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A fig tree in a concrete jungle: fine-scale population genetic structure of the cluster fig *Ficus racemosa* in an urban environment

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Abstract Urban vegetation is an essential requirement in cities for mitigating pollution, heat island effects and providing food and shelter to urban fauna. Efforts to conserve and augment green cover in cities, however, often lack data on the genetic diversity of urban trees, which could be crucial to the success of such programmes. We investigate the population genetics of the cluster fig *Ficus racemosa*, which occurs naturally in Indian cities and is a keystone species for urban fauna. Genetic analysis of 51 *F. racemosa* trees in urban Bangalore, India, shows no evidence of inbreeding; the overall inbreeding coefficient (F_{is}) across 12 microsatellite markers (0.0366) was non-significant with no evidence of heterozygote deficit. Spatial genetic structure (SGS) analysis of 47 trees showed an overall negative relationship between kinship coefficient and spatial distance, with strong SGS at distances <1 km. The absence of heterozygote deficit is likely due to the fig's obligate mutualistic association with fig wasps which pollinate their flowers even across long distances. However, the strong SGS at short distances could result from clumped seed dispersal close to natal trees. Therefore, the pattern of population genetics for *F. racemosa* from urban Bangalore likely results from short-distance seed dispersal and long-distance pollen flow. Despite the scattered and fragmented nature of green areas within cities, these gene mobility factors maintain robust population genetics in *F. racemosa* even at low population densities. The same may not apply for *Ficus* species that are planted as vegetative cuttings in cities and therefore may have limited genetic diversity.

Keywords Figs · Keystone resource · Pollen movement · Population genetic structure · Bangalore · India

Introduction

Urban areas need to replenish and preserve a minimum amount of their green cover to mitigate harmful pollution levels (Yang et al. 2008; Escobedo et al. 2011; Roy et al. 2012) and the urban heat island (UHI) effect (Tso 1996; Wong and Yu 2005; Edmondson et al. 2016) in cities. Urban vegetation also serves as an important source of food and shelter to a variety of vertebrate and invertebrate fauna (Corlett 2005, 2006; Acar et al. 2007; Somme et al. 2016) and large trees function as keystone resources in urban parks (Stagoll et al. 2012). However, current plans for the maintenance and expansion of urban green areas are often ineffective due to a lack of adequate data on the value and sustainability of different types of vegetation within an urban environment (Roy et al. 2012; Pataki 2013). The population genetic structure of trees in urban environments is likely to be one of the key pieces of data that could guide conservation and planting efforts in urban greening programmes. However, apart from a few studies that evaluate gene and pollen flow for trees across a fragmented and urbanised landscape (Nagamitsu et al. 2014; Davies et al. 2015; Noreen et al. 2016), few studies address this lacuna in our knowledge of urban vegetation.

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In Indian and Southeast Asian cities, an important component of urban vegetation consists of fig trees of the genus *Ficus* which are often planted and maintained for religious and aesthetic reasons. With over 800 species distributed throughout the tropical and warm temperate regions of the world, the genus *Ficus* Linn. (Moraceae) is one of the largest and most diverse genera of woody plants (Berg 1989). *Ficus* spp. are considered to be a keystone plant resource in forests in Southeast Asia and the Neotropics for many frugivores such as primates, bats, squirrels and hornbills (Borges 1993; Dew and Wright 1998; Shanahan et al. 2001; Muscarella and Fleming 2007). Apart from their keystone status in forests, figs also provide important resources to various fauna in urbanised areas (Corlett 2005, 2006; Lok et al. 2013). In Indian cities, several species of figs such as *Ficus benghalensis, Ficus religiosa* and *Ficus racemosa* are often found in parks, temples and road-sides, where they are planted and/or protected due to their religious significance. With their year-round fruiting cycles, large crops and nutrient rich fruits, these fig trees are likely to be important food resources for primates, bats, frugivorous birds and squirrels that have adapted to living in urban habitats in India. Furthermore, figs have an obligate mutualistic association with fig wasps (Hymenoptera: Chalcidoidea: Agaonidae) which pollinate their flowers, and the system also plays host to a variety of non-pollinating parasitic wasps (Cook and Rasplus 2003; Herre et al. 2008; Borges 2015). Therefore, the long-term survival of *Ficus* spp. in urban environments is essential for the future persistence of their associated wasp communities (Cook and Rasplus 2003), as well as for the many vertebrates relying on fig fruits as a food resource. Few studies currently document the population genetic structure of keystone trees such as those of *Ficus* spp. in urban areas, although such studies are likely to be useful for the long-term management and conservation of local tree cover, biodiversity and urban wildlife.

