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Conservation implications of fine scale population genetic structure of *Ficus* species in South African forests

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Abstract

Genetic considerations are rarely applied in forest conservation management strategies, but forest fragmentation can reduce pollen and seed dispersal both between and within isolated fragments. Gene flow and immigration rates determine the extent to which individual plants are related to each other at different distances from themselves. This gradation in relatedness is known as a population's fine scale spatial genetic structure (SGS). Specifically, reduced but clumped immigration from distant fragments reduces fine scale SGS, whereas reduced gene flow within fragments increases fine scale SGS. In addition, non-random mortality caused by post-dispersal ecological processes can also affect SGS. We studied the effects of fragmentation on the fine scale SGS of *Ficus* species with different habitat preferences and distributional ranges in an archipelago of South African forest patches. Significant fine scale SGS present in all three species suggests gene dispersal is restricted, even within forest fragments, probably due to localised seed dispersal. An endemic forest specialist, *F. bizanae*, has an unusually high fine scale SGS for a monoecious *Ficus*. This may be explained by several features that reduce pollen dispersal distances and are more typical of dioecious *Ficus*. A significant negative kinship coefficient in one *F. bizanae* population suggests that clumped long-distance immigration may have occurred in the past. Significant fine scale SGS in adult but not juvenile *F. craterostoma* suggests that recent population fragmentation has negatively affected long-distance immigration. Supplementation of *F. craterostoma* gene flow would maintain its genetic diversity. In contrast, the limited range of *F. bizanae* may result from its pollinator’s behavior, rather than specific habitat requirements and ensuring its long-term survival may require artificial introductions to other forests.

Keywords: fragmentation, *Ficus,* pollen dispersal, seed dispersal, *Sp* statistic, spatial genetic structure

1. Introduction

Forest habitats along the Eastern seaboard of Africa are restricted to an archipelago of montane fragments (White, 1983). These forests became fragmented millions of years ago due to climate change (Lawes, 1990), but current human activities have intensified the fragmentation (Hoffman, 1997; Lawes et al., 2007). In South Africa, forest fragmentation has reduced vertebrate diversity and caused local extinctions (mammals: Lawes, 2004; birds: Cooper et al., 2017), and future conservation of these forests, to ensure maintenance of their value as intact ecosystems (Watson et al., 2018), may require management intervention to mitigate ongoing anthropogenic mediated impact (Lindenmayer et al.,in press). The loss of forest-associated animal species means a loss of pollen and seed vectors and this decline in pollination and seed dispersal service providers will correspondingly reduce plant genetic connectivity between forest patches (Farwig and Berens, 2012; Acevedo-Quintero et al.,in press; Peña et al., in press).

If fragmentation is severe enough to sever gene flow between populations, genetic drift becomes a more important evolutionary force within the remaining forest fragments (Nielsen and Slatkin, 2013). Genetic drift allows the fixation of slightly deleterious mutations (Ohta and Kimura, 1969) and results in the loss of genetic variation (Nielsen and Slatkin, 2013). These phenomena lower the population's long-term survival because the accumulation of deleterious mutations weaken individuals

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Abbreviations: SGS spatial genetic structure and a lack of genetic variation prevents adaptation (Ohta and Kimura, 1969).

Fragmentation leaves genetic signatures that can be seen between populations (Nielsen and Slatkin, 2013) but also in the spatial arrangement of genetic variation within populations. Wright (1943) proposed that in a continuously distributed population, individuals are more likely to mate with close-by individuals than distant ones, due to limited dispersal. This results in fine scale spatial genetic structure (SGS) within populations (Wright, 1943; Sokal and Wartenberg, 1983; Epperson and Li, 1997; Smouse and Peakall, 1999; Rousset, 2000) that reflects the extent of gene flow (Hardy and Vekemans, 1999; Hardy et al., 2006).

For sessile organisms such as plants, gene dispersal is limited to pollen and seed dispersal (Heuertz et al., 2003; Hardy et al., 2006; Dick et al., 2008). In general, long distance dispersal of seed and pollen will result in weak or no fine scale SGS, while short distance dispersal of seed and pollen will lead to a strong fine scale SGS (Dick et al., 2008). Extensive seed dispersal with limited pollen dispersal tends to result in weak fine scale SGS, while the opposite normally generates strong fine scale SGS, since pollen contains only a haploid set of DNA whereas seed is diploid (Dick et al., 2008). However, occasional long-distance clumped seed dispersal, especially from a distant population, will also result in strong fine scale SGS (Pardini and Hamrick, 2008). This is the case because immigrants from a distinct gene pool will initially form a unique genetic cluster that will be homogenized through subsequent local admixture. In addition, temporal changes in gene flow can generate differences in fine scale SGS between age groups. Specifically, if long-distance gene flow is prevented, fine scale SGS will be reduced in younger age classes. Fine scale SGS can also be affected by post-dispersal ecological processes. For example, the SGS is affected by non-random mortality as a result of demographic thinning (Connell, 1971; Zhou and Chen, 2010; Ismail et al., 2012) and microenvironmental selection (Batista Leite et al., 2014; Berens et al., 2014; Helsen et al., 2015). Additionally, since gene flow can change over time and survival is inherently temporal, fine scale SGS can differ between age groups in plants (Connell, 1971; Ismail et al., 2012; Berens et al., 2014; Helsen et al., 2015). For instance, demographic thinning due to high mortality of *Ficus cyrtophylla* seedlings near their mother tree is known to progressively reduce the extent of fine scale SGS from seedlings to saplings to adults (Zhou and Chen, 2010).

