OXFORD

Original Article

Surrounded by concrete: genetic isolation of *Tillandsia recurvata* L. in an urban landscape in southeastern Brazil

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ABSTRACT

Increasing urban expansion has resulted in the decline of many natural and seminatural communities globally. However, the connectivity and genetic structure of species that survive in these urban landscapes have received little attention, especially with regard to epiphytic plants. This study aimed to describe and evaluate the connectivity and genetic structure of populations of *Tillandsia recurvata*, a highly abundant and widely distributed atmospheric epiphyte, amongst urban green spaces within a city. A total of 288 *T. recurvata* individuals were sampled across 65 trees throughout the city of Alfenas in South-East Brazil. We designed seven novel microsatellite markers and used four cross-amplified loci to determine the basic genetic structure of *T. recurvata*. All populations showed high global spatial genetic structure, which indicated low connectivity between urban populations. The findings of this study, as well as evidence from previous assessments of *T. recurvata* genetic structure, suggest that the combined effects of genetic drift, breeding system, and dispersal may have dictated the connectivity of these urban populations. This study represents an important step towards understanding epiphyte population structure within urban landscapes. Low connectivity across urban landscapes is likely to benefit epiphytes such as *T. recurvata*, due to their adaptability and high tolerance; this suggests a bleak future for many other more sensitive epiphytic species under predicted urbanization globally.

Keywords: epiphytes; urban; ecology; tropical forest; microsatellites; population genetics; bromeliads, canopy

INTRODUCTION

Predictions suggest that 66% of the global human population will occupy urban areas by the year 2050, an approximate increase of 2.5 billion people from current levels (United Nations 2018). This predicted expansion of urbanization has highlighted the need to anticipate and preserve species and habitat connectivity within urban green spaces, which are not well understood (Muderere *et al.* 2018, Yang *et al.* 2020). This lack of knowledge also presents a barrier to our current understanding of the implications of urbanization for biodiversity (Newbold *et al.* 2015). Current evidence suggests that urbanization may have severe consequences for habitat availability (Gaston 2010, Liu *et al.* 2016, Kondratyeva *et al.* 2020). Specifically, landscape fragmentation (i.e. the loss or alteration of habitat spatial configuration) (Fahrig 2003, Zambrano *et al.* 2019) has been shown to negatively affect the abundance and quality of remnant habitats within a previously continuous landscape, especially in the absence of a wide variety and abundance of urban arborization (Chetcuti 2020). Urbanization may thus result in the fragmentation of large and interconnected populations (Toledo-Aceves *et al.* 2014). The probability of a genetic bottleneck and genetic drift may subsequently increase in these smaller populations, as a result of reduced gene pool size (Ackerman and Zimmerman 1994, Ward 2006). Evidence has shown that inbreeding depression and population differentiation then become more likely, under the pressure of sustained dispersal limitations between isolated populations (Wright *et al.* 2013). As such, low connectivity would be expected in urban landscapes, as well as high spatial genetic structure (Chaves *et al.* 2021).

Plants present an ideal subject to explore the connectivity of urban landscapes, largely due to their strong sensitivity to landscape fragmentation (Fenster and Marten-Rodriguez 2007, Rios and Cascante-Marin 2016). Specifically, changes in

habitat conditions can lead to limited opportunity for colonization in fragmented landscapes (Bhatt *et al.* 2015), as well as altered pollen dispersal (Llorens *et al.* 2012). As such, urban ecosystems typically contain a lower diversity of plant species than primary forests or pasture trees (Poltz and Zotz 2011). Current evidence suggests that low plant diversity in urban landscapes has resulted from the dominance of opportunistic and horticulturally derived plants, which are better adapted to an urban environment and are able to capitalize on limited establishment opportunities (Williams 2015). Specialist adaptations within synanthropic plants can include self-fertilization (hereafter referred to as selfing) and rapid growth, both of which have been shown to offer reproductive assurance within fragmented landscapes (Agrawal and Whitlock 2012). Specifically, reproductive assurance describes the ability of selfing plants to persist in spite of low genetic diversity within small and isolated populations (Agrawal and Whitlock 2012). In the absence of the selfing strategy, inbreeding depression can reduce population fitness through the accumulation of recessive deleterious alleles in their homozygous state (Agrawal and Whitlock 2012). Selfing plants are able to lower the proportion of these accumulated lethal mutations over time, as a result of natural selection (Byers and Waller 1999, Bosse *et al.* 2018). As such, the selfing strategy of plants can prevent population decline (Eckert *et al.* 1996). This suggests that selfing plants may be able to retain low connectivity across highly fragmented and isolated urban ecosystems, although the extent of this connectivity has not been established.

Similarly low levels of connectivity can also be predicted for plants with an epiphytic life cycle. Angiosperm epiphytes, in particular, frequently express high selfing rates and rapid growth capability (Fenster and Marten-Rodriguez 2007, Rios and Cascante-Marin 2016). Furthermore, epiphytes are mechanically dependent on trees for physical support (Benzing 1990) and, as such, are highly susceptible to both direct (deforestation, large-scale disturbance events) and indirect (available colonization substrate, microclimate alterations—light, relative humidity, temperature) effects of landscape fragmentation (Benzing 1990, Ribeiro 2009, Larrea and Werner 2010, Zotz 2013). However, relatively little has been revealed about the genetic structure of this understudied group of plants within fragmented habitats, and even less so in urban ecosystems (Izzudin and Webb 2015). As a result, epiphytes are ideally suited to the study of plant connectivity in urban landscapes. They are also a globally very taxonomically diverse group of plants, accounting for 10% of all extant vascular plant species (Zotz *et al.* 2021). In addition, they are capable of reaching high levels of abundance in urban landscapes (Fenster and Marten-Rodriguez 2007, Chaves *et al.* 2021). Bhatt *et al.* (2015), for example, found a total of 30 epiphyte species from 12 families on 254 urban host trees in the eThekwini Municipal Area of South Africa. Equally, Alvim *et al.* (2020) found 56 species of epiphytes, belonging to 22 families, on 827 urban host trees in Juiz de Fora city in Brazil.