Studies on the population genetics of several *Ficus* spp. reveal that the spatial genetic structure (SGS) of any species of *Ficus* is likely to be dependent on its reproductive mode (Nazareno et al. 2013). Approximately half of all known fig species are monoecious, bearing figs which contain seeds and from which wasps also emerge (Berg 1989). In dioecious *Ficus* spp., however, male trees bear figs that produce only pollen and serve as breeding sites for pollinating wasps, while female trees bear figs that produce only seeds, not pollen, and in which wasps do not breed (Berg 1989). Dioecious fig species in general exhibit moderate to low genetic diversity (Chen et al. 2011; but see Dev et al. 2011), restricted gene flow among populations and high SGS (Wang et al. 2009; Dev et al. 2011; Chen et al. 2011; Yu and Nason 2013; but see Zhou and Chen 2010). These patterns of genetic diversity and structuring in dioecious *Ficus* spp. are attributed to the occurrence of these trees in relatively high densities, and the lower efficiency of long-distance dispersal in their pollinators as compared to monoecious fig species (Wang et al. 2009; Chen et al. 2011; Yu and Nason 2013; Nazareno et al. 2013; but see Yu et al. 2010; Zhou and Chen 2010). Monoecious *Ficus* spp. are known for their low population densities (Michaloud and Michaloud-Pelletier 1987; Borges 1993; Anstett et al. 1997; Nazareno and de Carvalho 2009; Wang et al. 2009) and long-distance pollen dispersal (Nason et al. 1996, 1998; Compton 2002; Zavodna et al. 2005; Harrison and Rasplus 2006; Ahmed et al. 2009). Despite low population densities, the long-distance pollen dispersal in monoecious figs is thought to limit genetic drift, homogenise inter-population genetic diversity and maintain high genetic diversity within populations, leading to low SGS (Nazareno and de Carvalho 2009; Nazareno et al. 2013; Bain et al. 2016).

Long-distance pollen dispersal in monoecious figs—the longest recorded distance being 160 km (Ahmed et al. 2009)—is possible as female pollinator wasps use wind-mediated dispersal to cover large distances over short periods of time (Compton et al. 2000; Compton 2002). Therefore, despite female pollinator wasps having life spans of no more than 24 to 48 h (Kjellberg et al. 1988; Dunn et al. 2008; Ghara and Borges 2010), pollen movement across long distances is common in monoecious figs. However, rising temperatures due to global warming have raised new concerns about the lowering of life-spans in pollinator fig wasps (Jevanandam et al. 2013), a phenomenon that could have important repercussions on the survival and genetics of *Ficus* populations world-wide.

In this study, we investigate the population genetic structure of the fig tree *Ficus racemosa* in a rapidly urbanising city, Bangalore, in India. Unlike avenue figs such as *F. benghalensis* which are specifically planted (probably from cuttings) along highways or near temples throughout India, figs that provide limited shade such as *F. racemosa* are not often planted, but are nurtured in situ wherever their saplings develop (RM Borges, pers. obs.). Over the last ten years, Bangalore has seen a huge upsurge in urbanisation. Despite rapid concretization, Bangalore still has several pockets of green areas which support wildlife in this urban area (https://www.google.co.in/maps/@12.9854033,77.6133353,22803m/data=!3m1!1e3). In these areas and in scattered locations across the city, *F. racemosa* trees provide important food and nesting resources to urban fauna which include meso-bats (*Cynopterus* spp.), mega-bats such as the Indian flying fox (*Pteropus giganteus*), funambulid palm squirrels, and birds such as barbets and rose-ringed parakeets. The main aim of this study is to record population genetic features for *F. racemosa* in a city, and to generate data that will serve as a baseline for future investigations on the effect of urbanisation on this important keystone species.

Materials and methods

Natural history of the study species

Ficus racemosa is a monoecious fig species that belongs to the subgenus *Sycomorus* and whose distribution spans the Indo-Australasian region. It is a common species found all over India, and is abundant in southern India, especially in the urban area of Bangalore where this study was conducted. The tree grows to around 10 m in height, and bears figs in cauliflorous bunches on the trunk and large tree limbs. *Ficus racemosa displays* synchronous or asynchronous flowering within trees (Krishnan and Borges 2014), with each crop developing over 2–2.5 months and trees producing on average 2–6 crops a year (Krishnan and Borges 2014). Since flowering is usually asynchronous among trees, fig production is spread across the year in the population. *Ficus racemosa* is pollinated by the agaonid wasp *Ceratosolen fusciceps* and is parasitized by six other non-agaonid species of non-pollinators (Ghara and Borges 2010). The fruits are largely dispersed by bonnet macaques, bats and squirrels in the urban environment.

The fig–fig wasp system is a brood-site pollination mutualism in which the fig, which is a globular enclosed inflorescence, serves as a site for seed production and as a nursery for pollinating fig wasps to breed in. A typical fig reproductive cycle consists of five phases (Galil and Eisikowitch 1968). 1) In the pre-floral phase, male and female flowers are undeveloped, and the fig is small, hard and green. 2) In the female reproductive phase, female flowers are pollen-receptive, figs are larger with softened ostioles (openings into the fig, through which female pollinator wasps can enter) and the fig trees emit volatile signals to attract female pollinator fig wasps (Ware and Compton 1994; Grison-Pigé et al. 2002). In a typical monoecious fig such as *F. racemosa*, pollinator female wasps enter the fig and pollinate some female flowers. The pollinators also oviposit into the ovules of other female flowers, causing these flowers to form galls, within which the pollinator offspring develop (Galil and Eisikowitch 1968). Foundress pollinator wasps die, generally within the fig, shortly after pollination and oviposition. 3) In the interfloral phase, seeds and fig wasp progeny develop within the galled flowers; the fig grows is size, but remains very hard and green. 4) In the wasp emergence/dispersal phase, the fig becomes soft and turns yellowish in color. The usually wingless male wasps emerge first, and mate with freshly eclosed females. Female pollinator wasps collect pollen from male flowers and leave the fig through an exit hole cut by the cooperative efforts of pollinator males who die within their natal fig. 5) In the post-floral phase, the fig changes color to become red/yellow/orange/purple and emits volatiles to attract seed dispersers (Borges et al. 2011).