Ficus species are pollinated by tiny fig wasps, which are known for their long-distance dispersal, especially those associated with monoecious *Ficus* species (Nason et al., 1996; Weiblen, 2002; Zavodna et al., 2005; Ahmed et al., 2009; Kobmoo et al., 2010; Heer et al., 2015). For example, Ahmed et al. (2009) found that the pollinator of *F. sycomorus* can disperse pollen 164 km between trees in a desert environment and the pollinators of monoecious rainforest species in Central America routinely disperse pollen between 5.9 and 14.2 km (Nason et al., 1996). Such long-distance gene dispersal of *Ficus* species may help to homogenize the genetic structure within populations, resulting in no or weak fine scale SGS. However, *Ficus* species and their fig wasps vary in traits crucial to gene flow (Harrison and Shanahan, 2005). In forests, pollen flow in monoecious and dioecious fig trees tends to be very different (Nazareno et al., 2013). Monoecious trees typically bear infrequent, large synchronous crops accompanied by pollinators that make use of passive wind dispersal by flying above the forest canopy (Nason et al., 1996; Compton et al., 2000; Harrison, 2003; Harrison and Rasplus, 2006). On the other hand, dioecious species often have small, more continuous and asynchronous crops and their pollinators fly more rarely above the canopy (Compton et al., 2000; Harrison, 2003; Harrison and Rasplus, 2006). Consequently, the latter must rely more on short distance active flight for pollination. As a result, dioecious *Ficus* species typically have a higher fine scale SGS than monoecious *Ficus* species (Dev et al., 2011; Nazareno et al., 2013).

Several *Ficus* studies reported fine scale SGS that may result from limited seed dispersal (Wang et al., 2009; Zhou and Chen, 2010; Dev et al., 2011; Heer et al., 2015; Krishnan and Borges, 2018). For example, SGS has been found within a 1 km radius in four monoecious *Ficus* species despite pollen-dispersal distances that exceed 1 km. Similarly, in *F. racemosa*, which has its pollen dispersed over long distances (Bain et al., 2016), strong spatial genetic structure was found within 1 km in an urban landscape, which suggested clumped seed dispersal close to parents (Krishnan and Borges, 2018).

Ficus species are often considered to be keystone species in South African forests (Bleher et al., 2003; but see Compton and Greeff, unpublished results) but this role could be adversely affected by fragmentation (Hoffman, 1997; Lawes et al., 2007). A generalist *Ficus* species that also occurs in the habitats between these forests (*F. sur*), shows little differentiation between populations, suggesting fragmentation has a small effect on this species (Deng et al., 2020). In contrast, genetic structure is higher between populations of two forest specialists (*Ficus craterostoma* and *Ficus bizanae*), suggesting an effect of long-term fragmentation (Deng et al., 2020). An isolation by distance pattern showed that although the fig wasps of *F. craterostoma* are able to transport pollen between nearby forests, distant populations are nonetheless more genetically distinct (Deng et al., 2020). The genetic structuring of *F. bizanae* populations is particularly high, and this possibly reflects its atypical and dioecious-like fruiting phenotype (Deng et al., 2020).

Intra-population SGS analyses can detect if gene flow is limited within forest populations and thus indicate whether *F. bizanae*'s pollinator disperses locally, as seen in pollinators of dioecious *Ficus* species. Further, by comparing SGS in different age classes, temporal changes in the extent of inter-population pollinator dispersal can also be assessed, though caution with interpretation is required because mortalities can generate similar patterns (Zhou and Chen, 2010; Helsen et al., 2015). Here we augment inter-population findings (Deng et al., 2020) with intra-population SGS analyses to answer the following questions 1) does the extent of fine scale SGS differ between three *Ficus* species found in Southern African forests? and 2) do different age classes of *F. craterostoma* vary in the extent of fine scale SGS? We then highlight the conservation and management implications of our findings.

2. Materials and methods

2.1. The study species

Three *Ficus* species with different life forms and habitat preference were compared. *Ficus bizanae* and *F. craterostoma* belong to section *Galoglychia* (subgenus *Urostigma*) while *F. sur* belongs to section *Sycomorus* (subgenus *Sycomorus*; Burrows and Burrows, 2003). *Ficus craterostoma* is a strangler while *F. sur* is usually a freestanding tree. *Ficus bizanae* is predominantly lithophytic and seldomly hemi-epiphytic. *Ficus bizanae* are frequently clustered on old scree slopes (S. van Noort, personal communications) and bears its fruit on the major stems well below the canopy. Its mature figs are 2.5-4.5 cm in diameter and green with a strong smell (Berg and Wiebes, 1992), suggesting that mammals may be its main seed dispersal vectors (Shanahan et al., 2001). *Ficus craterostoma* figs develop in the leaf axils of branches that normally protrude above the forest canopy. Its figs turn yellow with red spots or entirely reddish and measure 1.5-2.0 cm in diameter when ripe, indicating their main seed dispersal agents are likely to be birds (Shanahan et al., 2001). The figs of *F. sur* turn red or dark orange with a sweet smell and have a diameter of 2.0-4.0 cm when ripe (Berg and Wiebes, 1992). They are clustered on leafless branchlets on the trunk, located from the canopy to below the ground. A wide variety of birds and mammals are known to disperse its seeds (Thomas, 1988;

Deleted: and *F. bizanae* are

Shanahan et al., 2001).

