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Mating system variation in neotropical black mangrove, *Avicennia germinans*, at three spatial scales towards an expanding northern distributional limit

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Mating system variation in neotropical black mangrove, Avicennia germinans, at three

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| 2  | spatial scales towards an expanding northern distributional limit  |
|----|--|
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| 9  |  |
| 10 | Abstract   |
| 11 | Climate-driven range expansion of ecosystem-defining foundation species can have wide-                                 |
| 12 | reaching ecological consequences. Expansion may also result in mating system changes in these                          |
| 13 | foundation species because of the ecological characteristics of range margins, such as greater                         |
| 14 | conspecific isolation and reduced pollinator availability. It is important to understand how                           |
| 15 | mating systems may change during expansion due to their direct influence on intraspecific                              |

16 genetic and demographic dynamics. Here, we used 12 microsatellite loci to genotype progeny

arrays of the neotropical black mangrove (Avicennia germinans) at six collection sites (n = 23

18 maternal trees; 1,612 genotyped propagules) along a latitudinal gradient towards a northern

distributional limit on the Atlantic coast of Florida, USA  $(27.56 - 30.01^{\circ}N)$ , where mangroves

20 have expanded into salt marsh over the past several decades. We assessed mating system

21 variation at three spatial scales. First, at the species-distribution level, published outcrossing rates

22 for tropical conspecifics were more than two times higher than those for subtropical Florida *A*.

germinans, consistent with reductions in pollinator diversity and in mangrove abundance with 23 latitude. Second, at the population level, Florida outcrossing rates did not systematically decline 24 towards the northern range limit, but instead, a more open pollen-dispersal neighbourhood at the 25 transition from mangrove to salt marsh dominance may elevate outcrossing until conspecific 26 abundances become too low towards the range limit. Third, at the individual level, outcrossing 27 28 increased as conspecific cover increased at the Florida range margin, consistent with density-29 dependent plastic shifts in mating system. These findings suggest that ecological structure influences the A. germinans mating system at varying spatial scales. Further research needs to 30 evaluate the effect of A. germinans mating system variation on the survival and fitness of 31 32 offspring and on the extent of population-level local adaptation at expanding distributional limits. 33

*Keywords*: density-dependent; foundation species; outcrossing; pollinator diversity; range
expansion; self-fertilisation

36

## 37 Introduction

Climate-driven redistributions of species are now commonplace and can lead to important
changes in ecological communities, ecosystem function, and human well-being (Pecl et al.,
2017). For instance, range expansion of ecosystem-defining foundation species (e.g., long-lived
tree and shrub species) can result in entire biome shifts with wide-reaching ecological
consequences (Beck et al., 2011; Peñuelas and Boada, 2003; Saintilan et al., 2014).
Colonisation of new habitat has long been associated with greater self-fertilisation in plants

44 (i.e., Baker's Law; Baker, 1955) as characteristics of expanding range margins, such as greater

isolation among conspecifics (Eckert et al., 2010; Ghazoul, 2005) and reductions in pollinator 45 availability (Kalisz et al., 2004; Moeller et al., 2012; Yin et al., 2016), are known to select for 46 increased self-fertilisation (Hargreaves and Eckert, 2014). However, adaptive shifts in mating 47 system are not thought to be general attributes of the expansion of long-lived trees and shrubs 48 because these species generally maintain outcrossing independent of their environment (Barrett 49 and Harder, 2017). Instead, density-dependent plastic shifts towards greater self-fertilisation may 50 51 occur during initial colonisation (Morgan et al., 2005; Peterson and Kay, 2015), with subsequent changes post-colonisation as increased conspecific density favours shifts back towards greater 52 outcrossing (Pannell, 2015). Understanding mating system variation is important because of its 53 direct influence on genetic and demographic dynamics within a species (Barrett and Harder, 54 2017 and cites within). 55

Mangroves are an assortment of intertidal tree and shrub species that are ecologically-56 important coastal foundation species (Tomlinson, 1986). Mangroves originate in the tropics and 57 decline in abundance towards latitudinal range limits that correspond to ecological thresholds in 58 59 temperature and/or precipitation (Osland et al., 2017). The pantropical genus Avicennia consists of eight species, of which three are found in the Neotropics (Duke, 1992). Of these three 60 neotropical Avicennia species, Avicennia germinans (neotropical black mangrove) is the most 61 widespread, with a distribution across the tropics and into the subtropics (Lonard et al., 2017). 62 On the Atlantic coast of Florida (USA), mangroves are the dominant coastal foundation species 63 at lower latitudes where three mangrove species (A. germinans, plus Rhizophora mangle and 64 Laguncularia racemosa) form dense forests, but the higher-latitude mangrove range margin 65 66 consists of discrete patches of isolated individuals (almost exclusively A. germinans) in a salt marsh-dominated landscape (Kangas and Lugo, 1990). Lack of extreme winter freezes over the 67

past several decades have been linked to proliferation and expansion of *A. germinans* at this
northern distributional limit (Cavanaugh et al., 2019, 2014; Rodriguez et al., 2016) and further
expansion is forecast with climate change (Cavanaugh et al., 2019, 2015). This ongoing shift
from salt marsh to mangrove dominance at this expanding range margin will presumably lead to
significant changes in ecosystem structure and services essential to human well-being, including
nutrient storage, storm protection, and habitat availability for certain fauna (Doughty et al., 2017,
2016; Kelleway et al., 2017; Osland et al., 2018; Simpson et al., 2019).

