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Article:
Deng, J-Y, van Noort, S, Compton, SG et al. (2020) The genetic consequences of habitat specificity for fig trees in southern African fragmented forests. Acta Oecologica, 102. 103506. p. 103506. ISSN: 1146-609X

https://doi.org/10.1016/j.actao.2019.103506

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The genetic consequences of habitat specificity for fig trees in southern African fragmented forests

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ABSTRACT

Theory predicts that fragmentation will lead to reduced gene flow between populations, with losses of genetic diversity and increased population differentiation. However, these predictions may not always hold true, especially for long-lived woody plants and some fig trees (*Ficus* species) may not be affected by fragmentation because their fig wasps can transfer pollen for distances of over 100 km. Here we contrast the extent of genetic isolation caused by fragmentation among three southern African *Ficus* species with different habitat dependencies and distributional ranges. Two of the species are restricted to forest environments, which have been fragmented

since at least the Pleistocene, and provide an indication of the long-term genetic effects of forest fragmentation. The third species is less forest-dependent, with a more general habitat association and more continuous populations. We found significant population differentiation in all three species. Populations of *F. bizanae*, a forest specialist with a highly restricted distribution, displayed the most genetic structure, followed by the second forest specialist, *F. craterostoma*. Populations of the habitat-generalist *F. sur* were the least genetically structured. Forest specialist *Ficus* species are clearly not immune to habitat fragmentation, despite extensive pollen flow, and other southern African forest trees are likely to have experienced similar or greater effects of habitat fragmentation. The strong genetic structure of *F. bizanae* suggests a limited seed dispersal range and local dispersal by the fig wasp pollinator, a possible adaptation to the limited range of its host fig tree.

Keywords: Population differentiation, gene flow, genetic diversity, niche breath

1. Introduction

Gene flow in most plants is achieved by a combination of pollen movement and seed dispersal. Gene flow acts to homogenize populations and maintains genetic diversity. Fragmentation of habitats can erode the genetic diversity of populations even if gene flow is extensive (Albaladejo et al., 2012; Ismail et al., 2012; Leonardi et al., 2012; Morán-López et al., 2016; Sampson et al., 2014), but it can nonetheless buffer the effects of fragmentation brought about by factors such as climate change and other anthropogenic disturbances (Labra et al., 2006; Byrne et al., 2007; Llorens et al., 2012; Liu et al., 2013; Bai et al., 2014; Colabella et al., 2014;).

The genetic erosion caused by fragmentation is typically reflected in the fixation of some alleles and the genetic isolation of populations (Young et al., 1996). These effects eventually result in a loss of heterozygosity, which is really the last symptom of an ongoing process (Aguilar et al., 2008; Vranckx et al., 2012). Because the loss of alleles in response to population fragmentation is caused by drift, which is slow to act,

61 the genetic effects of fragmentation are only manifested generations after
62 fragmentation occurs (Young et al., 1996; Kramer et al., 2008; Bacles and Jump, 2011;
63 Vranckx et al., 2012). Furthermore, in long lived species such as trees we cannot hope
64 to evaluate the genetic effects of fragmentation experimentally (Kramer et al., 2008;
65 Bacles and Jump, 2011). To understand the impact of fragmentation it is therefore
66 important to study habitats that have been naturally fragmented for centuries or
67 millennia.
68 South African forests are a prime example of a long-fragmented habitat. Across
69 geological time scales, uplifting and warping facilitated formation of large internal
70 mountain ranges and basins that dramatically changed the landscape of South Africa
71 (van Zinderen Bakker, 1983). This facilitated expansion of arid areas, which split
72 habitats that had previously comprised continuous forests (Sepulchre et al., 2006). In
73 addition, around 20 glacial-interglacial cycles have occurred during the last two
74 million years (Lawes, 1990) and alternating colder and warmer cycles have led to
75 periodic contraction and expansion of forest cover (Eeley et al., 1999). During the
76 Last Glacial Maximum (LGM) (18 000 years ago), the forests shrank dramatically
77 and most were eliminated entirely (Eeley et al., 1999; Lawes, 1990; Lawes et al.,
78 2007a). As with eastern African coastal forests, some remnants may have moved to
79 lower elevations whereas inland forests retreated to higher elevations, contributing
80 further to their disjunctive distributions (Axelrod and Raven, 1978). Most recently,
81 human activities such as grazing, logging and agriculture have reduced the extent of
82 forest cover and further aggravated forest fragmentation across the sub-continent
83 (Hoffman, 1997; Lawes et al., 2004; 2007b). Currently, South African forests cover an
84 area of about 4981 km^2, which is only about 0.41% of the land area of the country.
85 Furthermore, they are divided into an estimated 19470 tiny fragments with a median
86 size of less than 0.02 km^2 and a mean of just over 0.25 km^2 (Mucina et al., 2006;
87 http://bgis.sanbi.org/vegmap).
88 The responses of species to forest fragmentation are highly variable (Henle et al.,

