sample size especially in the distance classes that mattered for our conclusions (≤ 3 km and ≤ 30 km, > 400 pairs of individuals, Fig. 2).

Second, we partitioned genotypic variation of individuals at the local spatial scale, within vs. among groups of trees separately for each sample site, using analyses of molecular variance (AMOVA) as implemented in ARLEQUIN, version 3.1 (Excoffier *et al.*, 2005). Thus, we calculated F_{ST}-values among groups of trees separately for each sample site (Table 1 a,b). We restricted the analyses to sample sites for which data for more than one group of trees were available (for each species seven sample sites). We then calculated the mean F_{ST}-value for each species over the seven sample sites, respectively. The F_{ST}-values and the proportion of F_{ST}-values significantly different from zero were compared between the two species using a *t*-test and chi-squared test for two-way tables, respectively.

Third, we partitioned genotypic variation of individuals at the regional spatial scale, within vs. among sample sites, again using AMOVA.

Finally, we compared the genetic variation between the local (among groups within sample sites) and the regional (among sample sites) spatial scale using hierarchical *F*-statistics (Weir & Cockerham, 1984) as implemented in ARLEQUIN, version 3.1

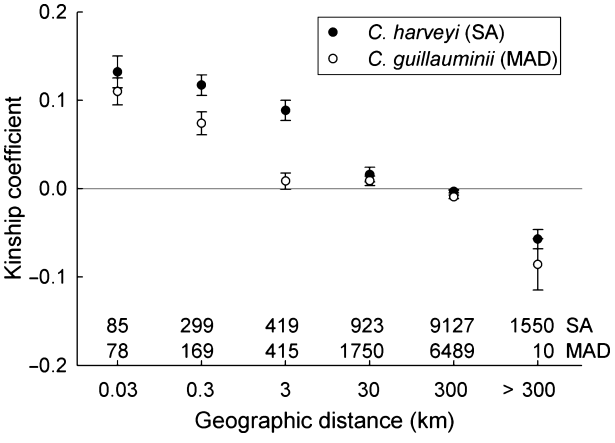

Figure 2 Kinship coefficient among 134 *Commiphora guillauminii* H. Perrier individuals in Madagascar and 158 *C. harveyi* Engl. individuals in South Africa per geographic distance class showing the multilocus jackknife values (mean ± 1 SE). Numbers at the bottom refer to the number of tree-pairs in the respective distance class in South Africa (upper row) and Madagascar (lower row).

Table 1 Groups and sample sites in (a) Madagascar with number of *Commiphora guillauminii* H. Perrier trees studied (*n*), number of fragments and private fragments, Nei's (1978) unbiased heterozygosity and percentage of polymorphic loci (99% criterion), for groups and sample sites, and (b) South Africa with number of *Commiphora harveyi* Engl. trees studied (*n*), number of fragments and private fragments, Nei's (1978) unbiased heterozygosity and percentage of polymorphic loci (99% criterion), for groups and sample sites.