Plant material

Leaf samples from trees were collected in the city of Bangalore, Karnataka, India, where they are patchily distributed and can be found growing in temples, residential areas, parks and campuses of educational institutions. A total of 51 trees were sampled in and around 4 main areas (Fig. 1): 6 trees from Gandhi Krishi Vijnana Kendra campus area (GKVK), 27 trees from the Indian Institute of Science campus area (IISc), 6 trees from Lal Bagh botanical gardens (Lal Bagh) and 12 trees from Gurupurahalli (Gph; the area is also called Kurubarahalli). The geographical coordinates of 47 of these trees were obtained using a Garmin GPS orientation system. Leaves (2–3 per tree) were collected in ziploc plastic bags and transported to the laboratory for DNA extraction. DNA was extracted from most samples on the same day of collection, though some samples were stored at −80 °C for 1–2 days before extraction.

DNA extraction

Total genomic DNA was extracted from 100 mg of fresh leaves using the Plant DNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. The DNA extracts were subjected to electrophoresis in 1.5% agarose gels to check their quality and quantified by comparison with a known concentration of lambda DNA (Genei, Bangalore). The DNA extracts were stored at −20 °C until required.

Microsatellite amplifications

Microsatellite markers are polymorphic DNA loci that contain repeated nucleotide sequences (Sunnucks 2000; Ellegren 2004). Variation in the number of repeats for a specific locus create alleles of varying lengths, though the length of the repeated unit for that locus is generally the same across most alleles. Microsatellites are a widely used tool for population studies such as linkage analysis and association studies, and can also be used to identify individual organisms (Sunnucks 2000; Ellegren 2004). Microsatellite analysis involves amplification of microsatellite DNA through a polymerase chain reaction (PCR) with fluorescently labelled primers. The labelled PCR products are then analyzed by capillary electrophoresis to separate alleles by size.

All PCRs were carried out in 10 μl reactions containing 20 mM Tris (pH 8.5), 50 mM KCl, 1.5 mM MgCl$_2$, 0.01% gelatin, 0.2 mM dNTPs, 0.5 μM labelled forward primer (Applied Biosystems), 0.5 μM reverse primer, 2 U Taq DNA polymerase (Genei, Bangalore) and ~20 ng DNA template, except for primer pair Frac110, for which PCRs were carried out with 1 mM MgCl$_2$ and 0.1% TritonX-100 instead of 0.01% gelatin. All forward primers were labelled at the 5′ end with the fluorescent dyes 6-FAM (Frub391, Frac83,
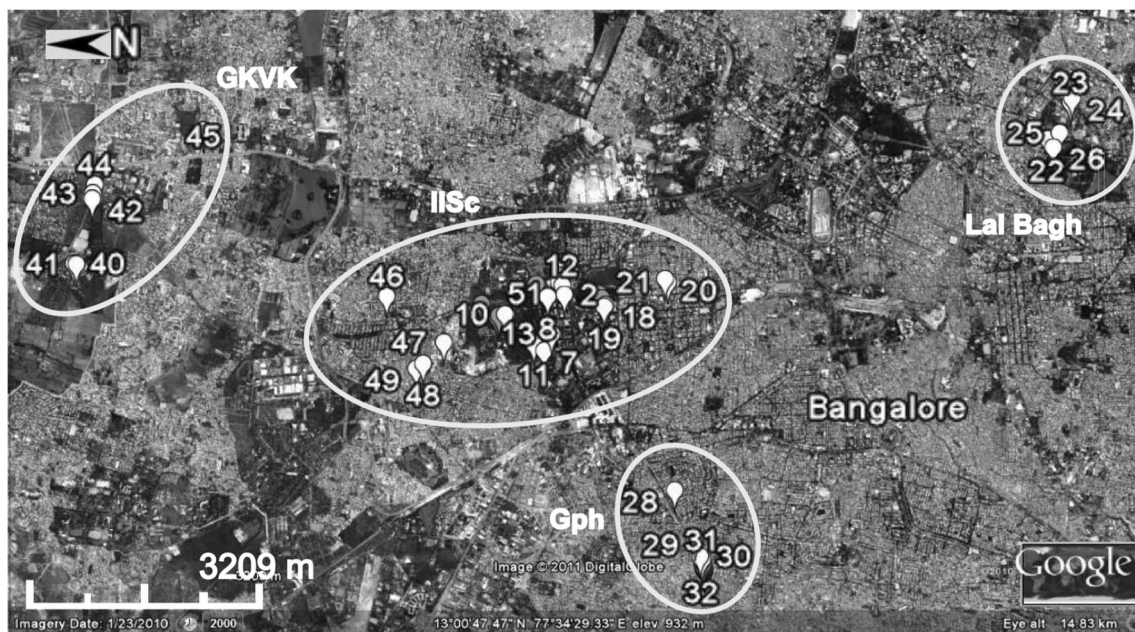
Fig. 1 The study site in Bangalore, India, showing the positions of trees sampled and the areas from which they were sampled. Gandhi Krishi Vijnana Kendra campus (GKVK), the Indian Institute of Science campus (IISc), Lal Bagh botanical gardens (Lal Bagh) and Gurupurahalli (Gph). All trees are denoted by numbers. Trees growing in very close proximity to each other may have some of their labels obscured by the others