Avicennia germinans is hermaphroditic with clusters of white flowers (each with one stigma 75 and four stamens) on axillary or terminal inflorescences (Lonard et al., 2017), and is recognised 76 77 as predominantly outcrossed via insect pollination (Tomlinson, 1986). However, higher-latitude mangroves may encounter far less diverse sets of pollinators than their tropical conspecifics 78 (Hermansen et al., 2014b). Few pollination studies exist for A. germinans, but those that do 79 demonstrate greater pollinator diversity at lower latitudes (Sánchez-Núñez and Mancera-Pineda, 80 2012) compared to higher latitudes (Landry, 2013). Consistent with this latitudinal decline in 81 pollinator diversity, indirect genetic evidence (via inbreeding coefficients, FIS) supports 82 predominant outcrossing in A. germinans populations closer to the range centre of this species 83 (Cerón-Souza et al., 2012; Mori et al., 2015; Nettel et al., 2008; Ochoa Zavala et al., 2019; but, 84 see Salas-Leiva et al., 2009), with observations of elevated inbreeding towards range limits 85 (Kennedy et al., 2020a; Mori et al., 2015; Ochoa Zavala et al., 2019). Direct evidence via 86 progeny arrays with A. germinans in Mexico also supports predominant outcrossing closer to the 87 range centre (Nettel-Hernanz et al., 2013), but we still lack equivalent direct estimates of mating 88 89 system towards A. germinans distributional limits.

90 Here, we used progeny arrays to estimate A. germinans outcrossing rates at six collection sites along a latitudinal gradient towards the expanding northern distributional limit of this 91 species on the Atlantic coast of Florida. We then assessed variation in mating system (i.e., 92 93 outcrossing versus self-fertilisation rates; Neal and Anderson, 2005) at three spatial scales. First, at the species-distribution level, we compared published outcrossing rates based on progeny 94 arrays from tropical conspecifics to rates observed here for subtropical A. germinans. Second, at 95 the population level, we assessed whether outcrossing rates decreased along the Florida 96 latitudinal gradient that reaches the northern range limit of this species. Third, at the individual 97 level, we assessed whether conspecific cover influences outcrossing rates of individual trees at 98 99 the expanding range margin. We tested the following predictions: (1) outcrossing rates will decline from the tropics to subtropics; (2) population-level outcrossing rates will decline along 100 the latitudinal gradient in Florida; (3) tree-level outcrossing rates will increase as conspecific 101 102 cover increases at the Florida range margin.

103

104 Materials and methods

## 105 2.1 Collection sites and sampling methods

106 On 7-8 October 2017, we collected ~100 propagules from and recorded GPS coordinates for 107 each of 30 maternal trees at six collection sites (n = 5 trees per site) along a latitudinal gradient 108 (27.56 – 30.01°N) on the Atlantic coast of Florida. We systematically collected propagules from 109 around the entire canopy of each maternal tree. A leaf was also collected from each maternal tree 110 and dehydrated in silica gel to obtain maternal genotypes. Atlantic Florida mangroves are 111 replaced by salt marsh as the dominant coastal foundation species at approximately 29 °N 112 (Spalding et al., 2010). Our three most southern collection sites (27.56 – 28.37°N) are areas

within the A. germinans continuous range core where mangroves are the dominant coastal 113 foundation species; whereas, our three most northern collection sites  $(29.41 - 30.01^{\circ}N)$  are areas 114 at the A. germinans range margin where salt marsh species are dominant and A. germinans exists 115 116 as discrete patches of isolated individuals (Fig. 1). Population genetic data for each of these collection sites was previously collected in 2015 (Table 1; Kennedy et al., 2020a). All 117 propagules from an individual tree were kept together in one plastic bag during field collections. 118 119 Propagules that were eventually genotyped had their pericarps removed and were stored at -20°C until DNA extraction. 120

Our study design reflects two principal factors: (1) Atlantic Florida A. germinans exhibits a 121 122 considerable reduction in genetic variation towards the northern distributional limit (Kennedy et 123 al., 2020a) and (2) limited polymorphism can restrict our ability to detect differences using molecular markers (Arnaud-Haond et al., 2005). We were concerned that reduced genetic 124 variation may inhibit our ability to quantify reliable outcrossing rates and, as such, we needed to 125 focus more sampling effort on the number of offspring per tree. This concern proved valid, as we 126 127 determined that a relatively large number of offspring were needed to obtain reliable tree-level outcrossing estimates (see 2.3 Descriptive analyses). Hence, for practical reasons, we were 128 limited in the number of sampled maternal trees that could be included in this study. We 129 included 23 maternal trees. From south to north, we genotyped progeny arrays from n = 5, 2, and130 2 maternal trees at each of the three range-core sites, and n = 4, 5, and 5 maternal trees at each of 131 the three range-margin sites (Table 1). We focussed more effort on the range-margin sites to 132 133 better address our third prediction (i.e., tree-level outcrossing rates will increase as conspecific 134 cover increases at the range margin). Although only two maternal trees were analysed at two of the range-core sites, we attempted to capture variation across as large of an area as possible. 135

136 These two forest patches (code: MH, U; Table 1) extend for approximately 2.3 and 3.9 km,

respectively, along the adjacent river channel, and we selected trees that were separated by 1.3

and 1.2 km, respectively (i.e., 56% and 31% of the linear extent of these collection sites).