2.2. The studied forests

Four of the five forests studied here are classified as scarp forests and one as a southern mistbelt forest (http://bgis.sanbi.org/vegmap, Table 1). The geographical isolation of these forest types predates the last glacial maximum (18 000 BP; Lawes et al., 2000; Busschau et al. in press; Kushata et al. in press), but the dry conditions during the last glacial maximum resulted in their fragmentation into an archipelago of tiny forest islands (Lawes, 1990; Mucina and Geldenhuis, 2006). Fire is likely to have played an important role in this fragmentation but subsequent wetter conditions led to forest expansion and the establishment of new tiny patches (Lawes, 1990; Lawes et al., 2000). These forests are typically smaller than 1 km^2 and are embedded in a matrix of other biomes such as grassland (Mucina and Geldenhuis, 2006). Assessment of SGS is only possible for larger areas of forest, and the five forests studied here are among the top 5% of the largest forests of South Africa despite them having areas of less than 25 km^2 (Table 1, http://bgis.sanbi.org/vegmap). This apparent relatively large size of the five studied forests is a distortion resulting from the mapping of thousands of very tiny patches (4 146 of 6 292 scarp and southern mistbelt forests are smaller than 0.1 km^2) that constitute less than 5% of these forests' total surface area. In fact, the average forest size, weighted by forest size, is 8 km^2. In other words, the average square meter of forest is in an 8 km^2 forest. Since, the forests sampled here are relatively large they are likely to be remnants from the last glacial maximum, rather than recolonizations. Although forest clearing by humans has caused further fragmentation during the last two centuries (Castley and Kerley, 1996), these were insignificant in comparison to the older changes generated by climate change (Lawes et al., 2000). Other human pressures such as collecting building material, firewood and medicinal plants would similarly have had limited direct impacts on fig trees as the trees are not widely used for firewood and only *F. sur* is used as medicine locally (van Wyk and Gericke, 2000). In contrast, human impacts on the functional diversity of avian and mammalian communities may have been severe, and losses of frugivores can adversely affect seed dispersal (Liu et al., 2019; Leaver et al., 2019).

Table 1 Sampled populations (with sample sizes of trees in parentheses). The three-letter code for each forest, the latitude and longitude of each forest are given. Forest classification and sizes are from http://bgis.sanbi.org/vegmap and defined in Mucina and Geldenhuis (2006).

Code	Populations	Forest classification	Species	Latitude	Longitude	Forest size (km^2)
ING	Ingeli	Southern mistbelt	*F. craterostoma* (110)	-30.530	29.689	7.18
MKA	Mkambati	Scarp	*F. bizanae* (54))	-31.297	29.979	1.26
MBO	Mboyti	Scarp	*F. bizanae* (32), *F. sur* (56)	-31.435	29.688	13.89
DWE	Dwesa	Scarp	*F. craterostoma* (48)	-32.280	28.848	21.46
MAN	Manubi	Scarp	*F. craterostoma* (30), *F. sur* (57)	-32.449	28.606	6.25

2.3. Sampling and genotyping

The study used the samples described in Deng et al. (2020), augmented with the locality data for each sample. Each tree's location was recorded using GPS for the fine scale SGS and for calculating the trees' densities. To make SGS analyses appropriate, only samples sites with 30 or more individuals of a species were considered. More specifically, we included 86 *F. bizanae* individuals from two populations, 188 *F. craterostoma* individuals from three populations, and 113 *F. sur* individuals from two populations (Fig. 1, Table 1). The three *Ficus* species were collected from four forests in the Eastern Cape Province and one forest in Kwazulu-Natal Province of South Africa (Fig. 1, Table 1). We also recorded the age class (saplings (DBH ≤ 10 cm) or adults (DBH>10 cm)) for the individuals of *F. craterostoma* in the Ingeli forest population because this sample was large enough (110 individuals) for a split analysis of the two age classes. Genotypes generated in Deng et al. (2020) were used. In brief, this involved genotyping *F. bizanae*, *F. craterostoma* and *F. sur* individuals for 8, 12 and 10 polymorphic fluorescently-labelled SSR markers, respectively (Deng et al., 2020).