Currently, fewer than 50 studies have been published globally on the genetic structure and connectivity of epiphytes. The majority of these papers have discussed epiphyte population genetics in the context of microsatellite marker cross-amplification and species biology. There has been considerably less evidence relating to the population genetics of epiphytes in relation to fragmented landscapes, such as rural sites (Aoki-Gonçalves *et*

al. 2020, Chaves *et al.* 2021), industrial mining sites (Lavor *et al.* 2014), and modified natural forests or reserves (Alcantara 2006, Goetze *et al.* 2015). Contrasting outcomes of landscape fragmentation were reported for epiphyte genetic structure across these papers, largely dictated by the variety of taxonomic groups and breeding strategies that have been described. For instance, selfing epiphytes typically showed population differentiation and high inbreeding in fragmented landscapes (Chaves *et al.* 2018, 2021). In contrast, self-incompatible epiphytes show low spatial genetic structure and high gene flow, although this connectivity appears to be dependent on an effective pollination strategy (Alcantara *et al.* 2006, Lavor *et al.* 2014, Goetze *et al.* 2015, Chaves *et al.* 2021). Therefore, the genetic structure and connectivity of epiphytes in fragmented landscapes appears to be determined by reproductive strategy and, as such, a similar driving factor can be predicted for epiphyte connectivity in urban landscapes. However, to the our knowledge, there are currently no direct assessments of epiphyte connectivity in planted trees within urban ecosystems.

Furthermore, amongst the identified studies that investigated epiphyte population genetics in fragmented landscapes, several taxonomic groups have received considerably more attention. Bromeliaceae, for instance, has been the focus of the majority of these studies (83%—five of six studies). This taxonomic bias is probably the result of the wide distribution and high abundance of many bromeliad species throughout the Neotropics (Paula *et al.* 2016). More specifically, *Tillandsia recurvata* L. has been featured most frequently in fragmentation studies (Chaves *et al.* 2018, 2021), as well as in broader investigations of epiphyte population genetics (Soltis *et al.* 1987, Solózano 2010).

This concerted focus on *T. recurvata* may be due to several key factors. For instance, *T. recurvata* has been found in high abundances throughout a wide geographical range, typically located within tropical and subtropical North and South America (Soltis *et al.* 1987), as well as deciduous forest, coastal sand dune scrub, and semideciduous forest (Bernal 2011). Moreover, this atmospheric bromeliad has been characterized as one of the most adaptable plants in the Western Hemisphere (Foster 1945), with valuable xerophytic qualities, such as small leaf size, Crassulacean Acid Metabolism (CAM) photosynthesis, and absorbent leaf scales. *Tillandsia recurvata* also exhibits opportunistic traits, such as selfing and high growth rates, which have allowed this species to reach extreme levels of abundance within fragmented landscapes, such as urban areas (Soltis *et al.* 1987, Benzing 1990, Chaves *et al.* 2021). As such, these traits make *T. recurvata* an ideal model to investigate the connectivity and genetic structure of epiphytic plants within understudied urban ecosystems.

In the absence of these empirical data to show the connectivity of *T. recurvata* (or other epiphytic species in urban landscapes), we are unable to make wider conclusions about the implications of urban ecosystems for epiphyte connectivity and diversity (Izzudin and Webb 2015). The consequences of this lack of evidence may be most keenly observed in the highly biodiverse tropics (Lewisohn and Prado 2005). Brazil, for example, harbours ~9.5% of all species worldwide (Lewisohn and Prado 2005), as well as one of the largest proportions of epiphytes and threatened plant species globally (4967 taxa in 1235 genera and 232 families) (Nieder 2001, Moraes *et al.* 2014, Freitas *et al.* 2016). In parallel with the current lack of evidence
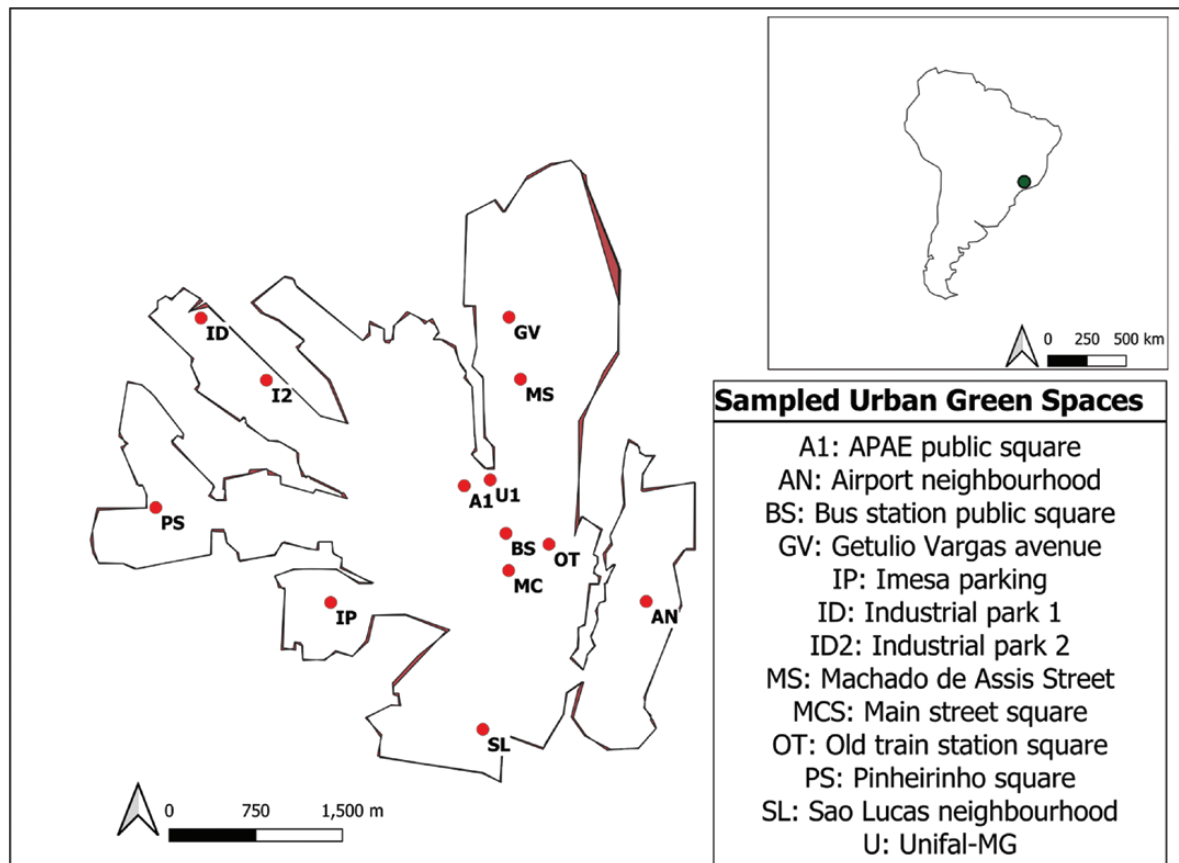
Figure 1. Position of each sampled urban green space across the city of Alfenas, Minas Gerais state, southeast Brazil. Each site is marked by an abbreviation. A1: APAE (Associação de Pais e Amigos dos Excepcionais) public square; AN: Airport neighbourhood; BS: bus station public square; GV: Getulio Vargas avenue; IP: Imesa parking; ID: industrial park 1; ID2: industrial park 2; MS: Machado de Assis Street; MCS: Main street square; OT: old train station square; PS: Pinheirinho square; SL: Sao Lucas neighbourhood; U: Unifal-MG.