89 2004), and may result in no discernable effects (Berens et al., 2014), a loss of genetic
90 diversity and increased population differentiation (Matolweni et al., 2000; McManus
91 et al., 2015) or even extinction of forest specialists (Lawes, 2004; Olivier et al., 2013;
92 Cooper et al., 2017;). The effects of fragmentation, although unlikely ever to be
93 beneficial, are therefore unpredictable and remain poorly understood.
94 Fig trees (*Ficus* spp., Moraceae) are a diverse and ecologically significant
95 component of the African flora (Berg and Wiebes, 1992; Shanahan et al., 2001;
96 Burrows and Burrows, 2003). Most species exhibit a monoecious breeding system
97 and they typically have wide distributions across the continent (Berg and Wiebes,
98 1992). The *Ficus* species present in South African forests vary in the extent to which
99 they are restricted to this habitat and in their overall area of distribution (Burrows and
100 Burrows, 2003). They are pollinated by small (1-2mm; Renoult et al., 2009) host
101 specific fig wasps (Agaonidae). The adults are short-lived (Warren et al., 2010) and
102 fly slowly, but can be carried passively by the wind over long distances (Ware and
103 Compton, 1994; Ahmed et al., 2009) and they are renowned for their dispersal ability
104 (Kobmoo et al., 2010; Liu et al., 2013; Bain et al., 2016). In a desert habitat one
105 species, *Ceratosolen arabicus* was recorded to disperse the pollen of its host *Ficus*
106 over a distance of 100 km (Ahmed et al., 2009). As a result, fig tree populations often
107 show little evidence of genetic isolation by distance (Liu et al., 2013; Yu and Nason,
108 2013; Wang et al., 2018; Yu et al., 2019), although smaller species, with more
109 aggregated populations, provide exceptions (Chen et al., 2011).
110 Long-term fragmentation might therefore be expected to have had less effect on the
111 population structure of fig trees than on other tree species in South African forests,
112 because as few as four immigrants per generation can effectively homogenize
113 populations (Hartl, 2000). It nonetheless remains unclear whether wasp-assisted
114 pollen flow can be sufficient to connect forest specialist fig trees into a single large
115 genetic unit across sub-continental scales, especially given that the dispersal of *Ficus*
116 seeds often extends for only short-distances, despite the services of strong flyers such

as frugivorous birds (Zhou and Chen, 2010). Seed dispersal distances in South Africa may have been reduced further by the loss of forest specialist birds and mammals after their own populations responded to fragmentation (Lawes, 2004; Olivier et al., 2013; Cooper et al., 2017).

Comparing allele numbers at microsatellite loci across species as a means of assessing genetic diversity is problematic because mutation rates differ between loci and even for the same locus, and ascertainment bias can also give misleading results (Jin et al., 1996; Kalia et al., 2011). An alternative approach is to estimate and compare genetic structure, because this is not prone to these shortcomings. We took this approach to describe the genetic effects of fragmentation on three related South African forest trees that differ in their habitat specificity and distributional ranges. Specifically, we asked whether two *Ficus* species that are restricted to forests (*F. bizanae* Hutch. & Burtt-Davy and *F. craterostoma* Midlbr. & Burret) exhibit a more developed genetic structure and less gene flow between populations than a habitat-generalist species (*F. sur* Forsk.), and whether autecological characteristics may modify the impact of fragmentation on individual species.