2.4. Spatial genetic structure

We analyzed the spatial genetic structure in each population of the three *Ficus* species using SPAGeDi version 1.5 (Hardy and Vekemans, 2002). For *F. craterostoma*, we compared separately the spatial genetic structure of saplings and adults. The kinship coefficient defined by Loiselle et al. (1995) was chosen because it does not assume Hardy-Weinberg equilibrium (Hardy, 2003; Vekemans and Hardy, 2004) and has been used widely in other *Ficus* studies (Wang et al., 2009; Dev et al.,

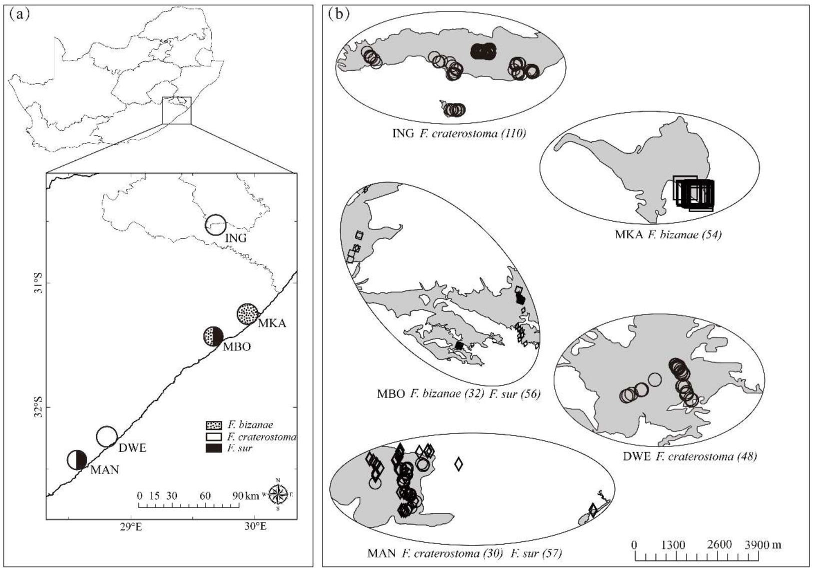

Fig. 1 Species sampled in each sample site (a) *F. bizanae* = dotted circles, *F. craterostoma* = empty circles, *F. sur* = circles with solid fill) and (b) the locations of individual trees of each species sampled in each forest with sample sizes given in parentheses (*F. bizanae* = squares, *F. craterostoma* = circles, *F. sur* = diamonds). Populations with less than 30 individuals in Deng et al., (2020) were excluded, e.g. *F. bizanae* at DWE (22). The grey areas indicate the forest patches of each population, which were downloaded from the website http://bgis.sanbi.org/vegmap on February 2019.

2011; Nazareno et al., 2013; Heer et al., 2015; Krishnan and Borges, 2018). The pairwise kinship coefficients (F_{ij}) (Loiselle et al., 1995) were estimated between individuals for each locus and then averaged over loci. We estimated the regression slopes (*b*) by regressing the pairwise kinship coefficients (F_{ij}) against the natural logarithm of spatial distance $\ln(d_{ij})$. Seven to fourteen distance intervals were defined (*F. bizanae*, Mboyti and Mkambati forest populations = 7; *F. craterostoma*, Dwesa forest population = 14, Manubi forest population = 9, Ingeli forest population (combined ages) = 12, (adults) = 12, (saplings) = 7; *F. sur*, Mboyti forest population = 9, Manubi forest population = 8, Table S1). We followed the suggestion of Cavers et al. (2005) that at least 30 comparisons per class are needed to achieve statistical robustness (Legendre and Fortin, 1989). The fine scale SGS was further visualized in a spatial autocorrelogram by plotting the kinship coefficient against geographic distance (Vekemans and Hardy, 2004). A total of 10 000 permutations of locations and genes were performed to test the significance and determine the 95% CI for the index in each distance class. The standard errors for each comparison were estimated by jackknifing over loci for each comparison. To compare the intensity of fine scale SGS among different species as well as between different ages of *F. craterostoma*, the *Sp* statistic was calculated as $Sp = -b/(1-F_1)$, where F_1 is the mean F_{ij} in the first distance class (Vekemans and Hardy 2004). In order to compare our results with those from other *Ficus* species, we searched for published fine scale SGS studies using '*Ficus*' and 'spatial genetic structur*' on Web of Science (http://www.webofknowledge.com accessed August 2019).

2.5. Typical tree densities

Although we only had enough data to do meaningful SGS analyses for a total of seven populations, we collected locality data from a further seven populations – totalling 108 *F. bizanae* from three populations, 247 *F. craterostoma* from five populations and 186 *F. sur* from six populations (available on Figshare data repository). Forests were not searched systematically; rather existing footpaths through the forests were followed. Here we calculated for each species the average distance to the ten nearest conspecific trees along the surveyed transects within each forest, which were determined by existing footpath trails, or access vehicle tracks.

3. Results

Significant fine scale SGS was found in all three *Ficus* species, with kinship coefficients decreasing significantly as geographic distance increased (Table 2, Fig.2). All three species showed significant positive kinship coefficients at their first distance class, suggesting local gene dispersal in all three species.