139



Fig. 1. Six collection sites along a latitudinal gradient  $(27.56 - 30.01^{\circ}N)$  on the Atlantic coast of Florida (USA) for *Avicennia germinans* progeny arrays. (a) The three most southern sites (blue circles) are areas within the continuous range core of this species, where mangroves are the dominant coastal foundation species. The three most northern collection sites (red circles) are

| 145 | areas at the range margin of this species, where salt marsh species are dominant and A.            |
|-----|--|
| 146 | germinans exists in discrete patches. Mangrove distribution is shown in green (Giri et al., 2011). |
| 147 | (b, c) Representative picture of range-margin and range-core community structure, respectively.    |
| 148 |  |
| 149 | 2.2 DNA isolation and microsatellite genotyping  |
| 150 | For leaves from the 23 maternal trees, genomic DNA was isolated from 20 mg of dry tissue with      |
| 151 | the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the standard protocol, with an       |
| 152 | extended incubation of 45 minutes. Trees were genotyped at 12 previously-developed nuclear         |
| 153 | microsatellite loci (Cerón-Souza et al., 2012, 2006; Mori et al., 2010; Nettel et al., 2005)       |
| 154 | following the protocol outlined in Kennedy et al. (2020a). We performed PCR on a Prime             |
| 155 | thermal cycler (Techne, Straffordshire, UK), analysed fragments on an Applied Biosystems 3730      |
| 156 | DNA Analyzer (Applied Biosystems, Foster City, California, USA) with LIZ 500 size standard,        |
| 157 | and scored alleles in the R-package Fragman (Covarrubias-Pazaran et al., 2016). We amplified       |
| 158 | and genotyped DNA from each maternal tree twice to ensure we had the correct multi-locus           |
| 159 | genotype.  |

For each propagule, we removed the cotyledons and extracted DNA from portions of the 160 hypocotyl and radicle, the eventual stem and root of the germinating seedling. Genomic DNA 161 from propagules was isolated from 50 mg of frozen hypocotyl/radicle tissue with the DNeasy 96 162 Plant Kit (Qiagen, Hilden, Germany) following the standard protocol. Subsequent genotyping 163 steps were identical to those for leaves, but we modified the PCR volumes outlined in Kennedy 164 et al. (2020a). Each of the two multiplex PCR contained a total volume of 6 µL with 2.5 µL 165 Multiplex PCR Master Mix, 0.5 µL primer mix, and 3 µL of genomic DNA. We also randomly 166

re-amplified and re-genotyped 5% of our propagule DNA samples to estimate a study error rate(Bonin et al., 2004).

169

170 2.3 Descriptive analyses: maternal genotypes and sample sizes

We performed a discriminant analysis of principal components (DAPC) (Jombart et al., 2010) in
the R-package adegenet 2.1.1 (Jombart and Ahmed, 2011) as a visual assessment of genetic
differences among the 23 maternal trees. We retained nine principal components, the minimum
number that explained ~90% of the total variance, identified two clusters, and retained three
discriminant functions. We extracted each individual's coordinates on the two principal axes of
the DAPC (i.e., ind.coord) and plotted them in ggplot2 (Wickham, 2011).

177 We performed an initial analysis to estimate how many genotyped propagules would be 178 needed to provide reliable tree-level outcrossing estimates. For each of two trees (code: MH1, MH3), we genotyped as close to 100 propagules as possible (n = 87, 82, respectively) and 179 estimated tree-level detectable outcrossing rates with the direct approach outlined below (see 2.4 180 *Outcrossing calculations*). We then reduced the number of propagules in the data set by 181 increments of five (i.e., n = 80, 75, 70, 65, 60, 55, 50, 45) and re-calculated outcrossing rates for 182 each of these new subsets. This approach enabled us to visualise how outcrossing estimates 183 changed as sample sizes decreased. Estimates of these subsets remained relatively consistent 184 with the initial estimates (MH1: 0.6 - 6.8% change; MH3: 2.5 - 9.3% change) until n  $\leq 55$  when 185 values became more variable (MH1: 7.7 – 13% change; MH3: 16.1 – 43.1% change) (Fig. 2). As 186 such, we determined that  $n \ge 60$  propagules per tree should be sufficient to estimate reliable tree-187 188 level outcrossing rates.



**Fig. 2.** Initial analysis to estimate an appropriate sample size of propagules to calculate tree-level outcrossing rates. Systematic reductions in sample size for two trees (MH1, MH3) continued to provide estimates of detectable outcrossing rate ( $t_d$ ) relatively consistent with initial estimates until n  $\leq$  55 when values became more variable (MH1: 7.7 – 13% change; MH3: 16.1 – 43.1% change). This threshold is shown with a vertical dashed line. We determined that n  $\geq$  60 propagules should provide reliable estimates of tree-level outcrossing rates.

197

## 198 2.4 Outcrossing calculations: population-level and tree-level

199 To assess population-level mating system variation towards the Florida A. germinans range limit,

- 200 we calculated multi-locus outcrossing rates  $(t_m)$  for each of the six collection sites with the
- 201 maximum likelihood-based MLTR (Ritland, 2002). We also calculated levels of biparental
- inbreeding  $(t_m t_s)$  and we used the proportion of offspring with the same father  $[r_p(m)]$  to

calculate the number of effective pollen donors  $[1 / r_p(m)]$  for each collection site. We used default parameters, 500 bootstraps to calculate standard errors, and resampled among individuals within families.