worldwide, the connectivity of Brazil's urban ecosystems has not been studied. This may have direct implications for the proportion of all species that are under threat from urbanization in Brazil (~10% of 1172 endangered species) (Bernard et al. 2019), especially where the urban human population is expected to rise by 170% before the year 2050 (Paiva et al. 2020). To effectively anticipate the implications of urbanization for Brazil's species diversity in the future, as well as in urban ecosystems across the globe, an improved understanding of connectivity in urban landscapes will be instrumental. To improve our understanding of the connectivity of remnant epiphyte populations in urban landscapes, this study aimed to describe and evaluate the connectivity (genetic structure and spatial genetic structure) of populations of *T. recurvata*, a highly abundant and widely distributed atmospheric epiphyte, amongst urban green spaces within the city of Alfenas in southeastern Brazil.

MATERIALS AND METHODS

Study species

Tillandsia recurvata is an exclusively wind-dispersed species, and produces a large number of seedlings with the ability to attach to a phorophyte, or nonorganic substrates such as power cables (Wester and Zotz 2010). *Tillandsia recurvata* shares this ability with other members of the genus *Tillandsia* (Wester and Zotz 2010). This species favours environments defined by low light, high humidity, and low airflow (Caldiz et al. 1993). The exact limit of its seed dispersal range is unknown. However, the small light seeds (~5.0 mm) and low seed terminal velocity suggest a high dispersal potential (Chilpa-Galván et al. 2018). This is likely to facilitate the wide geographical range of *T. recurvata* (Victoriano-Romero et al. 2017). This species exhibits both clonal growth and a selfing reproductive strategy (Birge 1911).

Study site

The city of Alfenas (21°25′59.99″S, 45°56′59.99″W) is located in the south of Minas Gerais state in southeast Brazil, situated 335 km from the state capital of Belo Horizonte and 300 km from São Paulo. The city covers an area of 849 km² at an elevation of 888 m a.s.l. (Fig. 1). The climate is humid subtropical, with an average annual temperature of 21°C. Average annual precipitation is ~1252 mm. The highest levels of rainfall occur in the summer months (December–February), resulting in a wet and a dry season (Arruda et al. 2013). Average wind speeds range between 5.9 and 7.7 km/h, typically moving in a southeast direction. Alfenas contains large urban developments that produce high levels of human-driven disturbance, with a human population of ~80 973 individuals and a population density of 95.21/km². This has resulted in the limited survival of small vegetation patches of trees within streets

and squares across the city, with only a small proportion of the city's population inhabiting rural areas (6.2%, 4598 of 80 973 individuals in the year 2021). Urban tree compositions in Alfenas are also quite distinct from the semideciduous natural vegetation, with the marked presence of exotic species. The functional diversity of Alfenas' urban trees was considered to be low, of small size, and exhibited entomophily, zoochory, evergreen leaves, and dry fruits (Monalisa-Francisco and Ramos 2019).

Sample collection

Thirteen urban green spaces were selected for sampling along streets and squares throughout Alfenas. The criteria for the selection of these urban green spaces were determined by the minimum number of trees per urban green space (five trees) and a minimum number of *T. recurvata* individuals per tree (more than three individuals per tree). All 13 of the urban green spaces were also located across an area of ~12.4 km²and the distance between green spaces in streets and squares ranged from 330 m to 5 km, which provided an adequate coverage of the entire city. Five trees within each of the 13 urban green spaces were randomly sampled (see Fig. 2 for typical examples of sampled trees). Wherever possible, five *T. recurvata* individuals were collected per tree in each urban green space and special care was taken to avoid the collection of samples when connected to other conspecifics. This yielded a combined total of 288 *T. recurvata* individuals that were collected across 65 trees. Fieldwork was conducted between April and June 2021. Samples were oven dried at 60°C for 48 h and stored in individual paper bags, before being transported to Edge Hill University, United Kingdom, for genetic analysis. Voucher specimens are currently held at the Biology Department at Edge Hill University.

Laboratory procedures

Genomic DNA was extracted from dry leaf tissue, using a modified Cetyltrimethylammonium bromide (CTAB) extraction method described by Tel-Zur *et al.* (1999). Purity of the DNA samples was indicated by the A_{260}/A_{280} ratio (1.56–2.29). Seven microsatellite markers were initially tested for amplification success within the *T. recurvata* populations of this study. These markers were originally designed for other bromeliad species and were reported to have been successfully cross-amplified for *T. recurvata* by Chaves *et al.* (2018) (CT5, E6B, VGC01, VS10, E6, PAD07, ngFOS_12).