		n		No. fragments		No. private fragments		Heterozygosity		Percentage of polymorphic loci	
Group	Sample site	Group	Site	Group	Site	Group	Site	Group	Site	Group	Site
(a)											
1a	1 Lonahena LA	4	8	51	65	0	0	0.0721	0.0749	18.43	26.27
1b		4		46		0		0.0535		15.21	
2a	2 South of Belo s. Tsiribihina SBO	4	4	61	61	0	0	0.0751	0.0751	22.12	22.12
3a	3 North of Berobuka NBA	2	17	34	77	0	1	0.0209	0.0741	4.15	32.72
3b		15		71		1		0.0747		29.95	
4a	4 Berobuka BA	13	13	80	80	1	1	0.0763	0.0763	33.64	33.64
5a	5 Kirindy KY	2	16	36	96	0	2	0.0348	0.0735	6.91	40.55
5b		3		47		0		0.0596		13.82	
5c		4		48		0		0.0444		12.90	
5d		7		64		2		0.0657		24.42	
6a	6 Andromena AA	10	10	67	67	0	0	0.0625	0.0625	25.81	25.81
7a	7 Kirindy-Mitea KM	4	15	40	57	0		0.0536	0.0600	12.44	23.04
7b		2		25		0		0.0162		3.23	
7c		6		41		0		0.0390		11.98	
7d		3		37		0		0.0361		8.76	
8a	8 Mukabe MKE	10	17	67	73	0	0	0.0637	0.0688	26.27	30.88
8b		7		43		0		0.0593		15.21	
9a	9 Manja MA	5	5	45	45	0	0	0.0607	0.0607	14.29	14.29
10a	10 Migamba MIG	8	15	44	74	0	0	0.0503	0.0610	14.75	29.03
10b		7		64		0		0.0620		22.58	
11a	11 Bevoy/Mangoky MY	4	4	41	41	0	0	0.0466	0.0466	11.98	11.98
12a	12 Antsakoabe TSK	8	10	61	68	2	2	0.0588	0.0605	22.58	25.35
12b		2		42		0		0.0395		7.83	
Total		134		217		6	6				
(b)											
1a	1 Ithala GR IAGR	4	10	51	76	0	0	0.0525	0.0776	15.82	33.90
1b		6		66		0		0.0859		27.68	
2a	2 Jozini Damm JD	7	9	54	64	1	1	0.0667	0.0728	19.77	26.55
2b		2		46		0		0.0670		13.56	
3a	3 Mkuze GR MEGR	6	6	58	58	1	1	0.0705	0.0705	21.47	21.47
4a	4 Montella MA	5	5	54	54	0	0	0.0725	0.0725	19.21	19.21
5a	5 Harold Johnson NR HJNR	14	14	80	80	0	0	0.0814	0.0814	37.29	37.29
6a	6 Burman Bush BBR	10	10	61	61	1	1	0.0619	0.0619	24.29	24.29
7a	7 Shongweni SGI	13	13	73	73	0	0	0.0802	0.0802	32.77	32.77
8a	8 Kranzkloof NR KFNR	8	16	69	80	0	0	0.0877	0.0956	28.81	36.16
8b		8		72		0		0.0880		29.38	
9a	9 Pietermaritzburg PMB	7	7	63	63	0	0	0.0774	0.0774	22.60	22.60
10a	10 Helas-Helas Richmond HHR	8	15	65	68	1	1	0.0979	0.0973	27.68	30.51
10b		7		58		0		0.0774		20.90	
11a	11 Montezuma MZ	6	11	59	72	0	0	0.0798	0.0888	23.16	33.33
11b		5		57		0		0.0749		21.47	
12a	12 Vernon Crookes NR VCNR	6	10	55	62	0	0	0.0711	0.0768	20.90	27.12
12b		2		44		0		0.0531		10.73	
12c		2		37		0		0.0559		11.30	
13a	13 Emizini NR EINR	11	11	64	64	0	0	0.0848	0.0848	27.68	27.68
14a	14 Oribi Gorge NR OGNR	5	14	60	76	0	1	0.0740	0.0912	23.73	36.16

Table 1 Continued

		n		No. fragments		No. private fragments		Heterozygosity		Percentage of polymorphic loci	
Group	Sample site	Group	Site	Group	Site	Group	Site	Group	Site	Group	Site
14b		5		54		0		0.0714		19.77	
14c		4		59		1		0.0818		22.03	
15a	15 Umatvuna NR UANR	7	7	62	62	4	4	0.0687	0.0687	23.16	23.16
Total		158		177		9	9				

Sample sites are ordered from north to south; for numbers refer to Fig. 1.

(Excoffier *et al.*, 2005). This analysis was again conducted at the level of tree groups. However, instead of calculating F_{ST}-values separately for each sample site (see second analysis), the genetic variance of all tree individuals was partitioned within one analysis into three distinct components (i.e. the variance among sample sites, the variance among groups within sample sites, and the variance within groups). Thus, introducing a hierarchical level allows us to distinguish on which spatial level the genetic differentiation occurs (i.e. among groups within sample sites or among sample sites).

