Evidence for the genetic similarity rule at an expanding mangrove range limit

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PREMISE: Host-plant genetic variation can shape associated communities of organisms. These community-genetic effects include (1) genetically-similar hosts harbouring similar associated communities (i.e., the genetic similarity rule) and (2) host-plant heterozygosity increasing associated community diversity. Community-genetic effects are predicted to be less prominent in plant systems with limited genetic variation, such as those at distributional range limits. Yet, empirical evidence from such systems is limited.

METHODS: We sampled a natural population of a mangrove foundation species (*Avicennia germinans*) at an expanding range limit in Florida, USA. We measured genetic variation within and among 40 host trees with 24 nuclear microsatellite loci and characterised their foliar endophytic fungal communities with ITS1 gene amplicon sequencing. We evaluated relationships among host-tree genetic variation, host-tree spatial location, and the associated fungal communities.

RESULTS: Genetic diversity was low across all host trees (mean: 2.6 alleles per locus) and associated fungal communities were relatively homogeneous (five sequence variants represented 78% of all reads). We found: (1) genetically-similar host trees harboured similar fungal communities, with no detectable effect of inter-host geographic distance. (2) Host-tree heterozygosity had no detectable effect, while host-tree absolute spatial location affected community alpha diversity.

CONCLUSIONS: This research supports the genetic similarity rule within a range limit population and helps broaden the current scope of community genetics theory by demonstrating that community-genetic effects can occur even at expanding distributional limits where host-plant genetic variation may be limited. Our findings also provide the first documentation of community-genetic effects in a natural mangrove system.

KEY WORDS

associated communities; *Avicennia germinans*; black mangrove; community genetics; endophytic fungi; foundation species; intra-individual heterozygosity; plant genetic variation

INTRODUCTION

Intraspecific diversity can shape the ecological dynamics of communities and entire ecosystems (Raffard et al., 2019). For instance, a central principle of community genetics is that genetic variation within a host plant can influence the structure and diversity of associated communities of organisms (Whitham et al., 2003). Empirical evidence of community-genetic effects is found across diverse systems, including terrestrial forests with low (Whitham et al., 2006) and high (Zytynska et al., 2011) species diversity, agricultural landscapes (Stevenson et al., 2017), and aquatic systems (Jormalainen et al., 2017). This pattern may be most prominent in systems dominated by a limited number of plant foundation species (Whitham et al., 2006), which define ecosystems with their physical structure and provide resources that directly influence diverse community assemblages (Ellison et al., 2005).

Community-genetic effects are measured both in terms of host-plant genetic similarity and diversity, plus spatial effects need to also be considered. First, genetically-similar host plants may harbour similar associated communities, a pattern known as the genetic similarity rule (Bangert, Allan, et al., 2006; Bangert, Turek, et al., 2006; Barbour et al., 2009; Kagiya et al., 2018). Second, increased genetic diversity at the population level may lead to concomitant increases in associated species diversity (Wimp et al., 2004; Crutsinger et al., 2006; Johnson et

al., 2006). Similar patterns are also found when considering the genetic diversity of individual host plants (i.e., heterozygosity) (Tovar-Sánchez et al., 2013; Valencia-Cuevas et al., 2018). This extension of community genetics theory is in line with extensive research on the link between intra-individual heterozygosity and fitness (reviews by Hansson and Westerberg, 2002; Szulkin et al., 2010). Lastly, in addition to host genetic variation, the spatial context of host plants, including their relative position in relation to neighbouring conspecifics and variation in environmental conditions, needs to also be considered as spatial effects can prove more influential (Tack et al., 2010; Gossner et al., 2015; Barbour et al., 2019; but see Bangert, Allan, et al., 2006; Lamit et al., 2015).

Community-genetic effects may also vary with the extent of genetic variation present in the host population. Plant systems with limited genetic variation are predicted to exhibit less prominent effects and, instead, environmental variation will exhibit a stronger effect on associated community structure (Bangert, Turek, et al., 2006). However, only one study has provided empirical evidence from such systems. Pohjanmies et al. (2015) documented that genetic variation within a tree foundation species correlates with the structure and diversity of associated herbivore communities at a distributional range limit. Range limits may exhibit limited genetic variation (Pironon et al., 2017) and are shifting for many species with anthropogenic climate change (Pecl et al., 2017). Further assessments of relationships between host-plant genetic variation and associated communities at range limits, especially those where foundation species are undergoing climate-driven range shifts, could help broaden the current scope of community genetics theory and provide insights into the ecological and evolutionary processes shaping these dynamic systems.

In this study, we evaluated relationships between genetic variation within a mangrove foundation species at its expanding distributional range limit and the structure and diversity of associated foliar endophytic fungal communities. Mangroves are (sub)tropical, intertidal woody plants that provide vital ecosystem services to coastal habitats worldwide (Lee et al., 2014). Mangrove forests consist of relatively few tree species (Alongi, 2009) and, as such, intraspecific differences may be particularly influential in shaping ecological dynamics in these systems (Farnsworth, 1998). Numbers of mangrove species are further reduced towards climate-sensitive, poleward range limits where generally only one predominant species exists (Osland et al., 2017) and often genetic variation is limited (e.g., Pil et al., 2011; De Ryck et al., 2016; Kennedy et al., 2017; Binks et al., 2019; Ochoa-Zavala et al., 2019).

Mangrove systems harbour numerous associated communities of both terrestrial and marine origin (Nagelkerken et al., 2008), including diverse fungal communities found on or within multiple mangrove tissues (e.g., Gilbert et al., 2002; Arfi et al., 2012; de Souza Sebastianes et al., 2013; Lee et al., 2019). Fungal endophytes are ubiquitous inhabitants within plant tissues, obtain shelter and nutrition from their host plant, and may influence plant health and function (Arnold, 2007; Porras-Alfaro and Bayman, 2011). Endophytic fungi in leaves and twigs vary among host genotypes of diverse plant species (Elamo et al., 1999; Pan et al., 2008; Lamit et al., 2014; Griffiths et al., 2020); however, whether intraspecific genetic differences among mangrove host trees correlates with the structure and diversity of their associated fungal communities remains unanswered.