To assess mating system variation among individual trees, we used a direct approach based 206 on allelic differences between maternal genotypes and their sampled offspring to calculate 207 apparent outcrossing rates (Cruzan et al., 1994). First, detectable outcrossing rates (t<sub>d</sub>) were 208 calculated as the number of propagules that possessed an allele not present in the maternal 209 210 genotype (i.e., detectable outcross event) divided by the number of propagules genotyped. However, there may also be a percentage of offspring that appear to be the product of self-211 212 fertilisation, but are instead the result of outcrossing to individuals with similar genotypes to the maternal tree (i.e., undetectable outcross event). Hence, maternal genotypes with high-frequency 213 alleles in a population will result in greater undetected outcrossing. Using allele frequency data 214 from each of these six collection sites in 2015 (Kennedy et al., 2020b), we calculated the 215 probability of an undetectable outcross event  $[P(u)_i]$  for each of the 23 maternal trees as the 216 217 product across loci of the frequencies of each maternal allele within the corresponding collection 218 site (Cruzan et al., 1994). For each maternal tree, we multiplied its specific  $P(u)_i$  by the number of propagules that were not identified as outcrossed to obtain an estimate of the number of 219 potential undetected outcross events. We then calculated apparent outcrossing rates  $(t_a)$  as the 220 number of detectable outcross events plus the number of potential undetected outcross events 221 divided by the number of propagules genotyped. We also estimated these tree-level outcrossing 222 rates with the maximum likelihood-based MLTR, as described above for population-level 223 224 estimates.

## 226 2.5 Estimates of conspecific cover at the range margin

227 It remains difficult to remotely assess mangrove cover at fragmented range margins (Bunting et al., 2018). However, mangrove patches within salt marsh can generally be manually identified 228 with relative ease in satellite images, while also aided by knowledge of the collection site. For 229 each of the three range-margin collection sites, we manually measured approximate estimates of 230 area covered with A. germinans within a 10 m radius circle around each maternal tree (n = 14 231 GPS-referenced trees) with the polygon function in Google Earth Pro 7.3.2.5776. We chose this 232 size because 10 m was the longest distance that permitted reliable estimates around maternal 233 trees adjacent to terrestrial hammock forest patches. 234

235

## 236 2.6 Statistical analyses

237 We performed all statistical analyses in R v3.4.2 (R Core Team, 2013). To test prediction 1 (decline in outcrossing from tropics to subtropics), we compared mean multi-locus outcrossing 238 rates  $(t_m)$  for these Florida collection sites (n = 6 sites) to published values for conspecifics in 239 Mexico (n = 3 sites; Nettel-Hernanz et al., 2013) with a two-sample t-test. To test prediction 2 240 (decline in outcrossing along the Florida latitudinal gradient), we evaluated the relationship 241 between  $t_m$  and latitude with a Spearman's rank correlation (n = 6 sites). To test prediction 3 242 (increase in outcrossing with increased conspecific cover at the range margin), we evaluated the 243 effect of A. germinans cover around each range-margin maternal tree on tree-level apparent 244 outcrossing rates  $(t_a)$  with a linear regression (n = 14 trees). We natural log-transformed A. 245 germinans cover to meet the statistical assumption of normality. 246

## 248 **Results**

249 3.1 Maternal genotypes, sample sizes and study error rate

- Each of the 23 maternal trees produced consistent multi-locus genotypes after being amplified
- and genotyped a second time. All 23 maternal genotypes were unique, with a range from 1 of 12
- to 10 of 12 matching loci, and exhibited a clear separation between range core and margin
- 253 genotypes (Fig. 3). We genotyped a total of 1,612 propagules, with a mean sample size per tree
- of 70.1  $\pm$  5.3 (SD; range: 64 87). We re-amplified and re-genotyped DNA from 87 propagules
- 255 (5.4% of all samples) and found an error rate of 0.01% (one error out of 1,044 locus
- comparisons). This locus-specific error was removed from the data set.



Fig. 3. Discriminant analysis of principal components (DAPC) of maternal tree multi-locus
genotypes. All 23 maternal trees possessed unique multi-locus genotypes and exhibited a clear
separation between range core and margin. Open shapes indicate range-core sites (A, Avalon;

- MH, Maritime Hammock; U, Ulumay); filled shapes indicate range-margin sites (N, North
  Peninsula; GS, GTM South; GN, GTM North).
- 264
- 265 *3.2 Population-level mating system variation*

Population-level multi-locus outcrossing rates ( $t_m$ ) in Florida ranged from 0.14 ± 0.02 (SE) to 0.49 ± 0.03 (Table 1). Florida  $t_m$  were, on average, more than two times lower than published values for tropical conspecifics in Mexico (mean  $t_m$  Florida: 0.32, Mexico: 0.71;  $t_{4.8} = -4.8$ , p = 0.006) (Fig. 4).

Population-level outcrossing (t<sub>m</sub>) did not systematically decline along the Florida latitudinal 270 gradient (Spearman's correlation,  $r_s = -0.20$ , p = 0.70). Instead, highest and lowest values were 271 observed in range-margin sites (Fig. 4). All collection sites exhibited low levels of biparental 272 273 inbreeding ( $t_m - t_s = -0.01 - 0.06$ ) (Table 1). MLTR could not calculate the proportion of offspring with the same father  $[r_p(m)]$  for one range-margin site (code: GS), presumably because 274 this site exhibited the lowest outcrossing rates (Table 1, 2). Estimates of  $r_p(m)$  were calculated 275 for all other collection sites and the number of effective pollen donors  $[1 / r_p(m)]$  varied from 2.5 276 to 20.4, with the lowest number of pollen donors at the northern range limit (code: GN) and the 277 highest number at the most southern range-margin site (code: N) (Table 1). 278