2. Materials and Methods

2.1. The study species

Ficus bizanae and *F. craterostoma* belong to section *Galoglychia* while *F. sur* belongs to section *Sycomorus* (Burrows and Burrows, 2003). These three *Ficus* species have different life forms, and they vary in their habitat preferences (Table 1, Fig. 1). *F. bizanae* usually grows as a free-standing tree and can reach a height of 18 m. It is usually associated with rocky habitats within coastal forest environments, where it favours gently sloping rocky scree slopes (Pers. Obs.). The figs of *F. bizanae* develop on major stems, usually in clusters of 2-3 figs emanating from a single boss. The figs reach 25- 45 mm in size. Although they do not dramatically change colour on ripening, they develop a strong smell when they turn from bright green to a pale

yellowish-green colour after the fig wasps have exited. These features suggest that both mammals and birds are likely to be attracted to the figs (Shanahan et al., 2001). *Ficus bizanae* is a local endemic that only occurs in a few populations. Its entire range is shown in Fig. 1. *Ficus craterostoma* can reach heights of up to 20 m, and often grows as a strangler of other forest trees. Its figs are located in the leaf axils, where they reach 15-20 mm in diameter and turn yellow with red spots or entirely reddish when ripe. Consequently, birds are likely to be their main seed dispersal agents (Shanahan et al., 2001). *Ficus craterostoma* has a wide distribution in Afromontane forests along the eastern side of Africa from just north of the equator to South Africa. It is also present in forests of central and West Africa. The third species, *Ficus sur,* is a large free-standing tree that grows up to 30 m in height. It is a habitat generalist, but is more common in forest and riparian habitats. The figs are produced in clusters on leafless branchlets arising from the trunk, from high in the canopy down to ground level. The figs reach 20-40 mm in diameter and turn red or dark orange with a pronounced sweet smell when ripe (Berg and Wiebes, 1992). The figs are likely to be dispersed by a wide variety of birds and mammals, including terrestrial non-volant species. *Ficus sur* has a wide distribution across Africa from the southern Cape coastal belt of South Africa northwards.

2.2. Sampling

We collected leaves or bark of the three *Ficus* species from six forests in the Eastern Cape and one forest in KwaZulu-Natal (Fig. 2 and Table 2). We dried and stored most samples in bags with silica gel crystals. Some samples were stored in a NaCL-CTAB-azide buffer (Bhattacharjee et al., 2009). Samples were collected from two types of forests as defined by Mucina et al. (2006): scarp and Southern mistbelt forests that occur as fragments along a southwest-northeast edge of southern Africa. Scarp forests are closer to the coast and mistbelt forests are more inland at higher elevations - usually above 1000 m. Montane mistbelt forests are botanically species-rich and contain subtropical floral elements. Scarp forests occur at lower

175 elevations and are characterized by high rates of endemism.

176 In total, we sampled 108 *F. bizanae* individuals from three populations, 247 *F.*
177 *craterostoma* individuals from five populations, and 186 *F. sur* individuals from six
178 populations (Table 2, Fig. 2).

179

180 *2.3. DNA extraction and genotyping*

181 Genomic DNA were extracted with the nucleoSpin, Plant II kit (Macherey-Nagel
182 GmbHandCo.KG, Düren, Germany) using standard protocols. We selected 49
183 microsatellite primer pairs that were developed for other *Ficus* species (Khadari et al.,
184 2001; Giraldo et al., 2005; Zavodna et al., 2005; Vignes et al., 2006; Ahmed et al.,
185 2007; Crozier et al., 2007; Heer et al., 2012; Tan et al., 2016). Amplifications for all
186 primers of samples of each species were ran with 15 ul of PCR mixture, including
187 ddH2O 10.6 ul, reaction Taq buffer 1.5 ul (10x), dNTP 0.3ul (10 mM), primers 0.2 ul
188 (10 uM), Taq polymerase (Takara) 0.2 ul (5U/ul) and DNA 2 ul (50 ng /ul). The
189 published cycle programs were used for each primer and suitable Tm were tested for
190 those primers showing clear bands in amplifications. We then amplified DNA using
191 all the primers again, but at their best Tms. The products were examined for
192 polymorphism on an 8% polyacrylamide gel with a 50bp DNA ladder stained with
193 silver nitrate. Twenty-three polymorphic SSR primer pairs were obtained and labelled
194 with fluorescent markers giving 8 for *F. bizanae*, 12 for *F. craterostoma* and 10 for *F.*
195 *sur* (Table S1). We genotyped the samples with these primers using an automated
196 sequencer (ABI 3730) and scored loci using GeneMarker HID v.2.05 (Holland and
197 Parson, 2011).