We sampled a natural population of neotropical black mangrove (*Avicennia germinans*) at a northern range limit on the Atlantic coast of Florida, USA. At this range limit, *A. germinans* is the predominant mangrove species (Lonard et al., 2017), exists as discrete patches within a

landscape dominated by salt-marsh vegetation (Kangas and Lugo, 1990), and exhibits reduced genetic variation (Kennedy, Preziosi, et al., 2020) and elevated levels of self-fertilisation (Kennedy et al., 2021). A lack of extreme freeze events for several decades has been linked to *A. germinans* proliferation (Cavanaugh et al., 2014; Osland et al., 2018) and further expansion is forecast with climate change (Cavanaugh et al., 2015, 2019), which may have wide-reaching effects on these coastal ecosystems (Kelleway et al., 2017). We genotyped *A. germinans* host trees with 24 nuclear microsatellite loci, characterised communities of endophytic fungi in their leaves with ITS1 gene amplicon sequencing, and accounted for potential spatial effects with host-tree GPS coordinates and inter-host geographic distances. We asked: (1) Do inter-host genetic similarity and inter-host geographic distance correlate with similarity among associated endophytic fungal communities? (2) Do host-tree heterozygosity and host-tree absolute spatial location correlate with alpha diversity of the associated endophytic fungal community?

MATERIALS AND METHODS

Study design

On 09 October 2017, we sampled from and collected GPS coordinates for 40 mature *A. germinans* trees, all approximately the same height (~2 m), at a single collection site (29.7284, -81.2425) near the Atlantic Florida range limit. Mangrove area has progressively increased for several decades at this site (Rodriguez et al., 2016) which is flanked by a brackish lagoon to the west and a fringe of terrestrial hammock forest to the east. Salinity during this time of the year (Sept–Nov) increases from west to east along the site (38 to 67 ‰), then decreases adjacent to the terrestrial fringe (40 ‰) (Guana Tolomato Matanzas National Estuarine Research Reserve, *unpublished data*; Fig. 1). Our sampling area covered ~0.1 km^2, which included most of the total

spatial extent of this *A. germinans* population, with a minimum inter-tree distance of 11 m and a maximum distance of 528 m (Fig. 1). For each tree, we sampled a total of three undamaged leaves, each from the first fully mature leaf pair on branches located in direct sunlight. We collected these leaves (generally the third leaf pair) to standardise leaf age and exposure to sunlight, both of which can influence fungal community structure (Koide et al., 2017; Younginger and Ballhorn, 2017). We placed leaves from each tree into separate, labelled plastic bags and stored them in a portable cooler with an ice pack during fieldwork and subsequent transport to the laboratory.

Sample processing and DNA isolation

Leaves were kept on ice and processed within 24 hours of sampling. We rinsed individual leaves under running tap water for 30 sec, then surface sterilised with sequential immersion in 95% ethanol for 10 seconds, 0.5% bleach for 2 minutes, and 70% ethanol for 2 minutes under a sterile hood (U'Ren et al., 2014). We allowed leaves to air dry and then used sterilised surgical blades to cut ~5 mm x 5 mm sections from the middle of each leaf at both sides of the midvein. We combined the cut sections from each of the three leaves per tree into a single microcentrifuge tube and isolated genomic DNA with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the standard protocol, with an extended incubation of 45 minutes. We also included two extraction blanks (negative controls) during this process. We quantified DNA extracts on a Qubit 2.0 Fluorometer (ThermoFisher Scientific, Waltham, Massachusetts, USA) and created standardised aliquots of 35 ng/uL to be used for both host-tree genotyping and fungal community sequencing. We stored DNA aliquots at -20°C until further processing.

Host-tree genotyping

We genotyped host trees at 32 nuclear microsatellite loci. Of this total, 12 loci were previously developed (Nettel et al., 2005; Cerón-Souza et al., 2006, 2012; Mori et al., 2010) and genotyped following the protocol outlined in Kennedy, Preziosi, et al. (2020). The remaining 20 loci were more recently developed (Craig, Feller, et al., 2020) and genotyped following the author's protocol. We performed PCR on a Prime thermal cycler (Techne, Straffordshire, UK), analysed fragments on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Foster City, California, USA) with LIZ 500 size standard, and scored alleles in the R-package Fragman (Covarrubias-Pazaran et al., 2016). We evaluated the presence of null alleles in MICRO-CHECKER 2.2.3 (van Oosterhout et al., 2004) and randomly amplified and genotyped 10% of our DNA samples (n = 4) a second time to estimate a study error rate (Bonin et al., 2004). We tested for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium, and calculated the number of alleles and observed and expected heterozygosity per locus in FSTAT 2.9.3.2 (Goudet, 2002).

We calculated five measures of host-tree heterozygosity (i.e., proportion of heterozygous loci, observed heterozygosity, expected heterozygosity, internal relatedness, homozygosity by loci) for each of the 40 host trees with the R-function GENHET (Coulon, 2010). We also manually calculated the number of alleles within the multi-locus genotype of each host tree. All six measures were highly correlated (Pearson's correlation, $r = 0.96–1.0$, $p < 0.001$). Hence, we present results only for homozygosity by loci (HL), an index that considers allelic variability at each locus to estimate heterozygosity and, based on simulations, correlates better than other measures with genome-wide heterozygosity (Aparicio et al., 2006). As this index varies from 0 (all loci are heterozygous) to 1 (all loci are homozygous), we used 1 – HL for statistical analyses

to provide more intuitive results (i.e., higher values represent higher heterozygosity). To evaluate genetic similarity, we calculated pairwise inter-individual genetic distances (as outlined in Smouse and Peakall, 1999) and geographic distances among the 40 host trees in GenAlEx 6.5 (Peakall and Smouse, 2012).