279**Table 1** Population-level genetic diversity and mating system parameters of *Avicennia germinans* from six collection sites on the280Atlantic coast of Florida (USA). AR, allelic richness;  $H_S$ , unbiased gene diversity; arrays, number of progeny arrays sampled; n, total281number of propagules genotyped;  $t_m$ , multi-locus outcrossing rate;  $t_m - t_s$ , biparental inbreeding;  $r_p(m)$ , proportion of offspring with the282same father;  $1 / r_p(m)$ , number of effective pollen donors. Standard error in parentheses. Note: estimates of  $r_p(m)$  for collection site GS283could not be calculated. <sup>†</sup>Data from Kennedy et al. (2020a) based on n = 30 adult trees per collection site genotyped at the same 12

|                  |      |          | genetic     | diversity <sup>†</sup> |        |     |      | p              | rogeny a       | arrays           |                |        |                      |
|------------------|------|----------|-------------|------------------------|--------|-----|------|----------------|----------------|------------------|----------------|--------|----------------------|
| Site             | Code | Latitude | AR          | H <sub>S</sub>         | arrays | n   |      | t <sub>m</sub> | t <sub>m</sub> | - t <sub>s</sub> | r <sub>p</sub> | (m)    | 1/r <sub>p</sub> (m) |
| Avalon           | А    | 27.5468  | 3.40 (0.64) | 0.42 (0.05)            | 5      | 336 | 0.37 | (0.03)         | 0.03           | (0.01)           | 0.16           | (0.04) | 6.2                  |
| Maritime Hammock | MH   | 27.9566  | 3.14 (0.48) | 0.42 (0.05)            | 2      | 169 | 0.22 | (0.04)         | 0.03           | (0.01)           | 0.17           | (0.07) | 5.8                  |
| Ulumay           | U    | 28.3756  | 2.33 (0.24) | 0.31 (0.05)            | 2      | 135 | 0.39 | (0.05)         | -0.01          | (0.02)           | 0.08           | (0.12) | 13.2                 |
| North Peninsula  | Ν    | 29.4096  | 2.83 (0.61) | 0.35 (0.06)            | 4      | 277 | 0.49 | (0.03)         | 0.00           | (0.02)           | 0.05           | (0.02) | 20.4                 |
| GTM South        | GS   | 29.7159  | 2.85 (0.42) | 0.40 (0.06)            | 5      | 342 | 0.14 | (0.02)         | 0.02           | (0.01)           | -              | -      | -                    |
| GTM North        | GN   | 30.0144  | 2.14 (0.29) | 0.21 (0.05)            | 5      | 353 | 0.31 | (0.02)         | 0.06           | (0.01)           | 0.41           | (0.07) | 2.5                  |

284 nuclear microsatellite loci used here.



**Fig. 4.** Population-level multi-locus outcrossing rates ( $t_m$ ) for tropical *Avicennia germinans* in Mexico (Nettel-Hernanz et al., 2013) and subtropical *A. germinans* in Florida (this study). Florida *A. germinans* mean  $t_m$  was more than two times smaller than their tropical conspecifics ( $t_{4.8} = -4.8$ , p = 0.006). Outcrossing ( $t_m$ ) did not systematically decline along the Florida latitudinal gradient ( $r_s = -0.20$ , p = 0.70). Non-significant correlation is depicted with a dotted line. *A. germinans* in Mexico is shown in black, in Florida range core is shown in grey, and in Florida range margin is shown in white. Population error bars are standard error. \*\*, p < 0.01.

## 294 *3.3 Tree-level mating system variation*

Tree-level detectable outcrossing rates (t<sub>d</sub>) ranged from 0.05 to 0.58 (Table 2). Probabilities of an 295 undetectable outcross event were generally low (0 - 0.09) and, as such, the number of potential 296 undetected outcross events was also generally low (0 - 4.7) (Table 2). However, there were two 297 notable exceptions with probabilities of 0.17 and 0.22, and a total of 9 and 12 potential 298 undetected outcross events (tree: GN1, GN4; Table 2). These two exceptions were trees at the 299 northern range limit, the least genetically-diverse collection site (Table 1). After incorporating 300 potential undetected outcross events into our calculations, tree-level apparent outcrossing rates 301 (t<sub>a</sub>) still ranged from 0.05 to 0.58 (Table 2) and were highly correlated with t<sub>d</sub> (Pearson's 302 303 correlation, r = 0.97, p < 0.0001). Tree-level estimates calculated with MLTR were also highly correlated with both  $t_d$  (r = 0.99, p < 0.0001) and  $t_a$  (r = 0.97, p < 0.0001). 304 305 Conspecific cover at the range margin, measured as the area covered by A. germinans within 306 a 10 m radius circle around each range-margin maternal tree, varied considerably from 15.5 to 177.4  $m^2$  (7 – 57% cover), with highest values at the most southern range-margin site (tree: N2-307 5; Table 2). Apparent outcrossing rates ( $t_a$ ) increased as conspecific cover increased ( $t_a = -0.46 +$ 308  $0.20*\ln(\text{cover})$ ,  $F_{1,12} = 21.0$ , p = 0.0006,  $r_{adj}^2 = 0.61$ ), with a 2% increase in outcrossing with 309

every 10% increase in A. germinans cover  $(m^2)$  (Fig. 5).