Frac113 and Frac202), VIC (Frac29, Frac244, and Frac61), NED (Frac13, Frac86) and PET (Frac110, Frac241, Frub398). All primers used were from Crozier et al. (2007), with the exception of the reverse primer for Frac202, which is a modified primer (sequence kindly provided by Ross Crozier, pers. comm.).

PCR conditions used were as follows: an initial 2 min denaturation step at 94 °C was followed by 35 cycles at 94 °C for 30 s for denaturation, annealing for 45 s and extension at 72 °C for 1 min; with a final extension for 10 min at 72 °C. Annealing temperatures for the different primers had to be standardised again and are mentioned in Table 1, as those mentioned in Crozier et al. (2007) did not give satisfactory amplification. All PCR products were visualised in a 2% agarose gel and then subjected to genescan using an automated ABI 3130 Genetic Analyser. The alleles were visualised and scored using the software PeakScanner v.10 (freely downloadable from Applied Biosystems (http://www.appliedbiosystems.com/absite/us/en/home/support/software-community/free-ab-software.html).

Data analyses

Overall allele frequencies at each locus, observed number of alleles (na), gene diversity (h) (Nei 1978), Shannon's genetic diversity index (I), observed (Ho) and expected heterozygosity (He) were measured using the software POPGENE v1.31 (Yeh et al. 1997). Linkage disequilibrium between different genes was checked using Genepop v4.0 (Rousset and Raymond 2007). Null allele frequencies were estimated using Microchecker v2.2.0 (Van Oosterhout et al. 2004). Wright's F-statistics were calculated and their significance tested through permutation tests (10,000 iterations) using the software SPAGeDi (Hardy and Vekemans 2002).

All spatial genetic analyses were carried out with data on the 47 trees for which geographical coordinates were available. Since the trees sampled were from 4 distinct areas (Fig. 1) that were separated from each other by distances between 4.5 km to 13.5 km, the hypothesis that these trees formed distinct sub-populations was tested using the software STRUCTURE v 2.3.3 (Pritchard et al. 2000). The analysis was done without information on the actual geographic locations of the individuals used. To detect the value of K (total number of clusters/sub-populations) that maximised the probability of the data, simulations were run for 20 replicates for each value of K from 1 to 8 with the following program settings: Admixture model, correlated allele frequencies, burn-in length = 10,000, and MCMC repetitions = 10,000. An assignment test was carried out to segregate all individuals into K sub-populations using the value of K that maximised the likelihood of the data. All individuals were assigned to the K populations probabilistically by using a burn-in = 100,000 and MCMC repetitions = 100,000.

The spatial genetic structure of the population was also analysed using an estimator of the kinship coefficient (F_{ij}) described by Loiselle et al. (1995). To investigate the relationship between kinship and geographic distance, 16 distance classes (ranging from 0.1 km till 14 km) were defined (such

Table 1 Details of microsatellite primers for *F. racemosa* (primers from Crozier et al. 2007)