The microsatellite loci were single-plexed and amplified using a PCR volume of 10 μL, containing: 1 μM DNA template, 5.0 μM MyTaq Plant-PCR Kit, 0.2 μM forward primer (tagged with individual fluorochromes—FAM, VIC, PET), 0.2 μM reverse primer, 0.4 μM $MgCl_2$ and 3.7 μM nuclease-free water. A Veriti 96-Well Thermal Cycler (Applied Biosystems) was used to perform a touchdown PCR programme, as described by Palma-Silva *et al.* (2007). Successful PCR amplification was verified through ethidium bromide (EtBr) gel electrophoresis, using 2% agarose gels.

Of the original seven microsatellites, only four were amplified with sufficiently high levels of success (>40%). Consequently,
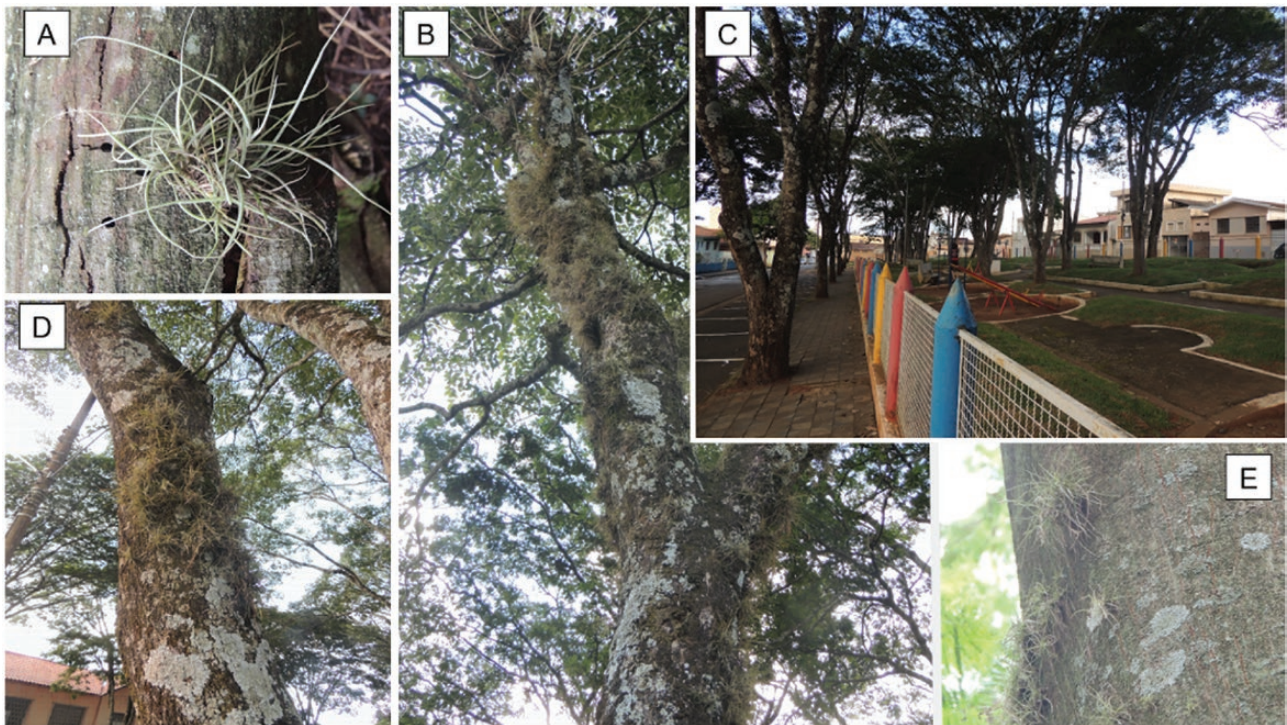

Figure 2. *Tillandsia recurvata* in Alfenas city. A single *T. recurvata* individual (A), an urban tree with a larger colony of *T. recurvata* individuals (B), an urban square (A1) with many large trees, almost all colonized by *T. recurvata* individuals (C), and trees with a smaller number of *T. recurvata* individuals (D and E) in Alfenas city. Photos by Flavio N. Ramos.

seven additional novel, species-specific, microsatellite markers were designed during this study to ensure sufficient levels of polymorphism for the detection of all multilocus genotypes (MLGs). Microsatellite candidate loci were selected from genomic short read sequence data from *T. recurvata* obtained from NCBI (7.2 Gb) (Sayers *et al.* 2022). The BioProject accession and accession numbers are PRJNA701548 and SRX10089449, respectively.

The microsatellite identification software Krait (0.5.2) (Du *et al.* 2018) was used to detect suitable microsatellites, both genome-wide and in the noncoding regions of *T. recurvata*. The single sequence repeats (SSRs) were refined to a minimum number of seven di-, tri-, or tetra-nucleotide repeat motifs. These sequences were further limited to SSRs of more than 100 bp in length and low GC content (<50%). Krait (0.5.2) (Du *et al.* 2018) was also used to design the primers for the selected SSR sequences, in conjunction with the integrated Primer3 software. The criteria for primer selection included: a primer length of 18–26 bp, an optimal melting temperature (T_m) of 54–59°C and GC content of <50%. These designed primers were single-plexed and amplified using the following PCR cycle: initial denaturation (95°C for 3 min), 34 cycles of 95°C for 30 s, annealing for 30s (JP01–JP12: 54°C, 4873TD + 35251TD: 56°C, 19286TD: 53°C, 5044TD + 186664TD + 214633TD: 58°C), 72°C for 1 min, and a final extension of 72°C for 5 min.