RESULTS

Descriptive population genetics

A total of 213 polymorphic loci were scored for the 134 Malagasy individuals, and 177 polymorphic loci were scored for the 158 South African individuals (Table 1 a, b). Of the 213 polymorphic loci in Madagascar, 2.8% were private within groups and sample sites (Table 1a). Of the 177 polymorphic loci in South Africa, 5.1% were restricted to single groups and single sample sites (Table 1b). The mean heterozygosity was 0.053 ± 0.003 (mean ± SE) within groups and 0.066 ± 0.003 within sample sites in Madagascar. In South Africa, the mean heterozygosity was 0.074 ± 0.003 within groups and 0.080 ± 0.003 within sample sites (for heterozygosity of single groups and sample sites, see Table 1a,b). Heterozygosity was significantly lower for groups and sample sites in Madagascar than it was in South Africa (Mann–Whitney *U*-test: groups: $S = 356$, $Z = -4.16$, $P < 0.001$, $n = 47$; sample sites: $S = 106$, $Z = -3.00$, $P < 0.003$, $n = 27$), which could be caused by more private fragments in Madagascar than in South Africa.

Kinship analyses

Our first analysis showed that in both species the average kinship coefficient decreased with geographical distance (Fig. 2). The kinship coefficient dropped to almost zero in the 0.3–3 km distance class in Madagascar, whereas in South Africa this occurred only in the distance class of 3–30 km (Fig. 2). Thus, trees seem to form a related group of individuals up to 3 km in Madagascar and up to 30 km in South Africa.

Local spatial scale

Second, the AMOVA at the local spatial scale revealed that mean genetic variation among groups of trees within sample sites was higher in Madagascar (variation among groups: mean = 6.9%, range = 0.0–10.9%, $n = 7$ sample sites) than in South Africa (mean = 2.1%, range = 0.0–5.1%, $n = 7$ sample sites). Mean F_{ST}-values for sample sites differed marginally significantly between Madagascar and South Africa (*t*-test: $t_{12} = 1.97$, $P = 0.072$). In addition, the proportion of significant F_{ST}-values was significantly larger in Madagascar than in South Africa (χ^2-test: χ^2-value = 7.19, d.f. = 1, $P = 0.007$). Nevertheless, for both species most of the genetic variation was attributed to the component within groups of trees [Madagascar (MAD): mean = 93.1%; South Africa (SA): mean = 97.9 %].

Regional spatial scale

Third, the AMOVA performed for the regional spatial scale demonstrated that differentiation among sample sites in Madagascar was low but significant ($F_{ST} = 0.053$). In South Africa, we found a stronger differentiation than for Madagascar, which was significant among sample sites ($F_{ST} = 0.163$). Again, in both species most of the genetic variation was found within sample sites (MAD: 94.7%; SA: 83.7%).

Hierarchical analysis

Finally, the hierarchical AMOVA showed that in Madagascar 6.0% of the genetic variation among groups of trees was attributed to differentiation at the local spatial scale (i.e. among groups within sample sites), and 1.6% of the genetic variation was explained by differentiation at the regional scale (i.e. among sample sites) (Table 2a). Only the differentiation among groups within sample sites was significantly different from zero (permutation test within the hierarchical AMOVA, 1023 permutations, Table 2a). In South Africa only 1.1% of the genetic variation among groups of trees was the result of differentiation at the local spatial scale (i.e. among groups within sample sites), and 15.6% of the variation was caused by differentiation at the regional spatial scale (i.e. among sample sites) (Table 2b). Here, only the differentiation among sample

Table 2 (a) Genetic variation among 23 groups of trees of *Commiphora guillauminii* H. Perrier in Madagascar at the regional spatial scale (among 12 sample sites) and at the local spatial scale (among groups within sample sites). Hierarchical AMOVA, $F_{ST} = 0.08$. (b) Genetic variation among 24 groups of trees of *Commiphora harveyi* Engl. in South Africa at the regional spatial scale (among 15 sample sites) and at the local spatial scale (among groups within sample sites). Hierarchical AMOVA, $F_{ST} = 0.17$.

Source of variation	d.f.	SS	Variance components	Percentage variation
(a)				
Among sample sites	11	153.68	0.15 n.s.	1.64
Among groups within sites	11	119.70	0.55***	6.00
Within groups	111	939.09	8.46***	92.36
Total	133	1212.47	9.16	
(b)				
Among sample sites	14	374.18	1.64***	15.62
Among groups within sites	9	83.56	0.11 n.s.	1.05
Within groups	134	1172.40	8.75***	83.33
Total	157	1630.14	10.50	

SS, sums of squares; n.s., not significant; ***$P < 0.001$.

sites was significant (permutation test within the hierarchical AMOVA, 1023 permutations). Thus, in Madagascar, differentiation among groups of trees at the local scale was higher than that at the regional spatial scale; in South Africa, variation at the regional scale was higher than that at the local scale (Table 2). Again, for both species most of the genetic variation was found within tree groups (MAD: 92.4%; SA: 83.3%).