198

199 *2.4. Confirming usability of loci*

200 We examined the presence of any genotypic errors due to stuttering, large allele
201 dropout and null alleles using Micro-checker v.2.2.3 (Van Oosterhout et al., 2004).
202 FSTAT v.2.9.3 were used to confirm that no loci were in linkage disequilibrium with

each other (Goudet, 1995). The deviation from Hardy-Weinberg equilibrium (HWE) in each population was examined by the multi-locus exact test in GENEPOP 4.0 (Rousset, 2008). A Markov chain method was used to estimate the P-value of the test with default settings.

2.5. Genetic differentiation and structure

Global and pairwise population differentiation indexes (F_{ST}) for each species were estimated in FSTAT, jackknifing over all loci and using 1000 permutations to test significance and confidence intervals were calculated based on mean and standard error (CI = mean $\pm$ 1.96 $\times$ SE). We standardized these estimates to correct for the maximum difference that could be obtained given the variation using $F'_{ST} = F_{ST}/F_{STmax}$ as suggested by Meirmans and Hedrick (2011). RecodeData (Meirmans, 2006) was used to convert the data and imported them into FSTAT to estimate global F_{STmax} and pairwise population F_{STmax}.

We used an AMOVA as implemented in ARLEQUIN 3.5 (Excoffier and Lischer, 2010) to partition the genetic variation of each species, into within and among population components with 1000 permutations to determine significance. In addition, for *F. craterostoma* and *F. sur*, we specified two groups of populations: montane (mistbelt forest) and coastal lowland forest (scarp). We used the AMOVA to partition the genetic variation into three components (within population, among populations and between groups). A 1000 permutations were used to estimate significance.

2.6. Isolation by distance

The effect of geographic distance on genetic differentiation between populations was measured using microsatellites. Specifically, we correlated pairwise estimates of genetic distances F'_{ST} against the corresponding geographic distance with a Mantel test using the R 3.3 package 'vegan' (http://www.r-project.org/). We determined significance with 1000 permutations.

3. Results

In total, we scored 541 individual trees: 8 loci in 108 *F. bizanae*, 12 loci in 247 *F. craterostoma* and 10 loci in 186 *F. sur* (Table 2, Table S1). We found no significant linkage disequilibrium. Several populations of each species deviated from HWE and some contained null alleles (Table S2). The excess of homozygotes in these populations may be due to inbreeding, a Wahlund effect, or the presence of null alleles (Hartl, 2000; Chapuis and Estoup, 2006).

All three species exhibited significant genetic structuring of their populations, with *F. bizanae* displaying the most structure and *F. sur* the least (Table 3). Similarly, among-population genetic variance was highest in *F. bizanae*, and lowest in *F. sur* (Table 3). Significant genetic difference ($F_{CT} = 0.03$, $p = 0.02$) was found between *F. craterostoma* in mistbelt (montane) and scarp (coastal lowland) forests, while no such difference was detected in *F. sur* ($F_{CT} = 0$, $p = 0.60$).

All three species had indications of an increase in genetic distance as geographical distance increased, but isolation by distance was only significant in *F. craterostoma* (Fig. 3). *Ficus bizanae* occurs in very few forest patches, which lowered the statistical power to detect a correlation, but its F'_{ST} was twice that of *F. sur* and nearly 1.5 times that of *F. craterostoma* (Fig. 3; Table 3). Despite occurring in many forests, we were unable to detect any significant isolation by distance in *F. sur*. These findings support the impression that genetic structure is highest in *F. bizanae* and lowest in *F. sur*.

4. Discussion

Despite the exceptional ability of some fig wasps to disperse pollen over long distances (Harrison and Rasplus 2006; Ahmed et al., 2009; Kobmoo et al., 2010; Nazareno et al., 2013), we found that natural long-term fragmentation of South Africa's forests has resulted in stronger genetic isolation of populations of two habitat-specialist *Ficus* species than in the habitat-generalist *F. sur*. This is likely to be because forest populations of *F. sur* are linked genetically via individuals growing in

other habitats between the fragmented forests. The extent of genetic isolation between populations was greater in the forest specialist species with a more limited distribution (*F. bizanae*) than in the more widely distributed *F. craterostoma*. This may be linked to differences in the distances that their seeds and pollen are dispersed.

Wright (1978) proposed that observed overall values of F'_{ST} can be characterized as belonging to one of three different qualitative categories, indicating very great divergence ($F'_{ST} > 0.25$), great divergence ($0.25 > F'_{ST} > 0.15$) and moderate divergence ($0.15 > F'_{ST} > 0.05$). Based on these criteria, great divergence was found in both *F. bizanae* ($F'_{ST} = 0.23$) and *F. craterostoma* ($F'_{ST} = 0.16$), while moderate divergence was revealed in *F. sur* ($F'_{ST} = 0.11$).