Associated fungal community sequencing

We performed ITS gene amplicon library preparation and sequencing at the University of Salford, UK. Fungal DNA was amplified at the ITS 1F-2 gene (White et al., 1990) with modified versions of the ITS1F (5'–CTT GGT CAT TTA GAG GAA GTA A–3') and ITS2 (5'–GCT GCG TTC TTC ATC GAT GC –3') primer set that included Illumina adapters, a linker, and unique barcodes (see Smith and Peay, 2014) as outlined in Griffiths et al. (2020). PCR products for our samples and those of 80 additional fungal samples, which consisted of ITS1 gene amplicons used for an unrelated study, were then pooled to equimolar concentrations. ITS1 gene amplicon sequencing was performed using paired-end reads with an Illumina v3 (2 x 300 bp) cartridge on an Illumina MiSeq (Illumina, San Diego, California, USA). Negative (extraction blanks) and positive (synthetic mock community with 12 mock isolates; Palmer et al., 2018) controls were also included in the sequence run.

We removed adaptor and primer sites from the ITS1 gene sequence data with cutadapt v2.4 (Martin, 2011), and performed all subsequent data processing and calculations in R v3.6.0 (R Core Team, 2020). A total of 275,829 raw sequences across our 40 samples were generated. We used the R-package DADA2 1.12.1 (Callahan et al., 2016) with default pipelines to perform quality filtering and taxonomic assignment with the UNITE v8.0 database (UNITE Community, 2019). Here, we analysed only forward sequence reads because lower quality and quantity of

reverse reads resulted in a nearly 50% reduction in total sequence reads after quality filtering of the assembled paired-end reads (Appendix S1a, see the Supplementary Data with this article). Discarding low-quality reverse reads is a common strategy that often provides better results than assembled paired-end reads (Nguyen et al., 2015; Pauvert et al., 2019). One chimera was removed. We then removed amplicon sequence variants (ASVs) with <100 reads across all samples as a conservative approach to deal with potential artifacts of high-throughput sequencing (Pauvert et al., 2019). Modal contig length was 225 bp (range: 153 – 251 bp). No contaminants were identified in the first negative control, and one ASV was identified in the second negative control, but was not found in other samples. All 12 expected ASVs were identified in the synthetic mock community. We did not further trim forward reads, we manually checked whether ASVs with identical taxonomic assignments were indeed unique sequences (i.e., did not simply vary at the start or end of the sequence), and all ASVs assigned as unidentified fungi were further checked with default blastn analyses on the UNITE website (Nilsson et al., 2019). We removed all ASVs that corresponded to the host-tree species (*A. germinans*), which included 64% of all sequence reads, and all additional unidentified fungi had significant alignments with public fungal ITS sequences (e-values = $1e^{-13}$ – $4e^{-88}$). The resulting data set consisted of 64,308 reads across 40 samples, with a median of 748 reads per sample (range: 104 – 9,314).

We exported the ASV table, taxonomy table, and sample identifications to the R-package phyloseq 1.28.0 (McMurdie and Holmes, 2013) for the following calculations. We calculated alpha diversity of fungal communities with Hill numbers (Hill, 1973) at the scales of q=0 (species richness), q=1 (exponential of Shannon index), and q=2 (inverse of Simpson index), which represent the effective number of species and put more weight on abundant species as the value of q increases (Chao et al., 2014). We performed these calculations with the raw count data

rarefied to a standardised number of reads equal to the sample with the lowest read count (104 reads; see Appendix S2a). Although read counts were limited for certain samples, asymptotes were reached in all rarefaction curves with few rank-order changes among samples past this lowest read count (Appendix S2a). As such, our sampling effort seems to have captured most diversity within these samples. Random sampling to generate rarefied counts can add noise to a data set and undermine the performance of downstream methods (McMurdie and Holmes, 2014); therefore, we also performed alpha diversity calculations and the subsequent statistical analyses with the raw count data and results were equivalent to those presented here (Appendix S1b). To evaluate community dissimilarity (beta diversity), we calculated Bray-Curtis dissimilarity with the raw count data converted to relative abundances. We also calculated Aitchison distance by centred log-ratio (clr) transforming the raw count data with the R-package microbiome (Lahti et al., 2017) and then calculating pairwise Euclidean distances in phyloseq 1.28.0 (McMurdie and Holmes, 2013). Aitchison distance accounts for the compositional nature of high-throughput sequence data, which makes this measure more appropriate than many standard measures (Gloor et al., 2017; Quinn et al., 2018).

Statistical analyses

We performed all statistical analyses in R v3.6.0 (R Core Team, 2020). To address our first question, we tested for an effect of inter-host genetic distance and a relative spatial effect of inter-host geographic distance on dissimilarity among associated endophytic fungal communities across all samples with ranked Mantel tests of correlation. As spatial effects may not be linear (Diniz-Filho et al., 2013; Legendre et al., 2015), we also performed multivariate Mantel correlograms to assess these patterns at five discrete distance classes. All analyses were