DISCUSSION

All analyses supported our expectation that genetic structure at the local spatial scale was stronger in Madagascar than in South Africa. Although sample sizes in our study within groups of trees were small, the congruent results of four separate analyses provided consistent evidence for this pattern. Kinship analysis suggested that gene flow between trees was restricted mostly to within 3 km in Madagascar and to within 30 km in South Africa. Furthermore, we recorded higher genetic differentiation among groups of trees within sample sites in Madagascar than in South Africa, in the separate AMOVAS within sample sites, as well as in the hierarchical AMOVA. At a regional spatial scale, however, the pattern was reversed, with higher genetic differentiation among sample sites in South Africa than in Madagascar, again using both the standard AMOVA and hierarchical AMOVA.

Descriptive population genetics

We found 213 loci in 134 individuals in Madagascar and 177 loci in 158 individuals in South Africa. These results are in the same range as in other AFLP studies on trees (Muluvi *et al.*, 1999; Wang *et al.*, 2003; He *et al.*, 2004). In both species, heterozygosity was low compared with other AFLP studies on trees in which heterozygosity ranged from 0.17 to 0.35 (Rivera-Ocasio *et al.*, 2002; Wang *et al.*, 2003; He *et al.*, 2004). However, Muluvi *et al.* (1999) recorded similarly low values of 0.026–0.099 in *Moringa oleifera*. The significantly lower heterozygosity in Madagascar than in South Africa could result from the smaller geographical range (Hamrick & Godt, 1989; Wolf *et al.*, 2000). The Malagasy species is narrowly distributed and restricted to the west coast of Madagascar (Fig. 1a), whereas the South African species has a much wider range along the east coast of southern Africa (Fig. 1a). A similar pattern with low genetic diversity in Madagascar and high diversity in continental Africa has been found for *Prunus africana* populations (Dawson & Powell, 1999).

Local spatial scale

The results at the local spatial scale confirm our hypothesis that short seed dispersal distances within forests in the Malagasy species led to low local gene flow and to high genetic differentiation among groups of trees within sample sites. Such a locally structured genetic pattern has also been found for other tropical trees with limited seed dispersal (Dutech *et al.*, 2002; Ng *et al.*, 2004). The genetic results are corroborated by the field observations. Böhning-Gaese *et al.* (1999) and Bleher & Böhning-Gaese (2001) recorded a very limited seed dispersal rate for the Malagasy species, with 92.1% of the seeds dropping under the crown of trees, and a median distance between a seedling and its mother, estimated from a simulation model, of only 0.9 m. As *C. nigra* is known to act as a pre-dispersal seed predator in other parts of Madagascar (Bollen & van Elsacker, 2004), the occasional destruction of seeds might further reduce the seed dispersal rate and increase local genetic structuring of the Malagasy species. In addition, this pattern fits to the strongly clumped spatial distribution of the adult trees. The median distance among conspecific trees within clumps of trees is only 11.9 m (Bleher & Böhning-Gaese, 2001). These clumps of trees could contain related individuals on a small spatial scale corresponding to the groups of trees identified in the genetic analysis. It should be noted, however, that the spatial pattern of the trees not only is related to seed dispersal distances, but is also influenced by various other factors, for example habitat heterogeneity.

In the South African species, the larger number of frugivorous birds that disperse the seeds, including long-distance seed dispersers such as hornbills, appears to cause high genetic exchange among groups of trees within sample sites. This is supported by the spatially uniform distribution of the adult trees (Bleher & Böhning-Gaese, 2001). The nearly random genetic structure in South Africa at the small spatial scale was also found in other forest tree species that are wind- or insect-pollinated and have efficient seed dispersal by birds (Hamrick *et al.*, 1993; Gibson & Wheelwright, 1995; Degen *et al.*, 2001; Hardy *et al.*, 2006). Thus, longer seed dispersal distances within forests seem to cause a higher relatedness of trees within sample sites in South Africa than in Madagascar. In contrast, in

Madagascar, only trees within groups of trees showed relatively high relatedness among each other, and genetic differentiation among groups of trees was already almost as high as that among sample sites.