A combined total of 11 successfully amplified microsatellites were selected from the cross-amplified and designed loci sets, which are detailed in Table 1. These microsatellites were subsequently fluorescently tagged. The PCR product sizes of these 11 microsatellites (CT5, E6b, VGC01, VS10, JP02, JP03, JP04, JP05, JP09, JP11, TD73) were evaluated through capillary electrophoresis across all 288 *T. recurvata* individuals. This study used an AB3500 Genetic Analyser (Applied Biosystems, Warrington, UK), 50-cm capillary array (Applied Biosystems). A 10-μL reaction volume was formulated from 8.9 μL Hi-Di formamide, 0.1 μL LIZ500 size standard (Life Technologies, Warrington, UK), and 1.0 μL of PCR product. Raw scoring data were processed using TANDEM (1.07) (Matschiner 2009) and peaks were binned to integer allele sizes, to ensure results were consistent with the repeat unit of each microsatellite. All primer information has been made available through the Dryad Digital Repository at the point of publication (https://datadryad.org/stash/share/YOiJjLCVX9hQcw4Z92qSjHrY0iuAECBGK7XuzFmeIuY).

Data analysis

Clonal replicates were detected using the R package 'poppr' (v.2.3.0) (RStudio Team 2021) (Kamvar 2004). The data were screened for the presence of repeated MLGs. An unbalanced number of samples and MLGs would indicate the presence of clonal replicates. Standard output for population genetics, to determine genetic variation and distribution within our *T. recurvata* populations, was then produced using the R packages 'gstudio' and 'diveRsity' (RStudio Team 2021). Results included average number of alleles per locus (A), allelic richness (A_r), observed heterozygosity (H_o), expected heterozygosity (H_e), Hardy–Weinberg equilibrium (HWE) and inbreeding

Table 1. Cross-amplified microsatellite markers, originally tested by Chaves *et al.* (2018) and designed microsatellite loci for *Tillandsia recurvata*. NA, not applicable as the information was unavailable. Locus name, primer sequence, annealing temperature (T_a, °C), repeat motif, base-pair range, alleles and original source are given.

Locus	Primer sequence 5′→3′	Repeat motif	Base pair range	Alleles	Reference
CT5	F:AATGAGTTTCAGTTTTAGAAGC R:CCAAGAAAAGAACGGATCA	$GA_{(25)}$	189	5	Boneh, Kuperus and Tienderen (2003)
e6b	F: CGTACGAAGGTAAGCACAA R: CCGTTGAAGAGGTTAGAGG	$CAA_{(12)}$	151	5	Boneh, Kuperus and Tienderen (2003)
VgC01	F: GCTAGGGTTTCACCCCAAAT R: TCAGCCTCTGATCCATCTCC	$CT_{(16)}$	208–218	6	Palma-Silva *et al.* (2007)
Vs10	F: GAATCGAGTCGGTGTGACCT R:CCATACCTCAATTCCTCATTCG	$AC_{(10)}CT_{(5)}$	187–196	12	Neri *et al.* (2015)
4873TD	F:TCTCGGTAGCTCAGTCCG R:AGAGAACGAGAGAAGAAGGG	TC	125	7	This study
JP02	F:AGTTAGCTGTAGTTCTTTCCA R:TGGAAGCAAGTAAAAGGGT	ATAG	120–140	NA	This study
JP04	F:CTGGAAGCAAGTAAAGGGT R:TGTAGTTTTCCAGCTTTCCA	ATAG	120–140	NA	This study
JP05	F: CGCAATTCAGTAGCTCATG R: TGAGCATGAGTCCGTGAT	ATAG	120–140	NA	This study
JP09	F:AGTAGCTCAGTTCTTTCTAGC R:ACGGTATGCCCACTAAGT	ATAG	120–140	NA	This study
JP10	F:GCTCATTTAGTTTTCCAGCTT R:CTGGAAGCAAGTAAAAGGGT	ATAG	120–140	NA	This study
JP11	F:CTCAGTCTCCCCACTAGG R:TGGGCATAGTAGAGTAGTTATTT	ATAG	120–140	NA	This study

coefficient (F_{is}). A chi-squared test (χ^2-test) with a Monte Carlo permutation procedure (999 replicates) was used to detect departures from HWE. Linkage disequilibrium (LD) was assessed with the 'poppr' package, which compared the relationship between loci (*rrd*) (Agapow and Burt 2005) against 999 permutations. A distance-based redundancy analysis (dbRDA) was performed using the 'vegan' package (Oksanen *et al.* 2017), v.2.4-2. This analysis was restricted by the geographical location of each sample, in order to calculate the proportion of the variation explained by distance (isolation by distance—IBD).

Spatial patterns of genetic variability were determined through a spatial principal component analysis (sPCA), using the package 'adegenet' (Jombart 2008, Jombart and Ahmed 2011), v.2.0.1. The sPCA scores were then multiplied by Moran's *I* to form a spatial autocorrelation, which summarized allelic differences between individuals. Global and local genetic structure were calculated through a Monte Carlo permutation, to indicate the degree of genetic similarity between plots. A neighbourhood-by-distance connection network was selected and all axes were retained. This was dictated by the equal variance explained across all axes (Jombart 2008). Ward hierarchical clustering was then used to assign each sPCA axis to a cluster group, using the function dist() to generate a distance matrix. The function hclust() was applied to build a hierarchy of clusters, with each individual sample allocated to a cluster. Each axis and subsequent cluster group explained a proportion of the variance observed. Cluster groups were then assigned a distinct colour and plotted, relative to each of the five trees within a population (hereafter defined as all of the *T. recurvata* individuals sampled within each individual tree) according to their geographical position within a map of the study site (QGIS, v.3.12.0-București, QGIS Development Team 2023).