Name	Primer sequence	Repeat Motif	Annealing Temp (°C)	Allele size range (bp)
Frub391	Frub391F: 5′ AGATGTCAAATAAGGTCAGCT 3′ Frub391R: 5′ AGATGCAGTTCCATACAATTC 3′	$(TG)_{19}$	54	142–170
Frac29	Frac29F: 5' CCAGGCATATGCATCTTGTGTA 3' Frac29R: 5' CTCGCAGCTTTCCTCGCA 3'	$(AC)_8G$ $(CA)_2(TA)_4$ $G(TA)_3$	54	178–209
Frac13	Frac13F: 5' CACGTTCACGCTGCAAACT 3' Frac13R: 5' GATAGAGAAGGCATATCCAGAG 3'	$(TC)_8CC$ $(TC)_4...$ $(AC)_{11}$	54	113–140
Frac110	Frac110F: 5' CCAGAACAGGTTGGACGTAAC 3' Frac110R: 5' GGATTACCCGCGCTATGAAGT 3'	$(CA)_{13}$	46	122–169
Frac83	Frac83F: 5' TGAACCTTCAATAACATCGGGTT 3' Frac83R: 5' CTCATGCAATCATAGCACTCA 3'	$(GACA)_3$ $G(AC)_5G$ $(TA)_4(CA)_5$ $... (AC)_{10}$	53	182–238
Frac244	Frac244F: 5' ACCTAGAATCATAACCCTTCA 3' Frac244R: 5' CCCATAAACTACGATACATAAGA3′	$(AC)_{17}$	54	275–306
Frac86	Frac86F: 5' TGTCACTGTTCTGTTTGTGC 3' Frac86R: 5' CAGCCAACCCTCAAGTATAAGA 3'	$(TC)_{13}(CA)_{10}$	54	154–193
Frac241	Frac241F: 5' GGCTCAAGCAAGGGATGGA 3' Frac241R: 5' CGAGCTCATTTGTTTATCGACC 3'	$(TG)_{19}$	54	254–283
Frac113	Frac113F: 5' CTGGGTCCTCTCTATTACCAAC 3' Frac113R: 5' GCTGATCTTCTCGGATGCT 3'	$(AC)_9GC$ $(AC)_4$	54	228–245
Frub61	Frub61F: 5' GTACACTCTCTTAGCTGCC 3' Frub61R: 5' TACCTTTCTCTGGACATTC 3'	$(TC)_{24}$	45	146–156
Frub398	Frub398F: 5' GTACCTTAGATTCTAGTGTGAG 3' Frub398R: 5' TGGGATCTCATGAACTATTTAC 3'	$(GT)_7AT$ $A(TG)_6C$ $(GT)_{13}$	54	196–223
Frac202	Frac202F: 5' AGAGATATGATGTTCTAGTGCA 3' Frac202R2: 5' ACCATGCTCTGCTTTAACTAGC 3'	$(TG)_{13}$	54	213–228

that each distance class had at least 25 pairs of individuals) and the average multilocus kinship coefficient (F_{ij}) per distance class was calculated. The significance of the relationship between the kinship coefficient (F_{ij}) and distance was tested with permutation tests (10,000 iterations). All analyses for investigating the spatial genetic structure were carried out using SPAGeDi (Hardy and Vekemans 2002).

Results

Every individual *F. racemosa* sampled had a unique multilocus genotype. The number of alleles per locus ranged from 8 to 27 alleles with a mean of 14.6 alleles across the 12 microsatellite markers used (Table 2). Details of allele size ranges are provided in Table 1. The mean values of Nei's gene diversity (h) and Shannon's diversity index (I) were 0.7985 (range = 0.5821–0.9321) and 2.0194 (range = 1.3059–2.9318) respectively (Table 2).

The observed heterozygosities (H_o) for all loci were found to be lower than the expected heterozygosities (H_e), except for loci Frub391, Frac244 and Frac241, where H_o was found to be greater than H_e. The inbreeding coefficients (F_{is}), for loci Frub391, Frac110, Frac83, Frac244 and Frac202 were found to deviate significantly from Hardy-Weinberg equilibrium as evidenced by permutation tests in SPAGeDi (Table 3). Loci Frac110, Frac83 and Frac202 showed significantly positive deviations from Hardy-Weinberg equilibrium, indicating an excess of homozygotes. The excess of homozygotes observed in loci Frac110 and Frac202 could be due to the presence of null alleles (Table 4). The other loci deviating significantly from Hardy-Weinberg equilibrium showed negative deviation, indicating an excess of heterozygotes. The global F_{is} incorporating all loci (Table 3) showed a low, non-significant positive value of 0.0366 ($p > 0.05$), with no evidence of heterozygote deficit.

Loci Frac29, Frac110 and Frac202 showed evidence of null alleles and the estimates of null allele frequencies by 4 different methods (van Oosterhout, Chakraborty, Brookfield1 and Brookfield2) are shown in Table 4. There was no evidence for scoring error or large allele drop out. Significant linkage disequilibrium occurred between 6 out of the 66 possible pairs of loci (Supplementary Information, Table S1). The loci pairs showing significant linkage disequilibrium are: Frac110 with Frac202 ($p < 0.01$), Frac86 with Frac29 ($p < 0.05$) and Frub61 ($p < 0.01$); and Frac83 with Frac244 ($p < 0.05$), Frub61 ($p < 0.05$) and Frub398 ($p < 0.05$).

Although the trees were sampled from 4 distinct areas (Fig. 1), analysis using STRUCTURE v. 2.3.3 showed that

Table 2 Genetic diversity parameters for *F. racemosa*

Locus	Observed number of alleles (N_a)	Nei's gene diversity (h)	Shannon's Diversity index (I)
Frub391	21	0.9202	2.7418
Frac29	8	0.7409	1.5766
Frac13	14	0.8747	2.257
Frac110	11	0.5821	1.3059
Frac83	15	0.8251	2.0579
Frac244	16	0.7403	1.8705
Frac86	27	0.9321	2.9318
Frac241	11	0.7472	1.7432
Frac113	13	0.812	1.9522
Frub61	8	0.7745	1.6209
Frub398	18	0.8845	2.4291
Frac202	13	0.7482	1.746
Mean	14.5833	0.7985	2.0194
Std Dev	5.4516	0.0982	0.4882

the value of K that maximised the likelihood of the data was found to be 2 (Fig. 2), indicating the presence of 2 sub-populations. The assignment test grouped nearly all of the trees from Gurupurahalli (Gph), three trees from GKVK, and three trees from Lal Bagh along with four trees from the IISc campus into one sub-population. All the other trees were assigned to the other sub-population, which consisted of almost all the trees from IISc campus, two trees from GKVK and three trees from Lal Bagh. Three trees – one each from Gph, IISc campus and Lal Bagh – showed a 50% probability of belonging to either population (Fig. 3).