In spite of the link between the seed dispersal distances and genetic structure of the two species, it is important to take into account that the genetic structure of trees is determined by a multitude of factors – in particular by pollen transport (Dick *et al.*, 2003; Heuertz *et al.*, 2003). At the range-wide scale, maternally inherited genomes experience considerably more subdivision than paternally or biparentally inherited genomes (Petit *et al.*, 2005). This suggests that historical levels of pollen flow are generally at least an order of magnitude larger than levels of seed flow (Petit *et al.*, 2005). At the local spatial scale, a number of studies on parentage analysis of seeds and seedlings of animal-dispersed trees have demonstrated that seed transport distances can be as high as (e.g. Hardesty *et al.*, 2006) or higher than (Godoy & Jordano, 2001; Garcia *et al.*, 2007) pollen transport distances. Most importantly, a recent analysis of the gene dispersal distances and genetic structure of Neotropical trees at the local scale strongly suggests that pollen and seed dispersal distances are correlated (Hardy *et al.*, 2006). As limited seed dispersal often leads to aggregated, clumped tree distributions, and pollen probably disperses less under high tree density (Stacy *et al.*, 1996; Vekemans & Hardy, 2004), limited seed dispersal is expected to result also in limited pollen dispersal and higher spatial genetic structure (Hardy *et al.*, 2006). Thus, limited seed dispersal not only has a direct effect on genetic structure, but might also have an indirect effect by limiting effective pollen dispersal distances (Hardy *et al.*, 2006).

Both the Malagasy and the South African species are dioecious, obligate outcrossing and pollinated by similar pollinator assemblages consisting of small unspecialized insect species. They have low pollination rates and low fruit sets (Farwig *et al.*, 2004; Voigt *et al.*, 2005). We do not have data on pollen transport distances of the two species. However, the spatial structure of the adult tree population is much more clumped in Madagascar than it is in South Africa (Bleher & Böhning-Gaese, 2001). If pollen dispersal distances were the same for the two *Commiphora* species or if pollen transport distances were higher than seed dispersal distances we would expect no difference in genetic structure between the two species on a local spatial scale (Hardy *et al.*, 2006). The fact that the genetic structure of the two species differs suggests that the Malagasy species not only has short seed but also short pollen transport distances. Future studies of the two species should include a larger sample size of trees to allow a more detailed analysis of genetic structure at the local spatial scale and especially should aim to measure pollen and seed transport distances directly.

Regional spatial scale

Compared with the genetic structure at the local scale, the pattern at the regional spatial scale was reversed, with higher genetic differentiation among sample sites and more private fragments in South Africa than in Madagascar. This pattern did not match our expectations based on field work performed on a local spatial scale (Böhning-Gaese *et al.*, 1999; Bleher & Böhning-Gaese, 2001). Since the seeds of the South African species are dispersed by large-bodied seed dispersers such as hornbills, we expected high gene flow also over larger distances at a regional scale and consequently low genetic differentiation among sample sites. This unexpected pattern could have been caused by the logistic difficulty in assessing the full tail of the leptokurtic curve of seed dispersal in Madagascar (Bullock & Clarke, 2000; Nathan & Muller-Landau, 2000). Thus, the seed dispersal distances estimated from simulated seedling-mother tree distributions do not take into account rare long-distance dispersal events and potentially long and fat tails (Cain *et al.*, 2000; Nathan & Muller-Landau, 2000). Therefore, we cannot exclude that, irrespective of the low dispersal distance assumed for the Malagasy species within forests, infrequent long-distance events occur, leading to gene flow over larger distances.