RESULTS

Eleven microsatellite loci revealed low levels of genetic diversity within populations. Polymorphism within the 11 sampled loci was sufficiently high to detect all MLGs (282) across 282
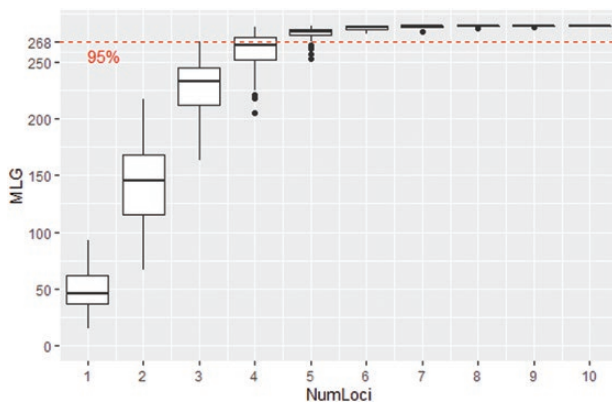

Figure 3. Genotype curve, assessing the ability of sampled loci to identify all multilocus genotypes (MLGs) within the population. The confidence cut-off point (95%) is indicated on the graph as a dashed red line. NumLoci (number of loci) ranges between one and 11. A minimum of five loci were required to identify all MLGs.

sampled individuals (Fig. 3), determined through power analysis. The equal number of MLGs and samples indicated an absence of clones within the data set, as anticipated from the experimental design.

The total number of alleles observed per population summed across all loci ranged from 50 to 105 (*A*, Table 2) and mean allelic richness (A_r) ranged from 3.02 to 4.88. Observed heterozygosity ranged from 0.25 to 0.4 across the majority of populations. Expected heterozygosity ranged from 0.59 to 0.74. Inbreeding coefficients (F_{is}) were not significantly different between populations, although the values were relatively high (range 0.41–0.63). All populations showed nonsignificant linkage disequilibrium, with the exception of populations GV ($rd = 0.04$, $N = 25$, $P \leq$ .01) and U ($rd = 0.025$, $N = 25$, $P \leq$ .01).

dbRDA indicated a nonsignificant and weak IBD pattern of IBD between populations ($R_{2adj} = .17$; $F = 1.08$, df = 2, $P = .16$). Significant global structure was detected through sPCA ($P \leq$ .0004, $\lambda = .0002$). No significant local structure was detected ($P = 1.0$, $\lambda = .00003$), meaning that the frequencies of alleles within populations were more similar at neighbouring sites. Eight distinct clusters of spatial genetic similarity were identified. These coloured cluster groups are illustrated in Figure 4, showing the arrangement of spatial genetic structure across each of the five trees per population.

DISCUSSION

This study applied empirical evidence to describe and evaluate the connectivity and genetic structure of populations of *T. recurvata*, a highly abundant and widely distributed atmospheric epiphyte, amongst urban green spaces within a city. *Tillandsia recurvata* populations exhibited high global spatial genetic structure and low genetic diversity within Alfenas city, which was typical of selfing epiphytes, as well as in relation to other assessments of *T. recurvata* genetic structure. However, this was not the result of IBD.

In this study, *T. recurvata* was found in small populations, isolated to multiple clusters of low tree density throughout Alfenas city. This species has also been reported to inhabit power cables and nonorganic substrates, although these populations are found in much smaller numbers (Wester and Zotz 2010). The low tree density in Alfenas city was driven by land-use change and deforestation for urban expansion, which probably led to the fragmentation of a previously continuous landscape (Defries *et al.* 2010), and also by the low number of planted trees in its streets and squares (Monalisa-Francisco and Ramos 2019). This trend has been mirrored across many urban areas (Arshad *et al.* 2020). Evidence from Toledo-Aceves *et al.* (2014) suggests that this low tree density may have reduced the size of a previously large and interconnected population, potentially due to dispersal limitations (Cascante-Marín *et al.* 2009). A genetic bottleneck probably occurred within these condensed *T. recurvata* populations, which translated into reduced genetic diversity as a result of small gene pool size (Ackerman and Zimmerman 1994). Genetic drift then possibly ensued from this bottleneck and altered the frequency of alleles over time within the finite *T. recurvata* populations (Chaves *et al.* 2021). This may have resulted in the high spatial genetic structure that was observed in this study and has indicated that genetic drift may have dictated the connectivity

Table 2. Summary of genetic variation in populations of *Tillandsia recurvata* in Alfenas city, southeast Brazil. A, APAE (Associação de Pais e Amigos dos Excepcionais) public square; AN, airport; BS, bus station public square; GV, Getulio Vargas avenue; IP, Imesa parking; ID1, industrial district 1; ID2, industrial district 2; MS, Machado de Assis street; MCS, main street square; OT, old train station; PS, Pinheirinho square; SL, São Lucas; U, unifal-MG. *N*, sample size; *A*, total number of alleles observed per population summed across all loci; %, percentage of total alleles observed across population samples per locus; *Ar*, allelic richness; *H*$_o$, observed heterozygosity; *H*$_e$, expected heterozygosity; *F*$_{is}$, relatedness of individuals under a model of random mating. *Significant Hardy–Weinberg equilibrium *P*-value from the χ^2 goodness-of-fit test.