Table 2 Tree-level outcrossing rates and conspecific cover around range-margin trees. n, number

of propagules genotyped;  $t_d$ , detectable outcrossing rate;  $P(u)_j$ , probability of an undetectable 312 outcross event; undetected, number of potential undetected outcross events; ta, apparent 313

outcrossing rate; cover, A. germinans cover within a 10 m radius circle around each maternal tree 314

at range-margin sites. Note: cover was not calculated for range-core collection sites. 315

316

| Tree | Latitude  | Longitude  | n  | t <sub>d</sub> | $P(u)_j$ | undetected | t <sub>a</sub> | cover |
|------|-----------|------------|----|----------------|----------|------------|----------------|-------|
| A1   | 27.560644 | -80.328730 | 72 | 0.33           | 0.000    | 0.0        | 0.33           | -     |
| A2   | 27.559835 | -80.329391 | 67 | 0.49           | 0.050    | 1.7        | 0.52           | -     |
| A3   | 27.559641 | -80.329857 | 66 | 0.42           | 0.002    | 0.1        | 0.43           | -     |
| A4   | 27.559870 | -80.330614 | 64 | 0.17           | 0.003    | 0.2        | 0.17           | -     |
| A5   | 27.557966 | -80.329410 | 67 | 0.42           | 0.014    | 0.6        | 0.43           | -     |
| MH1  | 27.958072 | -80.515093 | 87 | 0.24           | 0.004    | 0.3        | 0.24           | -     |
| MH3  | 27.951081 | -80.509048 | 82 | 0.20           | 0.001    | 0.1        | 0.20           | -     |
| U3   | 28.372275 | -80.684404 | 70 | 0.23           | 0.088    | 4.7        | 0.30           | -     |
| U5   | 28.380151 | -80.685456 | 65 | 0.52           | 0.003    | 0.1        | 0.52           | -     |
| N2   | 29.407971 | -81.099778 | 65 | 0.40           | 0.006    | 0.2        | 0.40           | 177.4 |
| N3   | 29.407679 | -81.098927 | 70 | 0.40           | 0.035    | 1.5        | 0.42           | 44.5  |
| N4   | 29.407872 | -81.099167 | 70 | 0.53           | 0.026    | 0.9        | 0.54           | 111.1 |
| N5   | 29.407941 | -81.099617 | 72 | 0.58           | 0.002    | 0.1        | 0.58           | 173.2 |
| GS1  | 29.729168 | -81.240662 | 65 | 0.05           | 0.005    | 0.3        | 0.05           | 28.2  |
| GS2  | 29.730158 | -81.240638 | 66 | 0.06           | 0.038    | 2.3        | 0.10           | 21.3  |
| GS3  | 29.730077 | -81.241591 | 70 | 0.10           | 0.016    | 1.0        | 0.11           | 32.9  |
| GS4  | 29.730190 | -81.241972 | 71 | 0.14           | 0.013    | 0.8        | 0.15           | 54.1  |
| GS5  | 29.729601 | -81.242441 | 70 | 0.31           | 0.079    | 3.8        | 0.37           | 87.1  |
| GN1  | 30.016524 | -81.345922 | 70 | 0.23           | 0.222    | 12.0       | 0.40           | 45.5  |
| GN2  | 30.016660 | -81.345954 | 72 | 0.43           | 0.014    | 0.6        | 0.44           | 39.7  |
| GN3  | 30.014781 | -81.344683 | 72 | 0.49           | 0.081    | 3.0        | 0.53           | 93.2  |
| GN4  | 30.014178 | -81.344754 | 69 | 0.23           | 0.171    | 9.0        | 0.36           | 91.2  |
| GN5  | 30.013645 | -81.345022 | 70 | 0.06           | 0.026    | 1.7        | 0.08           | 15.5  |



317

Fig. 5. Tree-level apparent outcrossing rates (t<sub>a</sub>) increased as conspecific cover [ln(cover)]
increased at the *A. germinans* range margin on the Atlantic coast of Florida (USA). Conspecific
cover was measured as the area covered by *A. germinans* within a 10 m radius circle around each
maternal tree at the three range-margin collection sites.

322

## 323 Discussion

We used progeny arrays to characterise mating system in *Avicennia germinans* at six collection sites along a latitudinal gradient towards the northern distributional limit of this species on the Atlantic coast of Florida ( $27.56 - 30.01^{\circ}$ N). We assessed variation in mating system at three

| 327 | spatial scales: (1) at the species-distribution level, (2) at the population level along the Florida |
|-----|--|
| 328 | latitudinal gradient, and (3) at the individual level among conspecifics at the expanding Florida    |
| 329 | range margin. First, published outcrossing rates for tropical conspecifics in Mexico were, on        |
| 330 | average, more than two times higher than those for subtropical Florida A. germinans. Second,         |
| 331 | population-level outcrossing rates did not systematically decline with latitude towards the          |
| 332 | northern range limit in Florida. Third, tree-level outcrossing rates increased as conspecific cover  |
| 333 | increased at the Florida range margin.   |

334

## 335 4.1 Reduced outcrossing from tropics to subtropics

In contrast to the prevailing idea that A. germinans is predominantly outcrossed, we found that 336 337 all Florida collection sites were predominantly self-fertilised. Tropical conspecifics in Mexico (14.61 – 15.95°N) exhibit predominant outcrossing (Nettel-Hernanz et al., 2013), with 338 339 outcrossing rates more than double our observations for subtropical Florida A. germinans. Predominant outcrossing has also been documented for a co-occurring neotropical congener, A. 340 341 schaueriana, at lower latitude (0.82°S) (Mori et al., 2015). Reduced outcrossing towards A. germinans distributional limits is consistent with a global decline in plant outcrossing rates 342 towards higher latitude (Moeller et al., 2017). However, Moeller et al. (2017) found that this 343 344 global pattern is mostly shaped by latitudinal changes in life history and growth form (i.e., lower latitude: greater frequency of perennial and tree species; higher latitude: greater frequency of 345 annual and herbaceous species), and propose that evaluations of mating system variation in 346 347 species with broad latitudinal distributions will help better understand the potential impact of plant-pollinator interactions in shaping latitudinal patterns in mating systems. 348