The kinship coefficient analysis showed a strong spatial structure in distance classes <1 km, with the first four distance

Table 3 Observed heterozygosity (*Ho*), expected heterozygosity (*He*) and fixation indices (*Fis*) for *F. racemosa* in the city of Bangalore, India

Locus	H_o	H_e	F_{is}
Frub391	1	0.9247	−0.0889*
Frac29	0.6383	0.7408	0.1323
Frac13	0.8511	0.8838	−0.0111
Frac110	0.4468	0.579	0.2153*
Frac83	0.7021	0.8332	0.1407*
Frac244	0.8723	0.7497	−0.1634*
Frac86	0.9149	0.9405	−0.0291
Frac241	0.8298	0.7433	−0.1243
Frac113	0.8085	0.83	0.0135
Frub61	0.7447	0.7904	0.0026
Frub398	0.8298	0.8993	0.058
Frac202	0.4444	0.7665	0.3747**
Mean	0.7569	0.8068	0.0366

* $p < 0.05$; ** $p < 0.01$

classes (0–0.1 km, 0.1–0.2 km, 0.2–0.5 km and 0.5–1 km) showing F_{ij} values of 0.0659, 0.0573, 0.041 and 0.0226 respectively (Fig. 4). Of the rest, 2 distance classes (4–5 km and 6–7 km) were significantly more negative than expected. Overall, the genetic correlation between individuals decreased with distance, resulting in a significant correlation between Loiselle's kinship coefficient and spatial distance. Regression with log distance explained the variance in kinship better than the regression with linear distance (SPAGeDi, log regression $r^2 = 0.015664$; linear regression $r^2 = 0.06233$). The mean slope of the regression with log distance was −0.01415 and with linear distance was −0.00279 ($p < 10^{-4}$ for both). Spatial analyses were re-run with the same dataset after all loci exhibiting null alleles were removed to ensure that the observed results were not due to a bias introduced by null alleles. The relationship was found to be significant with similar regression values (log regression $r^2 = 0.01729$, linear regression $r^2 = 0.05728$) and values of slopes (log distance slope = −0.0138, linear distance slope = −0.00298, with $p < 10^{-4}$) for both.

Discussion

Urban areas must protect, maintain, and if possible, expand their green cover to combat increasing levels of pollution and heat island effects. This involves conceptualising and implementing urban greening programs, many of which are ineffective due to a lack of information about the relative value and sustainability of populations of different types of trees in an urban environment (Roy et al. 2012; Pataki 2013). Although many studies focus on the advantages and disadvantages of having urban tree cover (Roy et al. 2012; Pataki 2013), information about the biology and population genetics of different plant species will also be important for designing tree-planting and conservation policies in cities.

Usually the provenance of plants, especially trees in cities is unknown, or they may come from a single local nursery that may have multiplied these plants by vegetative cuttings or from a localised seed bank. This raises two important issues for urban tree populations—monocultures and low genetic variability.

Monocultures of trees are a tempting option for urban beautification and greening projects due to reliability in form, colour, growth rate and predictability of suitable or unsuitable characteristics (Santamour 2004). In deforested tropical areas, industries and governments often encourage plantations of fast-growing monoculture stands to cater to the growing demands for wood products (Cossalter and Pye-Smith 2003). Despite their advantages, monocultures are likely to be more vulnerable to a variety of ecological disturbances, and are thought to have negative impacts on local environment factors such as soil quality and wildlife diversity (Cannell 1999;

Table 4 Null allele frequency estimates in *F. racemosa*

Locus	Null Present	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
Frub391	no	−0.0448	−0.0415	−0.0415	0
Frac29	yes	0.0748	0.0829	0.0652	0.0652
Frac13	no	0.0059	0.0069	0.0064	0.0064
Frac110	yes	0.1205	0.1269	0.0829	0.0829
Frac83	no	0.0547	0.0642	0.0546	0.0546
Frac244	no	−0.1021	−0.0764	−0.0704	0
Frac86	no	0.0057	0.0057	0.0055	0.0055
Frac241	no	−0.0705	−0.0603	−0.0549	0
Frac113	no	0.0184	0.0173	0.0153	0.0153
Frub61	no	0.048	0.0464	0.0387	0.0387
Frub398	no	0.0264	0.0239	0.0219	0.0219
Frac202	yes	0.1994	0.2716	0.1828	0.254

Cossalter and Pye-Smith 2003; Erskine et al. 2006; Piotto 2008). The widespread destruction of ash trees by emerald ash-borer beetles (Poland and McCullough 2006) and the depredations of the Dutch elm disease (Karnosk 1979) in urban USA and Canada, highlight the dangers of tree monocultures in urban areas.