243 performed in the R-package ecodist (Goslee and Urban, 2007). Significance for each analysis
244 was determined with 10^4 permutations, and p-values for correlograms were adjusted for multiple
245 comparisons with a false discovery rate correction method using the R-function p.adjust. For
246 both Mantel tests and Mantel correlograms, we first tested for a relationship between the two
247 predictor variables (i.e., inter-host genetic distance and inter-host geographic distance), then
248 performed separate tests between fungal community dissimilarity and each of the two predictor
249 variables, and finally performed partial analyses between fungal community dissimilarity and
250 inter-host genetic distance, while controlling for inter-host geographic distance.
251 To address our second question, we tested for an effect of host-tree heterozygosity and an
252 absolute spatial effect of host-tree spatial location on the alpha diversity of associated endophytic
253 fungal communities with multiple linear regressions. We fitted three additive models, with alpha
254 diversity of fungal communities at each Hill number (q = 0, 1, 2) as the response variable and
255 heterozygosity, longitude, and latitude of each host tree as predictor variables. We also tested full
256 models and subsets with interactions among two of the three predictor variables, but none of
257 these interactions proved statistically significant and none of these models provided better fits
258 based on the Bayesian Information Criterion (BIC; Schwarz, 1978). Hill numbers at q=1 and q=2
259 were natural log-transformed to meet the statistical assumption of normality, and we centred and
260 scaled the predictor variables to standardise regression coefficients.
261
262 **RESULTS**
263 **Host-tree genotyping**
264 We discarded seven of the 32 nuclear microsatellite loci that were monomorphic across all
265 samples, and discarded another locus that proved difficult to score. Our final host-tree genotypes

included 24 loci (Appendix S1c) with no missing data, and all 40 host-tree genotypes were unique. We found no evidence for null alleles and each of the four samples that were amplified and genotyped a second time produced consistent multi-locus genotypes. We found no evidence for linkage disequilibrium or deviations from Hardy-Weinberg equilibrium. Genetic variation was low across the 40 host trees, with 2.6 ± 1.4 (SD) alleles per locus and expected heterozygosity of 0.37 ± 0.20 (Appendix S1c). Host-tree heterozygosity (1 – HL) ranged from 0.06 to 0.81 (mean: 0.45 ± 0.15).

Associated fungal community sequencing

A total of 49 amplicon sequence variants (ASVs) were identified across the 40 host trees. Most ASVs were assigned to the phylum Ascomycota (35 of 49 ASVs, 87% of all reads) and 11% of all reads were assigned only to the level of kingdom Fungi (Appendix S2b). Less than half (47%) of all reads were assigned class level taxonomy, with the class Dothideomycetes as the most common (28% of all reads; Appendix S2c). The endophytic fungal community was relatively homogeneous, with one ASV (assigned taxonomy only to the level of phylum Ascomycota) representing 41% of all reads (Appendix S1d). The five most abundant ASVs represented 78% of all reads, and subsequent ASVs each represented ≤ 2% of all reads (Appendix S1d). Alpha diversity of fungal communities across the 40 host trees at q=0 (species richness) was 4.0 ± 1.7 (SD), at q=1 (exponential of Shannon index) was 2.8 ± 1.2, and at q=2 (inverse of Simpson index) was 2.5 ± 1.1.

Associated fungal community structure correlates with host-tree genetics

Genetically-similar host trees harboured similar associated fungal communities, with no detectable relative spatial effect of geographic distance among host trees both across all samples (Mantel tests) and at five distance classes (Mantel correlograms) (Fig. 2). For Mantel tests, the predictor variables (i.e., inter-host genetic distance and inter-host geographic distance) exhibited no relationship (Mantel correlation, $r_M = 0.05$, $p = 0.181$; Appendix S2d). Fungal community (Bray-Curtis) dissimilarity exhibited a weak, but statistically significant positive relationship with inter-host genetic distance ($r_M = 0.26$, $p = 0.002$), and no relationship with inter-host geographic distance ($r_M = 0.06$, $p = 0.164$) (Fig. 2a, b). Accounting for inter-host geographic distance did not impact the relationship with inter-host genetic distance (partial $r_M = 0.26$, $p = 0.002$). Community dissimilarity measured with Aitchison distance provided equivalent results (inter-host genetic distance: $r_M = 0.16$, $p = 0.041$; inter-host geographic distance: $r_M = 0.05$, $p = 0.188$) (Fig. 2e, f), with a weaker relationship with inter-host genetic distance (partial $r_M = 0.16$, $p = 0.043$).

Mantel correlogram results were equivalent to those of the Mantel tests, with no relationships between predictor variables ($r_M = -0.07 – 0.07$, $p \geq 0.568$; Appendix S2d), and community (Bray-Curtis) dissimilarity exhibited statistically significant positive relationships with the first two genetic distance classes ($r_M = 0.16$, $p = 0.002$; $r_M = 0.14$, $p = 0.050$; respectively), a statistically significant negative relationship with the fourth genetic distance class ($r_M = -0.16$, $p = 0.008$), and no relationships with inter-host geographic distance classes ($r_M = -0.06 – 0.03$, $p \geq 0.810$) (Fig. 2c, d). Accounting for inter-host geographic distances did not impact these relationships with inter-host genetic distance classes, except for the second genetic distance class that was now statistically non-significant ($p = 0.090$) (Appendix S2e). Community dissimilarity measured with Aitchison distance provided equivalent results (Fig. 2g, h), with

311 weaker relationships with inter-host genetic distance classes that were statistically significant at
312 only the first genetic distance class ($r_M = 0.13$, $p = 0.027$). Accounting for inter-host geographic
313 distances did not impact these relationships (Appendix S2e).
314
315 **Associated fungal community diversity correlates with host-tree spatial location**
316 Host-tree heterozygosity had no detectable effect on the alpha diversity of associated endophytic
317 fungal communities. Instead, the absolute spatial location of host trees affected these associated
318 fungal communities. Additive models explained limited variation in the alpha diversity of fungal
319 communities at each of the three Hill numbers. The model for q=0 was not statistically
320 significant ($F_{3,36} = 1.7$, $p = 0.195$, adjusted $r^2 = 0.05$) and models for q=1 ($F_{3,36} = 3.1$, $p = 0.038$,
321 adjusted $r^2 = 0.14$) and q=2 ($F_{3,36} = 3.4$, $p = 0.027$, adjusted $r^2 = 0.16$) were marginally
322 significant. Longitude was the only predictor variable to exhibit a significant partial regression
323 slope (for full model breakdown see Table 1). This increase in fungal community alpha diversity
324 with increased longitude (i.e., from the brackish lagoon to the landward margin) was statistically
325 significant at each of the three Hill numbers ($p = 0.043$, 0.009, 0.009, respectively; Table 1). Yet,
326 instead of a systematic increase, these effects seemed to be shaped primarily by the fact that
327 highest fungal alpha diversity was observed within trees closest to the landward margin (Fig. 3).
328
329 **DISCUSSION**
330 Community-genetic effects are predicted to be less prominent in plant systems with limited
331 genetic variation, such as those at distributional range limits. Yet, empirical evidence from such
332 systems is limited. Here, at the scale of an expanding range limit population of a mangrove
333 foundation species (*Avicennia germinans*), we found evidence for the genetic similarity rule