The most likely explanation for the low genetic structure in Madagascar at the regional scale, however, is the historical habitat distribution at this spatial scale. The Malagasy species occurs in deciduous dry forests along the western coast of Madagascar. Although the late Pleistocene vegetation of Madagascar's arid south-west is not known, the highly endemic taxonomic status of this region's flora suggests a long persistence of these ecosystems (Burney, 1997; Burney *et al.*, 2004). Wetter conditions than today in the mid-Holocene even suggest the occurrence of dry forests in the far south-west of Madagascar. Thus, the historic distribution of the Malagasy species in deciduous dry forests is expected to have been almost continuous along the western coast of Madagascar (Burney, 1997; Burney *et al.*, 2004). The recently observed fragmentation of forests increased for most parts of Madagascar only after enhanced human activity in 1600 when European settlers arrived (Burney, 1997) but might have not yet affected the genetic structure of mature trees, resulting in the observed low genetic structure among sample sites.

In contrast, the regional distribution of the South African species is naturally much more fragmented since it is restricted mostly to steep northerly slopes of gorges (MacDevette *et al.*, 1989). These scarp forests of South Africa persisted during the Last Glacial Maximum in isolated areas along the present scarp forest belt (Lawes, 1990; Eeley *et al.*, 1999; Griffiths & Lawes, 2006). A comparative study on life-history traits of South African tree species showed that species with abiotically dispersed fruits were correlated with scarp forests. This might indicate that, in general, zoochorous species had a low survival rate in the refugia during the glacial maxima and that colonization success in the isolated scarp forests was rather low (Griffiths & Lawes, 2006). Thus, even though gene flow within gorges appears to be high, gene exchange at the regional scale through seed dispersal between these isolated gorges or to new habitat patches might be restricted to rare long-distance dispersal events that may be caused only by very mobile seed

dispersers such as hornbills. In particular, the colonization of new habitat patches might result in genetic divergence as a result of founder effects (Barton, 1996; Whitlock & McCauley, 1999). Moreover, the colonization of new sites through propagule dispersal by individuals from different established populations can lead to high genetic structuring (Erickson *et al.*, 2004). Finally, habitat heterogeneity might lead to local adaptation and genetic differentiation through recombination and selection in newly colonized habitats (Hamrick, 2004). Thus, present and historical differences in habitat distribution might have caused the overall higher genetic differentiation among sample sites in South Africa than in Madagascar.

Genetic differentiation might further be augmented by non-overlapping flowering or fruiting periods, known to reduce gene flow between populations (Hall *et al.*, 1994; Jordano & Godoy, 2000). We observed such a shift in the flowering period of the South African species between the northern (e.g. Ithala Game Reserve, No. 1 in Fig. 1b) and the southern (e.g. Oribi Gorge NR; No. 14 in Fig. 1b) populations, with the northern populations starting to flower up to 3 weeks earlier.

Limitations and advantages of this approach

The limitation of our approach, comparing seed dispersal and the genetic structure of trees between different biogeographical regions, is that it is not possible to prove unambiguously that the differences between the seed dispersal distances of the Malagasy and South African species are the only factor causing the genetic patterns. Other factors such as pollen flow, habitat distribution, local adaptation and the biogeographical history of the study sites could potentially lead to similar results. One promising addition to strengthen the rigour of the results would be to combine our comparative approach with direct measurements of pollen and seed transport distances. Another approach would be to study additional tree species and, thus, to increase the sample size of the comparison.

The advantage of this comparative approach is that frugivore assemblages and seed dispersal distances differ between Madagascar and South Africa to a degree rarely found in other systems. Thus, our model system marks the most extreme consequences that a difference in seed dispersers can have on the genetic structure of trees. Furthermore, geological data indicate an ancient mid-Mesozoic separation of Madagascar from continental Africa (Rabinowitz *et al.*, 1983; Storey, 1995) and it was therefore possible to study the long-term effects of natural differences in seed dispersal on the genetic structure of mature trees. Finally, the results on the genetic structure of the trees fit with the dispersal rates, with the spatial distribution of seedlings and adult trees (Böhning-Gaese *et al.*, 1999; Bleher & Böhning-Gaese, 2001) and even with the dispersal syndromes of the tree communities observed in the field (Voigt *et al.*, 2004). Thus, the differences in frugivore assemblages and seed dispersal distances offer the most parsimonious explanation for the genetic structure of the two tree species at the local scale. This study further demonstrates that even the much lower seed dispersal distances and higher genetic structure at the local spatial scale in Madagascar did not lead to a high genetic differentiation at the regional scale. This suggests that seed dispersal influences tree genetic structure only at the local and not at the regional spatial scale.

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