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

3

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21 ABSTRACT

22

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

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

43

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

45

46 **1. Introduction**

47

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

56 The genetic erosion caused by fragmentation is typically reflected in the fixation of
57 some alleles and the genetic isolation of populations (Young et al., 1996). These
58 effects eventually result in a loss of heterozygosity, which is really the last symptom
59 of an ongoing process (Aguilar et al., 2008; Vranckx et al., 2012). Because the loss of
60 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 km², 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 km² and a mean of just over 0.25 km² (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

117 as frugivorous birds (Zhou and Chen, 2010). Seed dispersal distances in South Africa
118 may have been reduced further by the loss of forest specialist birds and mammals
119 after their own populations responded to fragmentation (Lawes, 2004; Olivier et al.,
120 2013; Cooper et al., 2017).

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

133

134 **2. Materials and Methods**

135

136 *2.1. The study species*

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

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

164

165 2.2. Sampling

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

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

207

208 *2.5. Genetic differentiation and structure*

209 Global and pairwise population differentiation indexes (F_{ST}) for each species were
210 estimated in FSTAT, jackknifing over all loci and using 1000 permutations to test
211 significance and confidence intervals were calculated based on mean and standard
212 error (CI = mean \pm 1.96 \times SE). We standardized these estimates to correct for the
213 maximum difference that could be obtained given the variation using $F'_{ST} =$
214 F_{ST}/F_{STmax} as suggested by Meirmans and Hedrick (2011). RecodeData (Meirmans,
215 2006) was used to convert the data and imported them into FSTAT to estimate global
216 F_{STmax} and pairwise population F_{STmax} .

217 We used an AMOVA as implemented in ARLEQUIN 3.5 (Excoffier and Lischer,
218 2010) to partition the genetic variation of each species, into within and among
219 population components with 1000 permutations to determine significance. In addition,
220 for *F. craterostoma* and *F. sur*, we specified two groups of populations: montane
221 (mistbelt forest) and coastal lowland forest (scarp). We used the AMOVA to partition
222 the genetic variation into three components (within population, among populations
223 and between groups). A 1000 permutations were used to estimate significance.

224

225 *2.6. Isolation by distance*

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

231

232 **3. Results**

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

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

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

252

253 **4. Discussion**

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

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

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

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

287 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

344 new populations. *Ficus* seed dispersal is influenced by species-specific fruit traits that
345 attract different animal species (Shanahan et al., 2001; Lomáscolo et al., 2008, 2010).
346 Primates may be the major seed dispersal vectors for *F. bizanae*, given the green
347 colour and the sweet smell of its mature figs (Shanahan et al., 2001; Lomáscolo et al.,
348 2008). Primates are usually not long-distance travelers and they frequently become
349 extinct in small patches in fragmented habitats (Lawes, 2004) so low rates of seed
350 dispersal, as well as short dispersal distances would not be surprising in *F. bizanae*.

351 In contrast, *F. craterostoma* trees, which bear large, dense and synchronous crops of
352 small figs in the leaf axils, frequently protrude above the forest canopy. Although *F.*
353 *craterostoma* figs are also known to be eaten by monkeys (Basabose, 2002; Linden et
354 al., 2015), their small size and the fact that they ripen to a bright yellow and
355 sometimes even to a reddish colour suggests that birds are important dispersers
356 (Lomáscolo et al., 2008), and figs in the canopy are more likely to attract birds that
357 disperse over long distances (Shanahan and Compton, 2001). Continuous forest cover
358 across parts of South Africa is believed to have started to fragment during the late
359 Pleistocene (Lawes, 1990; Lawes et al., 2007a; Eberle et al., 2017). The extent to
360 which the populations of the two forest-specialist fig tree species have diverged is
361 consistent with such long-term fragmentation of their forest habitat and agrees with
362 conclusions based on regional climatic conditions inferred from pollen studies (Scott
363 et al., 1997; Scott, 2002) and climatic reconstructions (Eeley et al., 1999; Eberle et al.,
364 2017). Divergence has occurred despite the extensive pollen flow that characterizes
365 monoecious *Ficus* species. Comparable genetic data is not available from other South
366 African forest trees, but our results suggest that many other tree species that are
367 restricted to South Africa's forests are likely to display similar or greater genetic
368 effects from fragmentation, because their gene flow is likely to be less extensive than
369 that of fig trees. Forest fragments form archipelagos in Afromontane areas across the
370 whole eastern side of Africa, and fragmentation effects are likely to be widespread
371 across a wide range of forest species (White, 1983).

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

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

386

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388

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399

400 **Appendix A. Supplementary data**

401

402 The result of additional analyses are given in supplementary tables.

403 The data used in this paper is available as a supplementary table. (These will be
404 uploaded in a final version)

405

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663
664

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

<i>Ficus</i> (pollinator)	Habitats	Life form	Seed dispersal vector
<i>F. bizanae</i> (<i>Courtella</i> 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
<i>F. craterostoma</i> (<i>Alfonsiella pipithiensis</i>)	Forest specialist ¹	Small tree (up to 5-10 m), shrub, lithophytic hemi-epiphytic (occasionally reaching to 20 m) ^{1,2}	Birds, small rodents and Samango monkeys ^{3,4}
<i>F. sur</i> (<i>Ceratosolen capensis</i>)	Forest, savanna and woodland ¹	Medium to large size 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	<i>F. craterostoma</i> (23), <i>F. sur</i> (13)	Southern mistbelt	-27.826	31.419
ING	Ingeli	<i>F. craterostoma</i> (125)	Southern mistbelt	-30.530	29.689
MKA	Mkambati	<i>F. bizanae</i> (54), <i>F. sur</i> (26)	Scarp	-31.297	29.979
MBO	Mboyti	<i>F. bizanae</i> (32), <i>F.</i> <i>craterostoma</i> (21), <i>F.</i> <i>sur</i> (56)	Scarp	-31.435	29.688
DWE	Dwesa	<i>F. bizanae</i> (22), <i>F.</i> <i>craterostoma</i> (48), <i>F.</i> <i>sur</i> (17)	Scarp	-32.280	28.848
MAN	Manubi	<i>F. craterostoma</i> (30), <i>F. sur</i> (57)	Scarp	-32.449	28.606
PIR	Pirie	<i>F. sur</i> (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.

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