349 Reduced outcrossing in subtropical A. germinans is consistent with reductions in pollinator diversity across this species' broad distribution range. At lower latitude, four highly-effective 350 pollinators were identified on the small, Caribbean island of San Andrés (12.54°N) (Sánchez-351 Núñez and Mancera-Pineda, 2012), and a diverse set of pollinators (including wasps, flies, and 352 bees) was also identified for the neotropical congener, A. schaueriana (7.68°S) (Nadia et al., 353 2013). At higher latitude, only one principal pollinator, the exotic European honey bee (Apis 354 355 *mellifera*), accounted for 87% of flower visits at a location in Southeast Florida (26.03°N) 356 (Landry, 2013). However, we cannot disregard additional factors that could also impact the A. germinans mating system in Florida, such as reduced mangrove abundances towards 357 distributional limits (Osland et al., 2017) and the highly-fragmented nature of Atlantic Florida 358 mangroves due to anthropogenic modifications of these coastal ecosystems (Brockmeyer et al., 359 1996). Outcrossing often increases with conspecific density and pollinator abundance, but these 360 361 two factors are not independent for animal-pollinated plants because dense groupings generally attract more pollinators (Ghazoul, 2005). Research near the southern latitudinal limits (33.94 -362 34.63°S) of a widespread Indo-West Pacific congener, A. marina, highlights this point. Exotic 363 European honey bees were also identified as the only significant pollinator for this species 364 (Hermansen et al., 2014b) and smaller, more fragmented stands attracted fewer pollinators with 365 altered foraging behaviour that may facilitate greater self-fertilisation (i.e., bees spent more time 366 foraging on individual trees) (Hermansen et al., 2014a). As a result, these smaller A. marina 367 stands exhibited reduced outcrossing compared to larger stands (Hermansen et al., 2015). Our 368 observation of reduced outcrossing in subtropical Florida A. germinans may be the product of the 369 interactive effects of reduced pollinator diversity and more fragmented mangrove area, that could 370 also reduce pollinator abundances, compared to tropical mangrove forests. Further research 371

| 372 | needs to directly link assessments of mating system with plant-pollinator surveys across the          |
|-----|---|
| 373 | broad, latitudinal distribution of A. germinans, as well as other Avicennia species, to provide       |
| 374 | definitive answers. In addition, research also needs to consider further environmental (e.g.,         |
| 375 | seasonality; Chybicki and Dzialuk, 2014; Yin et al., 2016) and biological factors (e.g., fecundity,   |
| 376 | phenology; Ghazoul, 2005; Kameyama and Kudo, 2015) that can shape mating system variation.            |
| 377 | Reductions in outcrossing are the product of a combination of biparental inbreeding and               |
| 378 | self-fertilisation. We found low biparental inbreeding at all collection sites, consistent with A.    |
| 379 | germinans progeny arrays in Mexico ( $t_m - t_s = 0.01 - 0.06$ ; Nettel-Hernanz et al., 2013).        |
| 380 | However, progeny arrays with the neotropical congener, A. schaueriana, exhibited a greater            |
| 381 | effect of biparental inbreeding ( $t_m - t_s = 0.15$ ; Mori et al., 2015), and even higher rates were |
| 382 | found at the southern latitudinal limits of the Indo-West Pacific congener, A. marina ( $t_m - t_s =$ |
| 383 | 0.29 - 0.53; Hermansen et al., 2015). Differences among these studies may be explained by             |
| 384 | interspecific variation in reproductive biology, variation in kinship structure among collection      |
| 385 | sites (Hasan et al., 2018), or simply methodological differences (e.g., number of progeny arrays,     |
| 386 | of propagules sampled, and of loci genotyped) that can impact these parameter estimates               |
| 387 | (Ritland, 2002). Our findings suggest that reduced outcrossing in subtropical Florida A.              |
| 388 | germinans is predominantly via self-fertilisation, either autonomous (within the same flower) or      |
| 389 | geitonogamous (among flowers on the same plant). Although we lack evidence for A. germinans,          |
| 390 | multiple Avicennia species are self-compatible, including the neotropical congener, A.                |
| 391 | schaueriana (Nadia et al., 2013), and three Indo-West Pacific congeners (Aluri, 1990; Raju et al.,    |
| 392 | 2012), with a much higher fruit set via geitonogamous compared to autonomous self-fertilisation       |
| 393 | for all species. Geitonogamous self-fertilisation is common in bee-pollinated plants (Harder and      |
| 394 | Barrett, 1995; Mitchell et al., 2004), is thought to aid mangrove colonisation (Primack et al.,       |

1981), and seems to be a likely mechanism in *A. germinans* as inflorescences have multipleflowers open simultaneously.