| Population	*N*	*A*	%	*A*$_r$	*H*$_o$	*H*$_e$	*F*$_{is}$	*F*$_{is}$ range
A	22.0	83	28.85	4.04	0.35	0.64	0.45*	0.39–0.51
AN	7.0	50	20.45	3.51	0.37	0.67	0.45*	0.25–0.57
BS	19.0	69	24.34	3.45	0.33	0.65	0.48*	0.38–0.58
GV	21.0	105	37.99	4.88	0.38	0.74	0.48*	0.40–0.54
ID2	20.0	81	29.7	3.7	0.32	0.66	0.51*	0.38–0.62
ID1	19.0	82	28.81	3.95	0.25	0.68	0.63*	0.55–0.69
IP	18.0	63	21.92	3.02	0.25	0.59	0.57*	0.45–0.67
MCS	9.0	65	22.93	4.21	0.4	0.68	0.41*	0.28–0.51
MS	19.0	72	29.37	3.63	0.36	0.62	0.42*	0.33–0.49
OT	17.0	86	31.52	4.32	0.37	0.73	0.49*	0.40–0.57
PS	19.0	91	32.07	4.07	0.37	0.69	0.46*	0.35–0.54
SL	19.0	87	32.79	4.07	0.37	0.69	0.45*	0.36–0.55
U	20.0	84	30.28	4.03	0.38	0.72	0.47*	0.41–0.53
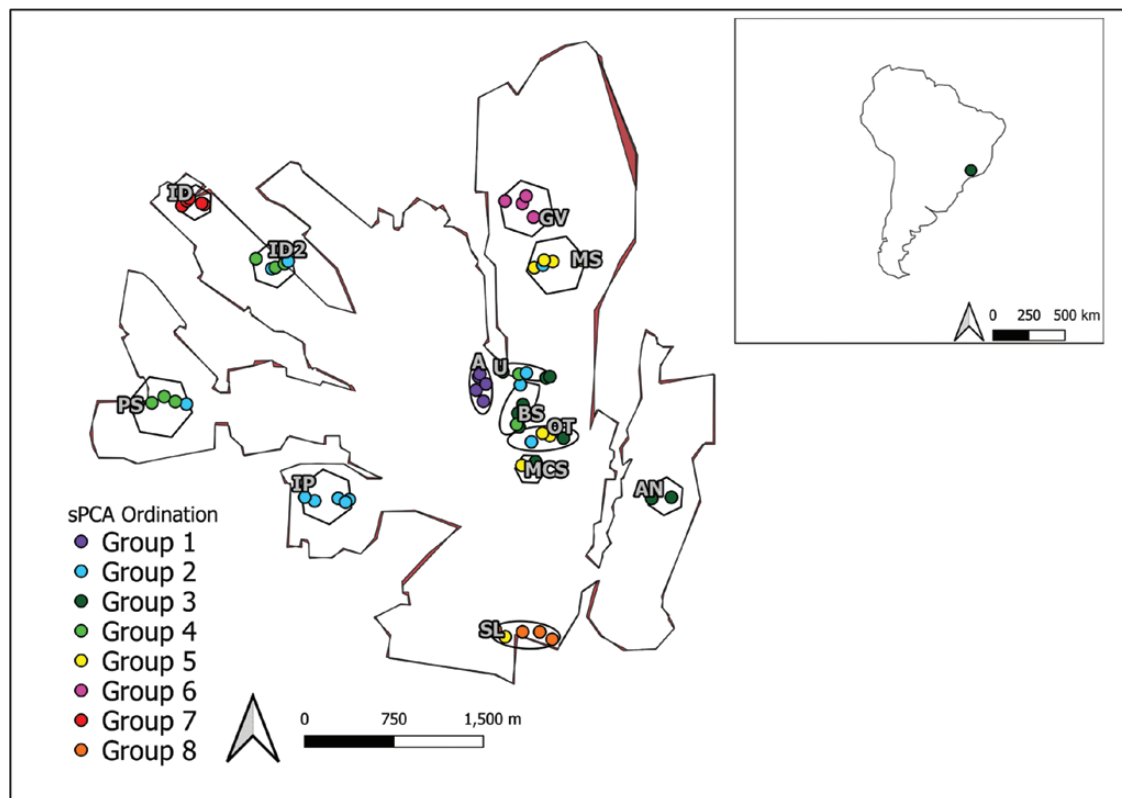
Figure 4. sPCA showing the respective cluster group for each population. The cluster group is represented by the colour of each point. Each of the 13 populations is indicated using a polygon and name label. The five points located within each polygon represent the five trees sampled per population. Each point is plotted relative to geographical location—Alfenas city, south-east Brazil. Populations: A, APAE public square; AN, airport neighbourhood; BS, bus station public square; GV, Giulio Vargas avenue; IP, I mesa parking; ID1, industrial district 1; ID2, industrial district 2; MS, Machado de Assis street; MCS, main street square; OT, old train station public square; PS, Pinheirinho square; SL, São Lucas neighbourhood; U, unifal-MG.

between urban *T. recurvata* populations. Furthermore, both the short life span of *T. recurvata* (~6 years; Chaves *et al.* 2021) and the extended period of urban expansion in Alfenas (founded in 1805) suggest that multiple generations of this epiphyte have experienced genetic drift over time and amplified the differentiation between populations (Nussbaum 2016).

Genetic drift and high spatial genetic structure all indicate low gene flow between the *T. recurvata* populations in this study. *Tillandsia recurvata* maintains gene flow through wind-dispersed seeds (Chilpa-Galván *et al.* 2018), since it has exclusive spontaneous self-pollination on its cleistogamous flowers (Bianchi and Vesprini 2014). The exact range of *T. recurvata* seed dispersal remains unquantified, although both the small, light seeds of this species and low seed terminal velocity are suggestive of a high dispersal potential (Chilpa-Galván *et al.* 2018). Nonetheless, the limitations of wind dispersal have been documented in other bromeliad species, specifically relating to seeds landing in close proximity to the mother plant (Paggi *et al.* 2010). Similar limitations have frequently been reported for wind-dispersed seeds in other plant groups within dense and enclosed landscapes over long distances (Nathan *et al.* 2002, Barrett and Harder 2007, Vergara-Torres 2010, Belinchón *et al.* 2017). As such, the confined and complex landscape of urban architecture within Alfenas may have limited *T. recurvata* seed dispersal in this study, amplified by the range between the urban tree patches (330 m to 5 km) and the low density of planted trees in the streets and squares (Ramos *et al.* 2016). For instance, the air currents surrounding moving traffic and buildings may have dictated the flow of wind-dispersed seeds (Lippe *et al.* 2013). Equally, human-mediated mechanical removal of this opportunistic epiphyte may have influenced population size and subsequent gene flow (Chaparro and Ticktin 2011). Under the pressure of sustained dispersal limitations, evidence has dictated that isolated species become more likely to experience a reduction in genetic diversity and population fitness (Wright *et al.* 2013).

The roles of low tree density and small population size on spatial genetic structure, as well as genetic drift, were reinforced through a recent simulation of *T. recurvata* spreading dynamics in an orchard landscape. Chaves *et al.* (2021) generated a TREC model that used an individual-based approach, with the simulated spreading dynamics of *T. recurvata* adjusted relative to empirical genetic data. The findings revealed high spatial genetic structure between *T. recurvata* populations, which the TREC model attributed to low tree density. A greater differentiation was also observed at the stage of early colonization. Specifically, they found that each tree formed individual habitat units, which encouraged distinct groups of MLGs through genetic drift, resulting from founder events on each host tree. The random combinations of alleles that formed these MLGs were sufficiently diverse for each population to persist in spite of low genetic diversity and mitigate strong evidence of IBD. The aforementioned findings of Chaves *et al.* (2021) were supported by the observations of this study and have reinforced the influence of genetic drift, after the founder events on each studied population, on the connectivity of urban *T. recurvata* populations.