The pairwise F'_{ST}'s of the three species nonetheless extend across Wright's categories, in response to the varying distances between populations (Fig. 3). More distant populations were more genetically dissimilar due to isolation by distance. This trend was only statistically significant for *F. craterostoma*, as a lack of statistical power limited our analysis of *F. bizanae*, despite it having greater genetic structure than *F. craterostoma*. For example, a comparison between the species shows that at a distance of 500 km the genetic distance between *F. craterostoma* populations is equivalent to that of *F. bizanae* populations located only 100km apart. The relationship between genetic and geographic distances between populations of the habitat generalist *F. sur* was not significant, suggesting that the gene flow is sufficient to homogenize samples that are over 600 km apart into one genetic unit (Fig. 3). This result is similar to that recorded for some other fig trees in Asia, including species closely related to *F. sur* (Kobmoo et al., 2010; Tian et al., 2015; Wang et al., 2018). The low gene flow of the two forest-specialists stems from the fact that these samples actually came from discrete populations, separated by 10's of kilometers rather than a semi-continuous distribution with individual trees more densely dispersed across the overall distributional range, as is the case for *F. sur* (Fig. 1).

Whereas *F. bizanae* was only recorded in scarp forests, *F. craterostoma* and *F. sur*

288 were recorded in both scarp and mistbelt forests. *Ficus bizanae* showed distinct
289 genetic structure within scarp forest. In *F. craterostoma*, the AMOVA revealed that
290 scarp and mistbelt forest populations were genetically distinct. Even so, pairwise F'_{ST}
291 indicate some gene flow occurs between the two forest types when subpopulations are
292 geographically close (ING and MBO). In contrast, in *F. sur* no significant difference
293 was found between scarp and mistbelt forests by AMOVA. Indeed, populations of this
294 species from distantly located forest fragments, as well as those from different forest
295 types can be genetically similar.
296 Working with animal rather than plant populations, Tolley et al. (2018) proposed an
297 idiosyncratic process of vicariance in South African forests. They concluded that
298 South African forests have undergone expansions, contractions and distribution shifts.
299 Despite our current results being insufficient to elucidate the underlying evolutionary
300 processes driving forest fragmentation in South Africa, we highlight dissimilar effects
301 of the fragmentation process on different plant species with different ecological
302 characters, as well as with some animal species.
303 The speciation process and evolutionary history of *F. bizanae* is currently
304 unresolved. In particular, it is unclear whether the species has had a wider distribution
305 in the past, or is a neo-endemic species that has always been restricted to the southern
306 coastal areas of South Africa. *F. bizanae*'s overall range is much more restricted than
307 that of *F. craterostoma* (Fig. 1; Burrows and Burrows, 2003). The greater genetic
308 isolation between its populations as compared to *F. craterostoma* suggests that the
309 ecology of the two species differs in ways that influence their response to habitat
310 fragmentation. Here we list a number of biological features of the two species and
311 then relate these to their dispersal ability. *Ficus bizanae*'s figs are borne on the trunks
312 (Burrows and Burrows, 2003) below the canopy of the forest. Phenology can be
313 asynchronous within a tree allowing wasps to disperse from mature figs to immature
314 figs on a single tree and thereby the plant can self-pollinate.
315 At a local population level, *F. bizanae*'s crop sizes are far smaller than those of the