The magnitude of fine scale SGS varied among species, with the strongest fine scale SGS intensity in *F. bizanae* (average $Sp = 0.025 \pm 0.011$), then *F. craterostoma* ($Sp = 0.008 \pm 0.005$) and finally *F. sur* ($Sp = 0.005 \pm 0.003$). This indicates that *F. bizanae* has much more local gene dispersal than the other two species. This

Table 2 Fine-scale spatial genetic structure parameters for each *Ficus* species. $b_{(\log)}$ represents the regression slope of kinship coefficient F_{ij} on log spatial distance with standard errors given in parentheses. F_1 indicates the average kinship coefficient between individuals within the first distance class with standard errors given in parentheses. Significance is indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Species	Population	$b_{(\log)}$	F_1	*Sp* ± 1.96SE
F. bizanae	MKA	-0.027*** (0.005)	0.144 (0.034)	0.031 ± 0.010
	MBO	-0.018** (0.006)	0.068 (0.026)	0.019 ± 0.012
	Average		0.106 (0.030)	0.025 ± 0.011
F. craterostoma	DWE	-0.007** (0.003)	0.017 (0.010)	0.007 ± 0.006
	ING (all)	-0.005*** (0.001)	0.022 (0.008)	0.005 ± 0.002
	MAN	-0.011** (0.004)	0.039 (0.013)	0.011 ± 0.008
	Average		0.026 (0.010)	0.008 ± 0.005
	ING (adult)	-0.006*** (0.001)	0.022 (0.007)	0.006 ± 0.004
	ING (young)	-0.002 (0.002)	0.010 (0.007)	0.001 ± 0.004
F. sur	MBO	-0.003* (0.001)	0.018 (0.004)	0.003 ± 0.004
	MAN	-0.006** (0.002)	0.024 (0.008)	0.006 ± 0.002
	Average		0.021 (0.006)	0.005 ± 0.003

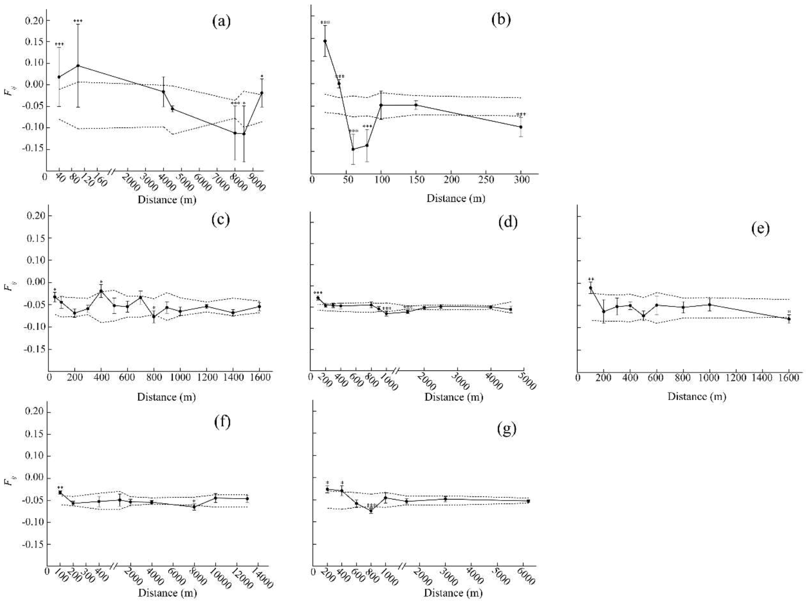

Fig. 2 Kinship coefficients in relation to geographic distances. *Ficus bizanae*: (a) & (b); *F. craterostoma*: (c), (d) & (e); *F. sur*: (f) & (g); MBO: (a) & (f); MKA: (b); DWE: (c); ING: (d); MAN: (e) & (g). The dotted lines are the 95% confidence intervals. The results of permutation tests are shown: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

difference also suggests that *F. bizanae* has restricted seed as well as pollen dispersal. Local gene dispersal in this species was also supported by the kinship coefficient of *F. bizanae* (F_1= 0.106) at the first distance class, which was almost five times larger than that of the other two species (*F. craterostoma*, F_1= 0.026; *F. sur*, F_1= 0.021). The recorded fine scale SGS intensities (*Sp* statistics) of monoecious *Ficus* species range from 0.003 to 0.031 with a median of 0.008. The recorded SGSs of dioecious

Ficus species are higher, ranging from 0.029 to 0.074, with a median of 0.033 (Table 3). The SGS intensity of *F. bizanae* is about three times higher than the median fine scale SGS intensity recorded for other monoecious species (Table 3) suggesting that *F. bizanae*'s pollinators predominantly disperse locally. On the other hand, the *Sp* statistics of *F. craterostoma* is lower and *F. sur* is the same as the median value for monoecious *Ficus* species (Table 3), suggesting that their gene flow is similar to that of other monoecious species.

Within each species, the intensity of fine scale SGS also varied across populations (Table 2). For *F. bizanae*, SGS was higher in the Mkambati compared to the Mboyti forest population. The higher fine scale SGS in Mkambati forest population was also indicated by the negative kinship coefficient of *F. bizanae* at the third (40-60 m) and fourth (60-80 m) distance classes (Fig. 2). These observations suggest that there has been clumped immigration by a number of seeds that were related to each other, but unrelated to the rest of the surrounding population.

In *F. craterostoma* from the Ingeli forest population, SGS was significant in adults (*Sp* = 0.006 ± 0.004, $P < 0.001$), but not in saplings (*Sp* = 0.001 ± 0.004, $P = 0.244$) (Fig. 3). This could be indicative of selective survival or a change in rates of immigration.

Table 3 *Sp* statistics of spatial genetic structure of *Ficus* species.