whereby genetically-similar host trees harboured similar associated endophytic fungal communities. In contrast, we found no detectable effect of host-tree heterozygosity on fungal community alpha diversity. This research demonstrates that community-genetic effects can occur even at expanding distributional limits where host-plant genetic variation may be limited, and provides the first documentation of these effects in a natural mangrove system.

Genetically-similar mangrove hosts harbouring similar endophytic fungal communities, with no detectable relative spatial effect, may be explained by the mode of fungal transmission and/or biotic filtering dictated by the physiology and anatomy of the host plant (Ricks and Koide, 2019). Horizontal transmission via airborne fungal spores is commonly observed in woody plants (Arnold and Herre, 2003 and citations within), although vertical transmission from parent tree to seed is also possible (e.g., Vega et al., 2010). Our studied species (*A. germinans*) produces cryptoviviparous propagules (i.e., embryos emerge from the seed coat, but remain within the fruit until abscission from maternal trees), with varying degrees of vivipary across many mangrove species (Tomlinson, 1986). This form of reproduction, where developing propagules remain attached to maternal trees for extended periods may lead to a greater contribution of fungal transfer from parent to offspring. Consistent with this hypothesis, endophytic fungi (Lee et al., 2019) and bacteria (Soldan et al., 2019) are found within surface-sterilised cryptoviviparous mangrove propagules collected directly from maternal trees. Host physiology may also dampen horizontal transfer in *A. germinans* as salt excretion through leaf glands (a mechanism to tolerate salt stress) can reduce foliar fungal colonisation (Gilbert et al., 2002). Fungal communities in trees also vary with differences in phenotypic leaf traits, such as internal chemistry and surface characteristics (Valkama et al., 2005; Kembel and Mueller, 2014). Additional research that compares fungal endophytes in both *A. germinans* maternal trees and

their offspring, with parallel leaf trait assessments, could evaluate the relative influence of fungal transmission mode and biotic filtering in shaping these associated communities.

We did not detect an effect of host-tree heterozygosity on fungal community alpha diversity. Instead, we found that alpha diversity varied with the absolute spatial location of host trees. Increased host-tree heterozygosity can lead to greater growth rates (Charlesworth and Willis, 2009) and greater foliar phytochemical diversity (Campbell et al., 2013), factors that may underlie increases in associated herbivore community alpha diversity observed elsewhere (Tovar-Sánchez et al., 2013; Valencia-Cuevas et al., 2018). We suggest that, within this mangrove population, the limited genetic variation present across host trees may not translate into large enough variation in host-tree phenotypic traits that would augment the alpha diversity of these associated communities. Rather, community alpha diversity increased with longitude across our collection site (i.e., from the brackish lagoon to the landward margin), an absolute spatial effect seemingly shaped by the fact that highest alpha diversity was observed within trees closest to the landward margin. Soil salinity increases with longitude across the site, but then declines at this landward margin adjacent to a fringe of terrestrial forest (Fig. 1). Salinity differences can impact fungal communities associated with the *A. germinans* rhizosphere (Vanegas et al., 2019), but their effect on foliar fungal communities remains to be formally tested. Higher soil salinity closer to the centre of the collection site will demand greater salt excretion through *A. germinans* leaf glands (Sobrado and Greaves, 2000; Suárez and Medina, 2008) that may further diminish foliar fungal colonisation in this species (Gilbert et al., 2002). In addition, as mangrove leaves may contain fungi predominately from terrestrial sources (Lee et al., 2019, 2020), the fringe of terrestrial forest is presumably a reservoir of unique fungal diversity. Therefore, within the mangrove population studied here, trees located nearest to this

landward margin may harbour slightly more diverse fungal communities than conspecifics elsewhere due to both reduced soil salinity and proximity to additional fungal sources. Whether this pattern extends to additional mangrove populations remains to be tested.

Pohjanmies et al. (2015), with their research at a distributional range limit, provided the first empirical evidence of community-genetic effects within a plant system with limited genetic variation. Our documentation of the genetic similarity rule at a mangrove range limit, where host trees possessed very limited genetic variation (on average, 2.6 alleles per locus), adds further support to these previous findings and strengthens the argument that correlations between genetic variation within foundation species and the dynamics of associated communities can occur even at distributional limits that may be genetically depauperate. These correlations, however, will ultimately depend on the strength of the community-genetic effect relative to the degree of environmental variation and how this relationship varies with spatial scale (Bangert et al., 2008). Both Pohjanmies et al. (2015) and our study assessed correlations between plant foundation species and their associated communities within single range limit populations. Environmental variation will inherently be small at this local scale compared to that across broader spatial scales where community-genetic effects may be less influential (Hughes and Stachowicz, 2009; Tack et al., 2010; Gossner et al., 2015; but see Bangert, Allan, et al., 2006; Davies et al., 2014; Lamit et al., 2015). Spatial effects on foliar endophytic fungal communities in mangroves are evident across greater geographic distances (Lee et al., 2019, 2020). As such, the relationship between mangrove host-tree genetic variation and associated fungal communities documented here may vary depending on the spatial extent under consideration and warrants additional research.