Genetic drift has also been recognized as a key factor in the differentiation of *T. recurvata* in the wider literature (Soltis *et al.* 1987), as well as in another species of *Tillandsia* (González-Astorga *et al.* 2004). Similarly, several other species of plants show evidence of differentiation as a result of genetic drift in isolated populations, many of which share comparable characteristics to those of *T. recurvata* (selfing, rapid and clonal growth, epiphytic life cycle, high abundance) (Trapnell 2004, Vekemans and Hardy 2004, Barluenga *et al.* 2016, Pettengill 2016, Atwater *et al.* 2018, Torres 2018, Mota 2020, Chaves *et al.* 2021). Therefore, genetic drift may have driven the high spatial genetic structure of *T. recurvata* populations in this study.

In order for *T. recurvata* to persist, in spite of low genetic diversity within a fragmented landscape or urban ecosystem, this epiphytic species must be adaptable to the consequences of small population size and genetic drift (Agrawal and Whitlock 2012). Inbreeding depression has the capacity to reduce population fitness through the accumulation of harmful homozygous recessive mutations, amassed across multiple generations of inbred progeny (Agrawal and Whitlock 2012). However, *T. recurvata* and other selfing plants are able to lower the proportion of accumulated lethal mutations, through the purging of recessive deleterious alleles in their homozygous state—as a result of natural selection (Byers and Waller 1999, Bosse *et al.* 2018). As such, the self-fertilization strategy of *T. recurvata* gives reproductive assurance (Ingvarson 2002), despite the constraints of potentially consecutive founder events (Eckert 1996). A number of papers have reinforced this concept across several species of selfing plants (Lloyd 1992, Johnston and Schoen 1996, Honnay and Jacquemyn 2007, Vandepitte *et al.* 2007, Aguilar *et al.* 2008, Cutter 2019, Lander *et al.* 2019). Thus, the ability of selfing plants to moderate the potentially lethal consequences of isolation offers a partial justification for the continued survival of this species within a fragmented urban landscape, regardless of the low genetic diversity that was found within the urban *T. recurvata* populations of this study. Likewise, the clonal growth strategy of this species may also have contributed to the continued survival of this species, despite habitat fragmentation, as a result of an increased probability of genet survival through an expansion of ramets within the population (Barrett 2015).

The reproductive assurance of self-fertilization has made this strategy a common and valuable characteristic amongst angiosperm epiphytes (Fenster and Marten-Rodriguez 2007, Rios and Cascante-Marin 2016). Thus, the response of several selfing species to urban ecosystems, especially those that share a similar life history to *T. recurvata*, can be inferred from the data of this study. For example, the maintenance of genetic diversity within isolated landscapes through reproductive strategy, as was previously discussed, suggests that selfing species are more likely to dominate epiphyte communities in urban areas—allowing greater reproductive success in fragmented landscapes (Herlihy and Eckert 2002, Goodwillie *et al.* 2005, Bhatt *et al.* 2015, Furtado and Neto 2015, Prather *et al.* 2018). The wider literature also suggests that some outcrossing epiphytes, in contrast, may be more likely to decline as a result of low genetic diversity and high spatial genetic structure in isolated urban landscapes, especially in the absence of an efficient pollination strategy and when compared to selfing species (Ksiazek-Mikenas *et al.* 2019).

CONCLUSION

This study has described and evaluated, for the first time, the low connectivity and high spatial genetic structure of *T. recurvata*, a highly abundant and widely distributed atmospheric epiphyte, amongst urban green spaces within a city. Genetic drift, breeding system and dispersal may have contributed to the low connectivity amongst the populations of this study. Likewise, the

self-fertilization strategy of *T. recurvata* gives reproductive assurance (Lloyd 1992), despite the constraints of low connectivity, low genetic diversity, and genetic drift (Eckert *et al.* 1996).

In the absence of the aforementioned reproductive assurance from self-fertilization or an efficient pollination strategy, the wider literature suggests that some outcrossing epiphytes may be more likely to decline in these conditions than selfing species, such as *T. recurvata* (Ksiazek-Mikenas *et al.* 2019). A comparison of the connectivity of outcrossing and selfing epiphytic plant species within urban landscapes would be useful to gain a greater understanding of epiphyte connectivity. More broadly, a replicated assessment of epiphyte population genetics across multiple urban landscapes would be an ideal next step to assess whether the conclusions drawn from this study, which was limited to one city, are also applicable to other locations.

This study represents an important first step towards understanding the connectivity of increasingly common urban landscapes, in the context of selfing epiphytes on planted urban green spaces. We also present novel microsatellite markers for the continued investigation of *T. recurvata* genetic structure elsewhere. According to the findings of this work, high spatial genetic structure and low connectivity across urban landscapes, dominated by opportunistic species such as *T. recurvata*, suggest a bleak future for epiphyte diversity under predicted urban expansion (Newbold *et al.* 2015).

DATA AVAILABILITY

All primer information and data will be available through the Dryad Digital Repository following the publication of this work: https://datadryad.org/stash/share/YOiJjLCVX9hQcw4Z92qSjHrY0iuAECBGK7XuzFmeIuY

COMPETING INTERESTS

The authors declare that they have no competing or conflict of interest.

AUTHOR CONTRIBUTIONS

M.R.Q., S.B. and F.N.R. contributed to the study conception and design. Material collection was performed by F.N.R. Data collection and analysis were performed by M.R.Q. T.D. and J.P. designed novel microsatellite markers for this study. J.C.B. and P.A. provided advice and guidance during data collection and analysis. The first draft of the manuscript was written by M.R.Q. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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