Sexual system	Species	N	*Sp*	Continent
Dioecy				
	F. hispida	50	0.0350	Asia[3]
	F. exasperata	45	0.0311	Asia[3]
	F. pumila	129	0.0742	Asia[1]
	F. cyrtophylla		0.0291	Asia[3]
	Median		0.0331	
Monoecy				
	F. citrifolia	46	0.0086	South America[4]
		82	0.0075	South America[4]
		49	0.0042	South America[4]
	F. citrifolia	52	0.0133	Central America[5]
	F. eximia	48	0.0063	South America[4]
	F. racemosa	47	0.015	Asia[6]
	F. yoponensis	37	0.0083	Central America[5]
	F. insipida	190	0.0034	Central America[5]
		155	0.0054	Central America[5]
	F. obtusifolia	59	0.0311	Central America[5]
	Median		0.0083	

[1] Wang et al., 2009; [2] Zhou and Chen, 2010; [3] Dev et al., 2011; [4] Nazareno et al., 2013; [5] Heer et al., 2015; [6] Krishnan and Borges, 2018;

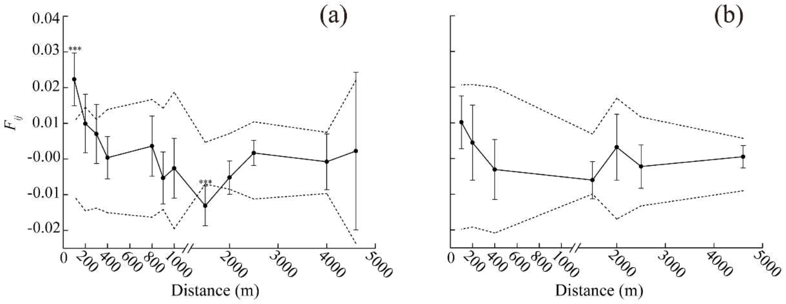

Fig. 3 Correlograms of average kinship coefficients of *F. craterostoma* (a) adults and (b) juveniles in the Ingeli forest (ING). The dotted lines are the 95% confidence intervals The results of permutation tests are shown: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Ficus bizanae trees were more spatially clustered than the other two species, with the first seven nearest neighbours of each individual located closer to each other (Fig. 4). *Ficus sur* was distributed more sparsely than the other two species (Fig. 4), with *F. craterostoma* intermediate in terms of nearest neighbours. This suggests that while *F. bizanae* frequently recruits close to parent trees, *F. sur* does not. It also suggests that pollen-donating trees are more likely to be close-by in *F. bizanae* and will usually be furthest away in *F. sur*.

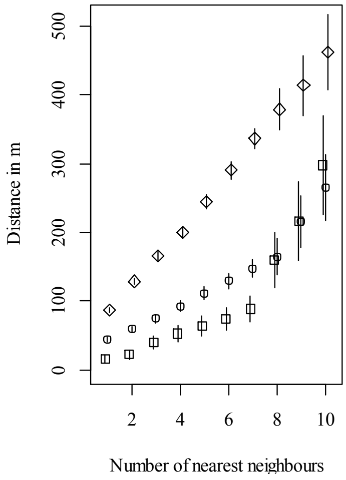

Fig. 4 The means ± the standard errors of distance to a certain number of nearest neighbours in the three *Ficus* species. *Ficus bizanae* = squares, *F. craterostoma* = circles, *F. sur* = diamonds.

4. Discussion

The forest fragments of all three *Ficus* species show some spatial substructure and suggest short distance gene flow, presumably through local seed dispersal increasing the clustering of related individuals. While two of these species were similar to other recorded monoecious *Ficus,* substructure was substantially stronger in *F. bizanae*, consistent with the limited dispersal of its pollinators and seeds suggested by Deng et al. (2020). The extent of fine scale SGS within populations of all three species also mirrored the genetic structure between populations (Deng et al., 2020), suggesting that some factors operate at both scales of diversity (Heer et al., 2015).

Variation in the degree of fine scale SGS among populations of the three species suggests either that an equilibrium had not been reached or that the processes involved vary between populations.

Ficus bizanae trees were the most clumped, with more conspecific trees close-by, while *F. sur* trees' nearest neighbours were the furthest apart. However, the *F. bizanae* clusters were composed of few trees, so its mean distances to nearest neighbours became indistinguishable from the other two species when ten neighbours were considered. Age-related differences in fine scale SGS were present in *F. craterostoma*, with fine scale SGS detected in adults but not in saplings. This is consistent with the view that human-induced fragmentation may have prevented recent long-distance clumped immigration into its populations.

All populations of the three species have significant fine scale SGS, suggesting that gene dispersal is frequently local. This may seem surprising for *F. sur* and *F. craterostoma* as they appear to be typical monoecious *Ficus* species, whose pollinators are known for their long-distance pollen dispersal (Nason et al., 1996; Zavodna et al., 2005; Harrison and Rasplus, 2006; Ahmed et al., 2009; Kobmoo et al., 2010; Bain et al., 2016). Therefore, the significant fine scale SGSs present in these two species are unlikely to be due to limited pollen dispersal and suggests that seed dispersal in all three species must be predominantly local. Local seed dispersal is known to increase fine scale SGS in *Ficus* (Wang et al., 2009; Dev et al., 2011; Nazareno et al., 2013; Heer et al., 2015; Krishnan and Borges, 2018) and this was

Deleted: Indeed, genetic variation in *F. craterostoma* populations suggests that forests as far as 242 km apart may be connected via pollen flow (Deng et al. in preparation). The genetic differentiation between *F. sur* populations is even less than that of *F. craterostoma* in forests that are equally distant from one another (Deng et al., 2020), suggesting even better gene flow in *F. sur*.

even inferred in a widespread South-East Asian *Ficus* (*F. racemosa*) that is pollinated by a fig wasp known to be widely-dispersing (Kobmoo et al., 2010). Genotyping of maternally inherited markers should answer this question.