Although we sampled a relatively small spatial area, this is the scale at which species expansion occurs as small isolated populations become colonised and begin to proliferate. This

process is particularly evident at the Atlantic Florida *A. germinans* range limit where initial colonisation may consist of a single individual (Kennedy, Dangremond, et al., 2020), and for the population studied here which has increased from only about 10% to 45% mangrove cover over the past several decades (Rodriguez et al., 2016). In this context, our research demonstrates that community-genetic effects can occur across the spatial extent of an expanding range limit population, with potential implications for host fitness and population resilience as endophytic fungi can vary greatly in function within plant hosts from latent pathogens to mutualistic symbionts (Porras-Alfaro and Bayman, 2011). Symbioses with endophytic fungi can contribute to plant adaptation to high-stress environments (Rodriguez et al., 2004), with evidence that variation in soil fungal communities can influence the fitness and susceptibility of *A. germinans* to cold stress (Chen et al., 2020), although fungal infections can reduce recruitment (Devaney et al., 2017). We documented a correlation between mangrove host-tree genetics and fungal community differences, but does this relationship generate variation in stress tolerance among mangrove hosts? If so, this insight could broaden the current discussion of how a shift from salt marsh to mangrove dominance may shape these coastal communities (e.g., Kelleway et al., 2017; Johnston and Gruner, 2018; Smith et al., 2019; Armitage et al., 2020) by including mangrove intraspecific variation as a factor that could influence population resilience at these high-stress range limits.

This research also provides the first documentation of community-genetic effects in a natural mangrove system. Does the genetic similarity rule apply elsewhere across the broad distributional range of mangroves and to further mangrove-associated communities? Experimental plantings demonstrate that mangrove maternal genotypic identity can impact the composition of associated soil microbial communities (Craig, Kennedy, et al., 2020), which

indicates that community-genetic effects can have a broader reach in mangrove systems than the more intimately associated endophytic fungal communities assessed here. Moreover, intraspecific differences in quantitative traits of mangroves, including trichome density (Piovia-Scott, 2011), plant architecture (Silva et al., 2017), and leaf chemistry (Erickson et al., 2004), can affect mangrove-associated communities. Heritable variation in these traits has been identified as a potential factor linking associated communities to host-plant genetics (Whitham et al., 2012). Assessments in additional mangrove-associated communities (of both terrestrial and marine origin) would further our understanding of how host-tree genetic variation may relate to the broader community of organisms associated with these plants, with direct implications for conservation and restoration practices.

CONCLUSIONS

We found evidence for the genetic similarity rule at an expanding mangrove range limit. This research helps broaden the current scope of community genetics theory by demonstrating that community-genetic effects can occur even at expanding distributional limits where host-plant genetic variation may be limited. Our findings also add to the growing number of diverse systems where associated communities vary with host-plant genetics. As community-level effects of host-plant genetic variation are found to be most prominent in systems dominated by few plant foundation species (Whitham et al., 2006), mangrove forests and their low tree species diversity may prove to be a system ripe for discovery.

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AUTHOR CONTRIBUTIONS

J.P.K., R.F.P. and J.K.R. conceived and designed the research. J.P.K. performed field collections, DNA extractions, and host-tree genotyping. R.E.A. performed library preparation and sequencing, and provided analysis tools. R.F.P. and J.K.R. supervised the research. J.P.K. conducted bioinformatics analysis and statistical analyses. J.P.K. wrote the manuscript with input from all co-authors.

DATA AVAILABILITY

Microsatellite genotype data are publicly available on figshare: https://doi.org/10.6084/m9.figshare.14252660.v1. Sequence data are deposited on the NCBI SRA database: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA643237/.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1 Supplemental Tables:

Appendix S1a. Summary of ITS1 gene sequence data sets using only forward sequence reads and using assembled paired-end reads.

Appendix S1b. Multiple linear regressions of associated endophytic fungal community diversity (calculated with the raw count data) as a function of the heterozygosity and absolute spatial location of host trees.

Appendix S1c. Genetic diversity of 24 nuclear microsatellite loci used for genotyping of *Avicennia germinans* host trees.

Appendix S1d. Endophytic fungal diversity identified with ITS1 gene sequencing.

APPENDIX S2 Supplemental Figures:

Appendix S2a. Rarefaction curves of observed amplicon sequence variants (ASVs) in sampled *Avicennia germinans* trees.

Appendix S2b. Relative abundance across all sequence data of fungal phyla for the forward-reads data set.

Appendix S2c. Relative abundance across all sequence data of fungal class for the forward-reads data set.

Appendix S2d. Graphical representation of Mantel test and Mantel correlogram between inter-host genetic distance and inter-host geographic distance.

Appendix S2e. Graphical representation of partial Mantel correlograms between fungal community dissimilarity, measured with Bray-Curtis dissimilarity and Aitchison distance, and inter-host genetic distance.

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Table 1. Multiple linear regressions of alpha diversity of associated endophytic fungal communities as a function of the heterozygosity and absolute spatial location of host trees. Alpha diversity of associated communities was calculated with Hill numbers at the scales of q=0 (species richness), q=1 (exponential of Shannon index), and q=2 (inverse of Simpson index), which put more weight on abundant species as the value of q increases. Bold values indicate statistical significance ($p < 0.05$).