Low genetic variability is another grave concern in the long-term future of urban tree species. Since the genetic variability of a population helps it survive and adapt to habitat changes, preserving genetic diversity is essential to survive ecological challenges such as diseases, parasites, and environmental changes (Lande 1988; O'Brien and Evermann 1988). Inbreeding, which is measured by heterozygote deficit, can cause loss of fitness and increase the risk of extinction for small populations (Frankham 1995; Carr and Eubanks 2002). In urban environments, most plant populations experience fragmentation and patchy distributions that can hinder gene flow and lead to heterozygote deficit, resulting in the negative impacts of inbreeding (Van Rossum 2008; Wang et al. 2010; Wodkiewicz and Gruszczyńska 2014; Bartlewicz et al. 2015).
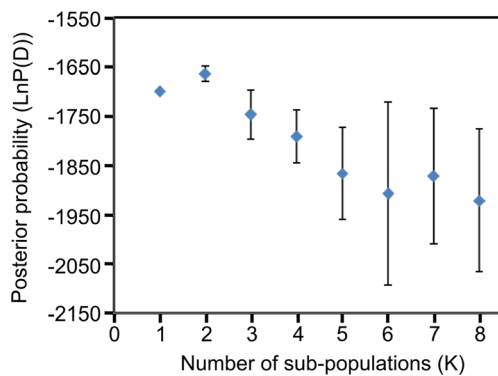
In urban India, monoecious fig trees, especially the cluster fig *F. racemosa*, are good candidates for urban greening programs for several reasons. Despite the scattered and fragmented nature of green areas within cities, the long-distance pollen dispersal mechanisms of monoecious fig species (Nason et al. 1996, 1998; Compton 2002; Zavodna et al. 2005; Harrison and Rasplus 2006; Ahmed et al. 2009) allows them to maintain robust population genetics even at low population densities (Nason et al. 1998; Nazareno and de Carvalho 2009; Nazareno et al. 2013; Bain et al. 2016). Besides this, *Ficus racemosa* trees with their large crops of nutrient-rich fruit, year-round fruiting capacity with 2–6 fruiting cycles per year and unique hymenopteran assemblage (Ghara and Borges 2010; Krishnan and Borges 2014) can serve as a keystone resource for a variety of urban vertebrate and invertebrate fauna (Ranganathan and Borges 2009; Ranganathan et al. 2010; Borges et al. 2011). The negative effects of monocultures on wildlife diversity may even be offset in *Ficus* monocultures due to their keystone status, though there have been no studies to prove this. Due to their religious significance (Shanahan 2016), these trees are occasionally planted in parks and temples and are less likely to be felled than other non-fig trees, an important consideration in Indian cities such as Bangalore, where much of the green cover is lost due to tree felling for development projects.
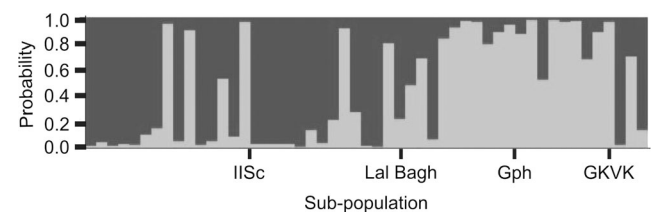

Fig. 2 Mean posterior probabilities (LnP(D)) ± SD over 20 runs for each value of K (number of sub-populations). The value of K that maximises the probability of the data (highest mean posterior probability or LnP(D)) is considered to be the number of sub-populations in the given dataset

Fig. 3 Genetic structure of the sampled *F. racemosa* population as defined by STRUCTURE v.2.3.3. Each vertical box represents one of the sampled trees and the probability of each individual being assigned to a particular population is denoted by partitioning the corresponding box into different colors to denote the different populations