The intensity of fine scale SGS varied among the three South African forest species, suggesting there may be variation in the extent of restriction to seed dispersal among the three *Ficus* species. These differences should stem from different dispersal abilities of the predominant seed vectors of each species and different responses to fragmentation by the vectors. Fruit characteristics tend to fall into discrete syndromes (Lomáscolo et al., 2008; Lomáscolo et al., 2010): birds are particularly associated with small and colored fruits such as *F. craterostoma*, while bats and other mammals prefer large fruit with a strong scent like *F. bizanae* (Lomáscolo et al., 2008; Lomáscolo et al., 2010). The large figs of *F. sur* attract a wide variety of both birds and mammals, including bats (Berg and Wiebes, 1992; Shanahan et al., 2001). The lower SGS of this species and the probably bird-dispersed *F. craterostoma*, agree with predictions that birds and bats can disperse seeds over considerable distances (Laurance, 1991; Shilton et al., 1999; Davies et al., 2000; Henle et al., 2004).

The degree of clustering of trees reflects these views on dispersal. *Ficus bizanae*'s small clusters suggest that offspring are frequently recruited close to the mother, aided by the clustered boulder substrate. Local recruitment seems less common in *F. craterostoma* and very unlikely in *F. sur*. This clustering and the SGS pattern may also be related to germination patterns. For instance, if germination sites are diffuse, as in the case of hemi-epiphytes, seedlings tend not to germinate in close proximity to each other (Michaloud and Michaloud-Pelletier, 1987; Heer et al., 2015). This may explain why the hemi-epiphytic *F. craterostoma* has such a low SGS.

Habitat fragmentation affects the migration of vectors between patches (Henle et al., 2004; Kramer et al., 2008). This is especially important in the context of South African forests, some of which became fragmented millions of years ago (Van Zinderen Bakker, 1983; Lawes, 1990; Sepulchre et al., 2006; Lawes et al., 2007). Mammals such as primates may be more vulnerable to fragmentation than birds and volant mammals (Laurance, 1991; Davies et al., 2000; Lawes et al., 2000; Henle et al., 2004). Since its fruit characteristics suggest that mammals are the dominant seed dispersal vectors of *F. bizanae* (Lomáscolo et al., 2008; Lomáscolo et al., 2010), forest fragmentation is expected to have limited its seed dispersal more strongly and this is reflected in it having the strongest SGS among the three species.

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The pollen dispersal distances of monoecious fig trees are expected to be larger and more varied than those of dioecious species because they are typically a) more sparsely distributed (Harrison, 2003; Heer et al., 2015), b) grow taller (Harrison and Shanahan, 2005) c) bear infrequent, large synchronous crops (Harrison and Shanahan, 2005), and d) their pollinators often travel above the canopy and over long distances (Compton et al., 2000; Harrison, 2003; Compton et al., 2005; Harrison and Rasplus, 2006). In contrast, it is expected that dioecious *Ficus* should have shorter and more uniform pollen dispersal distances because they more frequently a) occur in dense stands (Harrison, 2003), b) are small trees or shrubs far beneath the canopy (Harrison and Shanahan, 2005), with c) frequent, small and asynchronous crops (Harrison and Shanahan, 2005) and d) have pollinator fig wasps that often fly beneath the canopy (Harrison, 2003; Harrison and Rasplus, 2006).

Ficus sur and *F. craterostoma* fit this general expectation of extensive pollen-mediated gene flow in monoecious *Ficus* species, but *F. bizanae* does not. *Ficus bizanae* has a higher *Sp* statistic and kinship coefficient at the first distance class than the other species. This indicates that in addition to limited seed dispersal, its pollen dispersal is also likely to be localized. Such limited pollen dispersal may stem from some features that *F. bizanae* shares with dioecious *Ficus* – namely clusters of nearby trees with asynchronous crops that should result in pollination by nearby trees. Third, their figs are produced in the lower strata of the forest, meaning that pollinator fig wasps will be released underneath the canopy. Despite South African forests having low canopy heights (with ranges from 10-25 m, (Mucina and Geldenhuys, 2006)), having release sites closer to the ground, if it is combined with active avoidance of flying upwards into the general air column by the fig wasps, is likely to reduce the distances that pollen is moved (Ware and Compton, 1994; Compton, 2002). We expect *F. bizanae*’s pollinators to avoid going above the canopy since *F. bizanae* is a forest specialist with a very limited distribution (Burrows and Burrows, 2003). Therefore, like so many wingless island species (Macarthur and Wilson, 2001), natural selection may have favoured *F. bizanae* pollinators that avoid dispersal away from their native forest because such dispersal guarantees failure to reproduce (Gates and Nason, 2012). This idea could be tested by germinating and genotyping seeds and using it to identify pollen donors (as in the study of Ahmed et al., 2009).