Response	Predictor	Estimate	SE	t	p
q=0	Heterozygosity	-0.01	0.16	-0.12	0.909
	Longitude	0.37	0.18	2.10	**0.043**
	Latitude	-0.26	0.18	-1.47	0.150
q=1	Heterozygosity	-0.08	0.15	-0.55	0.588
	Longitude	0.47	0.17	2.76	**0.009**
	Latitude	-0.11	0.17	-0.65	0.520
q=2	Heterozygosity	-0.10	0.15	-0.66	0.515
	Longitude	0.46	0.17	2.74	**0.009**
	Latitude	-0.05	0.17	-0.29	0.772

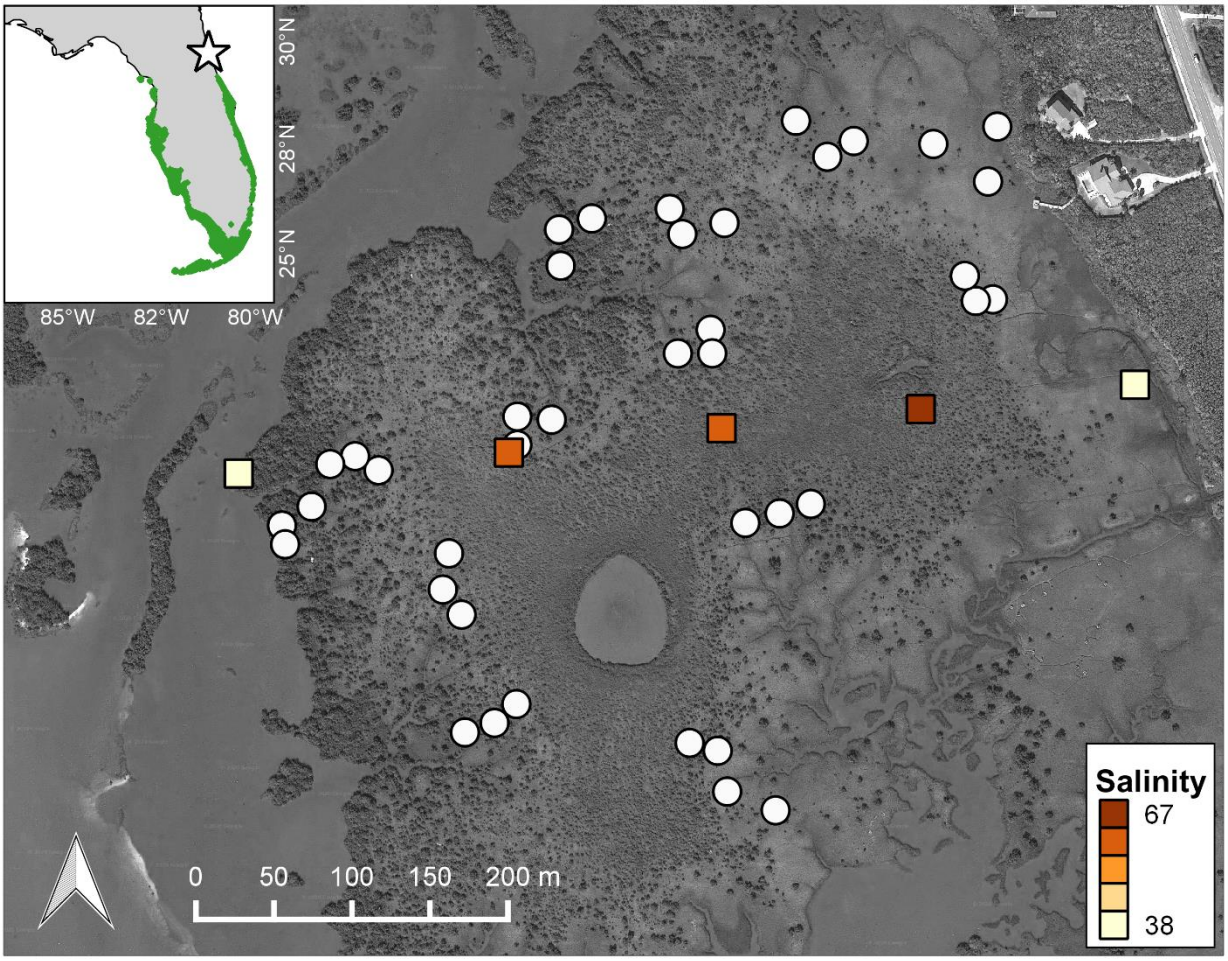
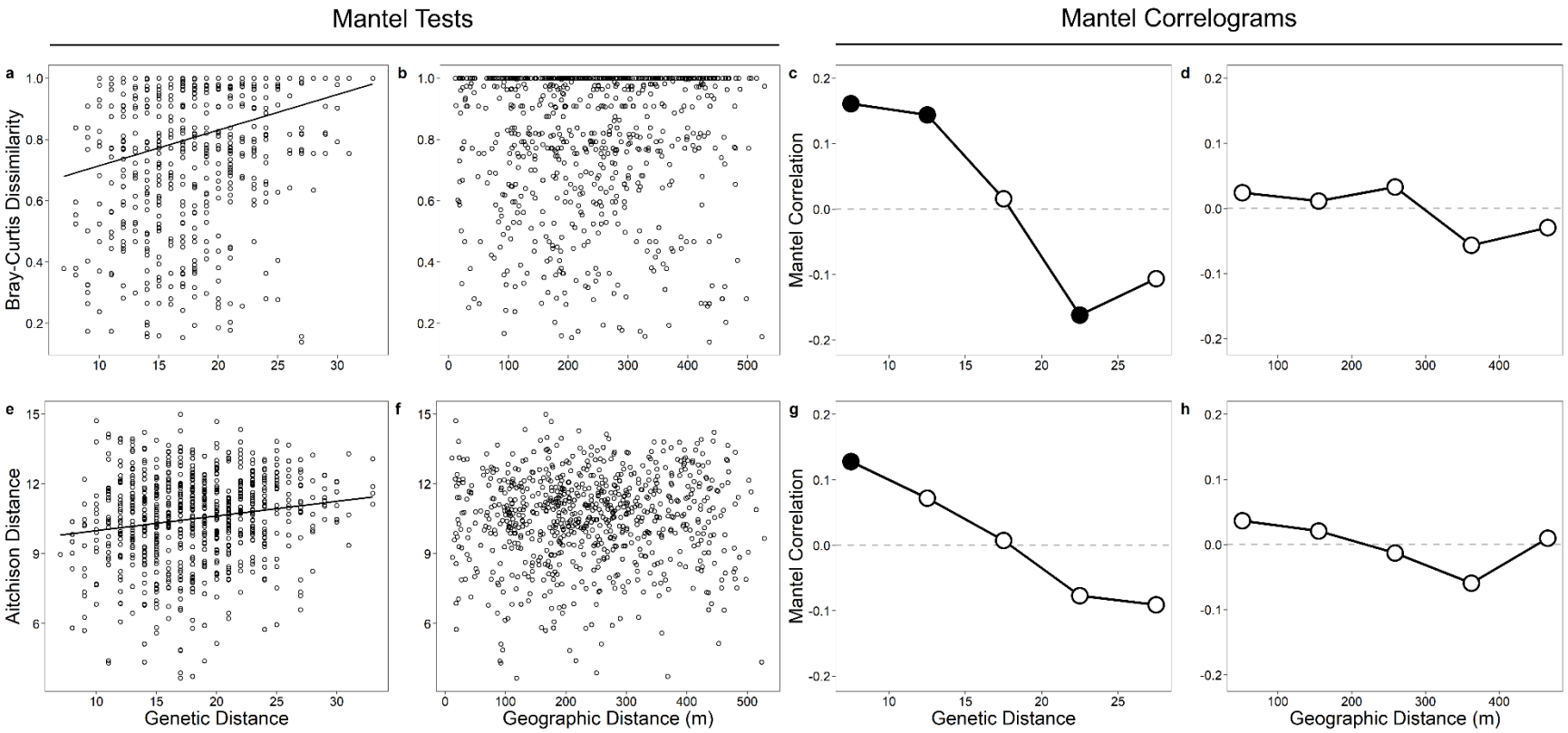