316 other two species, and closely situated individual trees often simultaneously bear figs
317 at different stages of development. These factors may favour more local dispersal of
318 both pollen and seeds and could explain the stronger genetic structure of *F. bizanae*. *F.*
319 *bizanae*'s pollinators are produced by figs situated underneath the canopy and few or
320 no pollinators may venture above the canopy to disperse passively over long distances
321 using wind currents (Compton, et al., 2000; Harrison and Rasplus, 2006). Intermittent
322 asynchrony of *F. bizanae* crops will be conducive to pollinator cycling on the same
323 tree, and could result in selfing. This was never the case in *F. craterostoma* where
324 within-crop development is highly synchronised (Hossaert-McKey and Bronstein,
325 2001). Selfing should result in detectable inbreeding. However, if inbreeding
326 offspring suffer from inbreeding depression it will reduce or even result in a negative
327 inbreeding coefficient. In all the studied populations (bar one) of all three *Ficus*
328 species, the inbreeding coefficient was significantly larger than 0. However, the
329 excess homozygosity may be due to true inbreeding, null alleles, or Wahlund effect.
330 Parentage analysis will be required to identify the cause of the observed excess
331 homozygosity.
332 General island biogeographic effects may have selected for more localized
333 movements of *F. bizanae* pollinators as seen in the reduced dispersal exhibited by
334 animals on small islands (Macarthur and Wilson, 2001), and parasitoid wasps whose
335 hosts are highly restricted to a particular habitat (van Noort et al., 2014). *Ficus*
336 *bizanae*'s restricted range means that long distance pollinator dispersal will always be
337 unsuccessful and these pollinators may thus have evolved a reduced affinity to fly
338 upwards towards strong wind currents, thereby avoiding the risk of ending up outside
339 a forest (Smith and Bronstein, 1996; Gates and Nason, 2012; Fagan et al., 2014). Such
340 local wasp dispersal may also be responsible for restrict *F. bizanae*'s ability to found
341 new populations because mature trees in newly established forest populations will
342 never be pollinated.
343 Other factors may be restricting the ability of *F. bizanae* to colonise and establish

new populations. *Ficus* seed dispersal is influenced by species-specific fruit traits that attract different animal species (Shanahan et al., 2001; Lomáscolo et al., 2008, 2010). Primates may be the major seed dispersal vectors for *F. bizanae,* given the green colour and the sweet smell of its mature figs (Shanahan et al., 2001; Lomáscolo et al., 2008). Primates are usually not long-distance travelers and they frequently become extinct in small patches in fragmented habitats (Lawes, 2004) so low rates of seed dispersal, as well as short dispersal distances would not be surprising in *F. bizanae.*

In contrast, *F. craterostoma* trees, which bear large, dense and synchronous crops of small figs in the leaf axils, frequently protrude above the forest canopy. Although *F. craterostoma* figs are also known to be eaten by monkeys (Basabose, 2002; Linden et al., 2015), their small size and the fact that they ripen to a bright yellow and sometimes even to a reddish colour suggests that birds are important dispersers (Lomáscolo et al., 2008), and figs in the canopy are more likely to attract birds that disperse over long distances (Shanahan and Compton, 2001). Continuous forest cover across parts of South Africa is believed to have started to fragment during the late Pleistocene (Lawes, 1990; Lawes et al., 2007a; Eberle et al., 2017). The extent to which the populations of the two forest-specialist fig tree species have diverged is consistent with such long-term fragmentation of their forest habitat and agrees with conclusions based on regional climatic conditions inferred from pollen studies (Scott et al., 1997; Scott, 2002) and climatic reconstructions (Eeley et al., 1999; Eberle et al., 2017). Divergence has occurred despite the extensive pollen flow that characterizes monoecious *Ficus* species. Comparable genetic data is not available from other South African forest trees, but our results suggest that many other tree species that are restricted to South Africa's forests are likely to display similar or greater genetic effects from fragmentation, because their gene flow is likely to be less extensive than that of fig trees. Forest fragments form archipelagos in Afromontane areas across the whole eastern side of Africa, and fragmentation effects are likely to be widespread across a wide range of forest species (White, 1983).

Given the results of our study, it would be beneficial to both forest specialist *Ficus* species if nearby forest populations were linked genetically by transplanting seeds and seedlings. The artificial transfer of *F. bizanae* to other forest patches would also make populations of this rare endemic more resilient. The limited range of *F. bizanae* might nonetheless suggest that it has highly specific habitat requirements, but this remains to be confirmed.

In conclusion, we used a naturally fragmented population to study the effect of millennia of repeated forest contraction and expansion. It is clear that even for *Ficus*, with its high pollen dispersal ability, populations have become differentiated over time. Such differentiation is the result of a reduction of gene flow, which was more marked in forest specialists that are physically more isolated. The isolation was also more marked in the range-limited forest species where seed and pollen dispersal distances may both be reduced, which emphasizes the importance of individual species ecology.