We detected a significant SGS in *F. craterostoma* adults, but not in saplings. Higher SGS among older plants has been found in many other studies (Jones and Hubbell, 2006; Batista Leite et al., 2014; Berens et al., 2014; Helsen et al., 2015), including other *Ficus* species (Heer et al., 2015). In addition to the cessation of clumped immigration, microenvironmental selection and overlapping generations may also explain this age-related SGS structure. One mechanism that can increase SGS is relatively recent immigration into a population by kin-groups unrelated to the local population. This can result from occasional long-distance seed dispersal. However, older *F. craterostoma* trees in the Ingeli forest population show a higher SGS than younger trees, which suggests that some of the adults originated through immigration that has not been taking place more recently when the saplings were generated. Increased fragmentation during more recent decades may have been so severe as to sever immigration into the Ingeli forest population.

Several other ecological factors can nonetheless result in variation of SGS across different life stages (Chung et al., 2003; Jones and Hubbell, 2006; Berens et al., 2014). For instance, better survival of heterozygous individuals (Ledig et al., 1983; Cohas et al., 2009), demographic thinning due to density-dependent predation, pathogen pressure, or higher mortality of juveniles germinating close to their mother will reduce SGS by the time that trees become adults (Janzen, 1970; Connell, 1971; Ismail et al., 2012). On the other hand, if such a Janzen-Connell effect only developed recently, it can reduce juvenile SGS in comparison to that of adults. However, *F. craterostoma* is a strangler and successful germination sites will not be clustered within local areas of a forest (Michaloud & Michaloud-Pelletier, 1987; Heer et al., 2015). This brings into question the existence of a Janzen-Connell effect for this species.

The establishment of new forests by humans will affect SGS. Given that the forests we studied here are most probably remnants from the last glacial maximum, the patterns likely reflects a long-term equilibrium. It is unclear how these patterns will manifest in patches that are smaller (5 874 patches smaller than 1 km^2, 4 146 patches smaller than 0.1 km^2) and that may in addition, have been established recently.

The gene flow patterns we recorded among *Ficus* species have implications for the ecology of South African forests in general. Firstly, they have indirectly revealed the loss of forest functional connectivity, given that gene flow plays an important role in maintaining interconnectivity between forest patches (Calviño-Cancela et al., 2012; Villard and Metzger, 2014). Second, the local seed dispersal in all three *Ficus* species may also be the case for other animal-dispersed plant species. This will reduce natural regeneration by reducing colonization (Howe and Smallwood, 1982; Nathan and Muller-Landau, 2000; Anzures-Dadda et al., 2011) and increasing mortality of clumps of juveniles close to the mother (Janzen, 1970; Connell, 1971; Ismail et al., 2012). Third, a recent reduction of gene flow suggests that human activities such as logging, road construction and commercial timber plantations may have intensified the effects of fragmentation on frugivore communities. Lastly, the most affected species may be range-limited species as their pollen and seed dispersal may be curtailed. This isolation results in a loss of genetic variation and functionality that can culminate in local extinction (Young et al., 1996; Honnay and Jacquemyn, 2007). Since plants are defining components of forest ecosystems, such a loss may cause cascading effects throughout the food web (Souza et al., 2016; Watson et al., 2018; Peña et al., in press; Lindenmayer et al., in press).

While functional connectivity can fail at ecological time-scales, drift is slow and the impact of current fragmentation on long-lived species such as trees will take many human generations to manifest (Aguilar et al., 2008; Heinken and Weber, 2013). This provides opportunities for management of genetic connectivity because we can ensure the retention of genetic variation, the effectivity of natural selection and the long-term survival of specialist forest fig species by transplanting seeds and even extending natural ranges of species with restricted ranges, such as *F. bizanae*. The unusual biology of fig trees suggests that their conservation may not be as hampered by normal genetic threats as is the case for many other species. Specifically, *F. craterostoma*'s extensive pollen flow means that the typical genetic threats posed by transplantation, such as cyto-nuclear mismatches, local adaptation and male-sterility should be absent. Therefore, seed transplants have the potential to reverse recent fragmentation without the normally associated risks. On the other hand, *F. bizanae* has substantially lower gene flow and some of these genetic impediments may be at work. However, its local gene flow may well be the result of its unusual biology adapted over millions of years of fragmentation. The long-term survival of *F. bizanae* could be improved by translocations that generate artificial range extension, if suitable areas for transplantation of this unusual *Ficus* species can be identified.

CRediT authorship contribution statement

Jun-Yin Deng: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Simon van Noort**: Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Stephen G. Compton**: Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition. **Yan Chen**: Methodology, Resources, Writing - review & editing, Project administration, Funding acquisition. **Jaco M. Greeff**: Conceptualization, Methodology, Resources, Data curation, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Conflict of interests

No competing interests.

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Data availability statement

Sampling location, microsatellite genotypes of each individual: Research data repository-Figshare DOI: https://doi.org/10.25403/UPresearchdata.11900007.v2

Locality data for three fig species from seven forests: Research data repository-

Figshare DOI: https://doi.org/10.25403/UPresearchdata.12235379.

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