Figure 1. Collection site at the Atlantic Florida, USA, northern distributional limit of *Avicennia germinans* with locations of the 40 sampled *A. germinans* trees. This site is flanked by a brackish lagoon to the west and a fringe of terrestrial forest to the east. Soil salinities (‰) are mean values measured between September and November (2012–2017) (Guana Tolomato Matanzas National Estuarine Research Reserve, *unpublished data*). Upper panel shows the location of the collection site (with a star) and the Florida mangrove distribution in green (Giri et al., 2011).



847

848 **Figure 2**. Genetically-similar mangrove host trees harboured similar associated endophytic fungal communities, independent of
849 geographic distances among these host trees. Panels show graphical representations of the relationships between fungal community
850 dissimilarity (measured with Bray-Curtis dissimilarity and Aitchison distance) and each of the two predictor variables (inter-host
851 genetic distance and inter-host geographic distance) across all mangrove host trees (Mantel tests) and at five distance classes (Mantel
852 correlograms). Statistically significant ($p < 0.05$) correlations between fungal community dissimilarity and inter-host genetic
853 distance(s) are depicted with solid lines for Mantel tests and with black circles for Mantel correlograms.

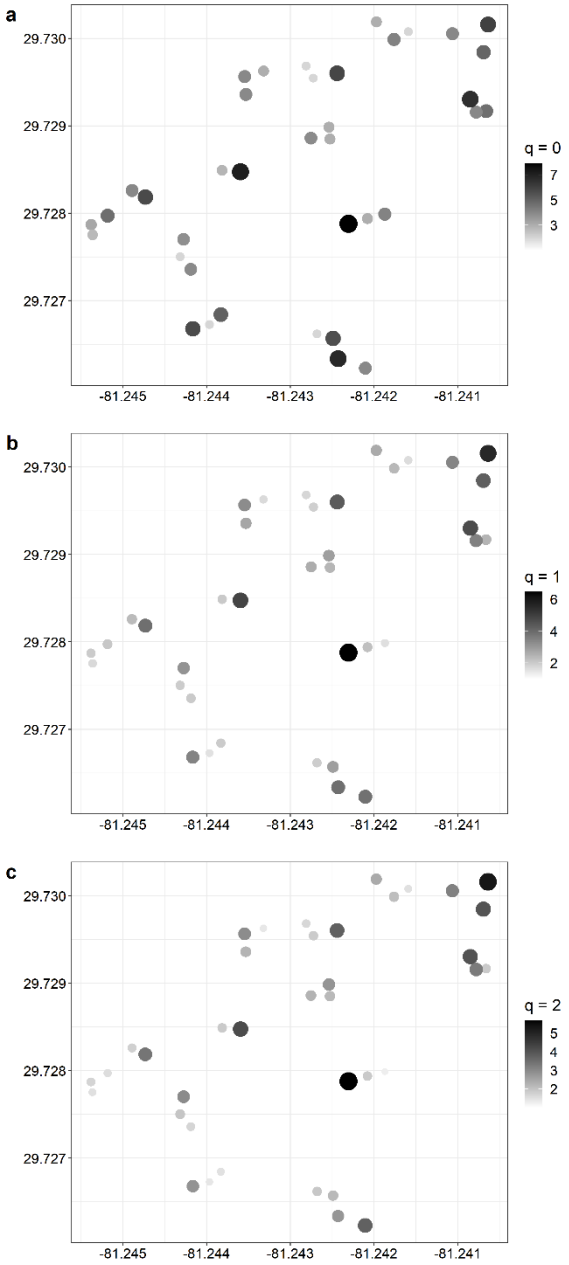


Figure 3. Spatial distribution of the alpha diversity of associated endophytic fungal communities within 40 *Avicennia germinans* trees across a collection site at the northern distributional limit of this species. Alpha diversity was calculated with Hill numbers at the scales of (a) q=0 (species richness), (b) q=1 (exponential of Shannon index), and (c) q=2 (inverse of Simpson index), which put more weight on abundant species as the value of q increases. In the figure, values of fungal alpha diversity for each tree increase with colour (from white to black) and with the size of the data point.