Acknowledgements

We are grateful to Michael Cherry for running the FBIP program, Andrew Wannenburgh for providing forest shape files, Zander Myburg for allowing us to use his plant extraction facility. Chuan Yuan, Huai-Min Zhao and Rong-Hua Fu provided help in the Lab and with creating the maps. We thank the referee for constructive comments. Sample collections were covered by permits issued by the Department of Agriculture, Forestry & Fisheries, Republic of South Africa. This work was supported by the National Research Foundation of South Africa under FBIP grant number 98871 "The effect of habitat fragmentation on faunal diversity of Eastern Cape forests". JYD was supported by a grant-holder NRF bursary from this FBIP grant. SGC was supported by the SARCHI initiative at Rhodes University.

Appendix A. Supplementary data

The result of additional analyses are given in supplementary tables.
The data used in this paper is available as a supplementary table. (These will be uploaded in a final version)

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664

665 Table1. The habitat preferences, life forms, South African pollinators and recorded
666 seed dispersal vectors of three South African *Ficus* species.
667

Ficus (pollinator)	Habitats	Life form	Seed dispersal vector
F. bizanae (Courtella sp.)	Forest specialist[1]	Tree (up to 15-18 m), lithophytic or hemi-epiphytic[1,2]	No records, probably understory birds, bats and mammals such as monkeys
F. craterostoma (Alfonsiella pipithiensis)	Forest specialist[1]	Small tree (up to 5-10 m), shrub, lithophyticor hemi-epiphytic (occasionally reaching to 20 m) [1,2]	Birds, small rodents and Samango monkeys[3,4]
F. sur (Ceratosolen capensis)	Forest, savanna and woodland[1]	Medium to large size tree tree (up to 12 m in open woodland or to 30 m in forest) [1,2]	Birds, bats, rodents, primates (monkeys or baboons) [5,6]

668 [1] Burrows and Burrows (2003)
669 [2] Berg and Wiebes (1992)
670 [3] Gautier-Hion et al. (1985)
671 [4] Basabose (2002)
672 [5] Blecher et al. (2003)
673 [6] Linden et al. (2015)
674

675

676 Table 2. Sampled populations and sample sizes in parentheses. The three letter code
677 for each forest, the forest type and the latitude and longitude of each forest are given.

Code	Populations	Species	Forest types	Latitude	Longitude
NGO	Ngome	*F. craterostoma* (23), *F. sur* (13)	Southern mistbelt	-27.826	31.419
ING	Ingeli	*F. craterostoma* (125)	Southern mistbelt	-30.530	29.689
MKA	Mkambati	*F. bizanae* (54), *F. sur* (26)	Scarp	-31.297	29.979
MBO	Mboyti	*F. bizanae* (32), *F. craterostoma* (21), *F. sur* (56)	Scarp	-31.435	29.688
DWE	Dwesa	*F. bizanae* (22), *F. craterostoma* (48), *F. sur* (17)	Scarp	-32.280	28.848
MAN	Manubi	*F. craterostoma* (30), *F. sur* (57)	Scarp	-32.449	28.606
PIR	Pirie	*F. sur* (17)	Southern mistbelt	-32.739	27.298

678

679 Table 3. Global F_{ST} with 95% confidence interval in parentheses, F'_{ST} and among
680 population percentage of variation for each species.

Ficus species	*Global* F_{ST}	F'_{ST}	AMOVA: Percentage variation among populations
F. bizanae	0.12 (0.07-0.17) ***	0.23	11.85
F. craterostoma	0.05 (0.01-0.09) ***	0.16	6.25
F. sur	0.04 (0.03-0.05) ***	0.11	3.89

681 *** $p < 0.001$
682
683
684 **Figure legends**
685
686 Fig. 1 The distributions of *F. craterostoma* (horizontal lines, a) and *F. sur* in Africa
687 (horizontal dashed lines, b). The distributions of *F. bizanae* (filled circles, its entire
688 range), *F. craterostoma* (horizontal lines) and *F. sur* (horizontal dashed lines) in
689 southern Africa (c). Country borders in solid black bold lines and provincial borders
690 in grey lines.
691
692 Fig. 2. A more detailed depiction of the eastern part of Fig. 1 indicating sampled
693 forests. Species sampled in each location are indicated (*F. bizanae* = dots filled, *F.*
694 *craterostoma* = empty, *F. sur* = black filled) and forest distribution is given in black
695 pixels.
696
697 Fig. 3. The relationships between genetic distances (pairwise F'_{ST}) and geographic
698 distances between population pairs. *Ficus bizanae* = squares and solid line; *F.*
699 *craterostoma* = circles and dashed line; *F. sur* = diamonds and dotted line.