Fig. 4 Variation of Loiselle's pairwise kinship coefficient over mean distances (km) in *F. racemosa*. Dashed lines indicate the 95% confidence intervals and all points outside these intervals are significant. Significance values and number of pairs of individuals in each distance class are also indicated. A steep drop in the kinship coefficient is observed in the distance intervals <1 km
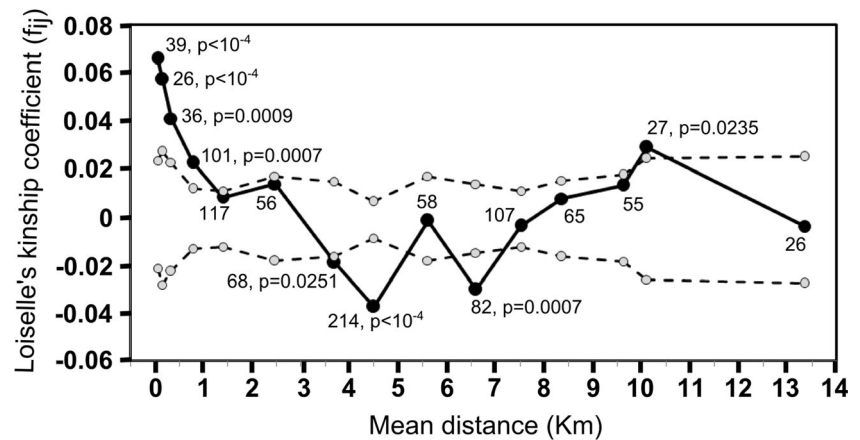
Although much is known about the biology of *F. racemosa* within urban environs (Wang et al. 2005), especially within Bangalore (Ranganathan and Borges 2009; Ranganathan et al. 2010; Ghara and Borges 2010; Borges et al. 2011; Krishnan and Borges 2014; Yadav and Borges 2017), and a recent study documents the genetic diversity of the species across south and south-east Asia with a very limited sample in this city (Bain et al. 2016), there have been no investigations on the spatial genetic structure of *F. racemosa* in Bangalore or indeed any other city in its worldwide range. The estimates of genetic diversity as reported in Bain et al. (2016) indicate that the *F. racemosa* population in Bangalore exhibits a positive inbreeding coefficient suggesting a significant heterozygote deficit. However, in our study, the overall inbreeding coefficient (F_{is}) across 12 microsatellite markers for the *F. racemosa* population in Bangalore showed a low, non-significant positive value of 0.0366, with no evidence of heterozygote deficit. This is similar to the patterns of genetic diversity observed in other monoecious fig species (Hamrick et al. 1991; Nazareno and de Carvalho 2009; Nazareno et al. 2013). This difference in the estimate of inbreeding coefficient between our current study and Bain et al. (2016) could stem from differences in sampling; while Bain et al. (2016) use only 19 individuals from a single location in Bangalore (from within the IISc campus), we have used 51 individuals from across 4 different locations in the city.

Although we have sampled in 4 different locations across the city, an analysis to detect sub-sets within our data (using STRUCTURE v 2.3.3, Pritchard et al. 2000) indicated that the sampled trees could be divided into 2 subpopulations, and assignment tests grouped most of the trees from the IISc campus to one subpopulation, while the trees from Gph formed a major part of the second subpopulation. Trees from the other two sampled areas, Lal Bagh and GKVK, were equally divided between these two subpopulations. It is interesting to note that although the trees from Gph and IISc campus form two sub-populations, these two locations are closest to each other (~4 km apart) compared to all other sampled locations. Spatial

correlation analyses reveal that pairwise kinship coefficients (Loiselle et al. 1995) were independent of geographic distances above 1 km, indicating the lack of spatial genetic structuring at this scale. At distances below 1 km, however, pairwise kinship coefficients showed a strong negative correlation with distance, suggesting that individuals spatially close to each other were more related than those farther away at this distance scale. Since most of the trees at the IISc campus are located within 1 km of each other, it is likely that they form a highly related sub-population of *F. racemosa* in Bangalore. In the light of these results, it is clear that the estimates of inbreeding obtained by Bain et al. (2016) for the Bangalore population of *F. racemosa* were high because of their sampling from only within the IISc campus.

Unlike avenue figs such as *F. benghalensis* which are planted along highways and temples mostly from cuttings, figs such as *F. racemosa*, which provide limited shade, are not often planted but protected and cared for wherever their saplings grow (RM Borges, pers. obs.). The strong negative correlation between kinship and distance exhibited by *F. racemosa* at short distance scales is similar to the SGS pattern exhibited by several other dioecious fig species such as *F. hispida, F. exasperata* and *F. hirta* (Wang et al. 2009; Dev et al. 2011; Yu et al. 2010; Chen et al. 2011). Unlike these dioecious fig species, however, *F. racemosa* does not exhibit SGS even at very large distance classes (Bain et al. 2016). This pattern of strong SGS at short distances in *F. racemosa* is surprising given that long distance pollen flow is prevalent in monoecious figs. However, such an SGS pattern in figs could arise from short-distance or clumped seed dispersal (Zhou and Chen 2010) with seedlings germinating close to their natal trees (Nason and Hamrick 1997). *Ficus racemosa* fruits are eaten by fruit bats (Tang et al. 2007), several species of which are abundant in Bangalore, and by small rodents such as palm squirrels that often deposit clumps of seeds directly under or very close to natal trees (A. Krishnan, pers. obs.). Therefore, the pattern of SGS observed in this study for a population of *F. racemosa* from urban Bangalore, is likely to

be due to short-distance seed dispersal and long-distance pollen flow.

Fig trees serve as keystone resources in a variety of ecosystems, and monoecious figs could be especially well adapted to surviving and enriching urban vegetation. It is therefore important to assess the importance and advantages of including monoecious fig trees in urban greening programs, and to monitor the health and genetic features of such trees in urban areas to maintain strong and thriving populations. In our study, we show that *F. racemosa* trees in urban Bangalore show spatial genetic structuring at fine-scale distances, and that genetic estimates indicate that the population is robust and shows no signs of inbreeding.

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