397

## 398 4.2 Density-dependent mating system variation towards range limits

We did not observe a systematic decline in outcrossing rates towards the expanding A. 399 germinans northern range limit in Florida, consistent with the prevailing view that adaptive shifts 400 in mating system are not common during range expansion of long-lived trees and shrubs (Barrett 401 and Harder, 2017). Instead, density-dependent plastic shifts in mating system may occur, with 402 403 transitions between increased self-fertilisation at low density and mixed mating at higher density (Morgan et al., 2005; Peterson and Kay, 2015). We found lowest outcrossing at the range 404 405 margin, but we also observed highest outcrossing at the most southern range-margin site (Fig. 4). While low density and greater spatial isolation among conspecifics at range margins can reduce 406 407 outcrossing (Ghazoul, 2005), the same can also be true in areas with higher species diversity that may increase competition for pollinators (Vamosi et al., 2006). Higher-density forests can also 408 409 restrict routes of pollen vectors and lead to declines in the diversity of tree-level pollen donors (González-Varo et al., 2009). Lower-latitude Florida mangroves consist of dense forests with A. 410 germinans and two other mangrove species (Rhizophora mangle, Laguncularia racemosa), with 411 evidence of competition for pollinators between A. germinans and L. racemosa (Landry, 2013). 412 In contrast, higher-latitude range-margin Florida mangroves are patches of almost exclusively A. 413 germinans individuals surrounded by lower-stature salt marsh species (Kangas and Lugo, 1990). 414 415 A more open pollen-dispersal neighbourhood and limited interspecific competition at the higherlatitude range margin could facilitate inter-tree pollination among A. germinans, consistent with 416 our observation of highest outcrossing and largest number of effective pollen donors at the most 417

southern range-margin site. However, this greater potential for inter-tree pollination would be
overshadowed farther north where limited numbers of conspecifics would provide very few
potential pollen sources, as evidenced by the lowest number of effective pollen donors at the
northern range limit.

At the range margin, tree-level outcrossing increased as conspecific cover increased. Lowest 422 outcrossing ( $t_a = 0.05$ ) at an A. germinans cover of 28.2 m<sup>2</sup> (7% of surrounding area covered by 423 A. germinans) was more than ten times smaller than highest outcrossing ( $t_a = 0.58$ ) at a cover of 424 173.2 m<sup>2</sup> (57% covered by A. germinans). We presume that continued proliferation of A. 425 germinans at this range margin, forecast with climate change (Cavanaugh et al., 2019, 2015), 426 427 will drive further mating system changes as increased conspecific density shifts mating systems towards greater outcrossing (Pannell, 2015). As A. germinans becomes more abundant, inter-428 individual distances will decline, presumably these denser patches will attract more pollinators, 429 and tree-level outcrossing will increase. Hence, documented loss of genetic diversity towards A. 430 germinans range limits (Kennedy et al., 2020a; Mori et al., 2015; Ochoa Zavala et al., 2020, 431 2019; Sandoval-Castro et al., 2014), and for other Avicennia species (Arnaud-Haond et al., 2006; 432 Binks et al., 2019; De Ryck et al., 2016; Maguire et al., 2000), may be the product of founder 433 effects combined with density-dependent plastic shifts towards greater self-fertilisation. Areas 434 that experience subsequent proliferation and immigration will then transition towards greater 435 outcrossing and increased offspring genetic diversity, but will exhibit unique genetic signatures 436 because of the disproportionate contribution of initial colonisers to the gene pool (e.g., Goldberg 437 and Heine, 2017). 438

Plastic shifts towards greater self-fertilisation at range margins can be advantageous as a
form of reproductive assurance (Hargreaves and Eckert, 2014) and tend to elevate seed

production when pollinators or mates are unreliable (Morgan et al., 2005). However, these 441 advantages may be offset by the genetic costs associated with inbreeding depression (e.g., 442 reduced offspring survival and fertility) (Charlesworth and Willis, 2009). These costs are 443 documented near the southern latitudinal limits of the Indo-West Pacific congener, A. marina, 444 where smaller, less-outcrossed stands exhibited reduced reproductive success, reduced propagule 445 size, and reduced seedling recruitment compared to larger stands (Hermansen et al., 2017). 446 447 However, our finding of predominant self-fertilisation in A. germinans runs contrary to mangrove performance at the Atlantic Florida range margin. These mangroves have undergone 448 proliferation and expansion for several decades (Cavanaugh et al., 2019, 2014; Rodriguez et al., 449 2016), with evidence from a co-occurring mangrove species, Rhizophora mangle, of precocious 450 reproduction and increased propagule size (Dangremond and Feller, 2016) and greater 451 reproductive success (Goldberg and Heine, 2017) compared to Florida conspecifics farther south. 452 453 Characterisation of A. germinans mating system coupled with assessments of offspring performance at this range margin is needed to garner insights into the potential influence of 454 mating system on these expanding populations. 455

## 456

## 457 *4.3 Considerations*

458 Mating system assessments are a balance between the number of progeny arrays and the number 459 of offspring genotyped per progeny array. Here, we focussed our efforts more on genotyping 460 larger numbers of offspring per progeny array. Our sampling design was shaped by our concern 461 that reduced genetic variation towards this northern distributional limit could inhibit our ability 462 to quantify outcrossing rates. This concern was valid as we found that a substantial tree-level 463 effort ( $n \ge 60$  propagules per tree) was likely needed to obtain reliable estimates. In addition,

although the probability of an undetectable outcross event was low across most maternal trees,
two trees at the northern range limit exhibited relatively high probabilities, with 9 and 12
potential undetected outcross events. Estimates based on smaller sample sizes per tree,
comparable to research in the tropics (14-18 propagules on average per tree; Nettel-Hernanz et
al., 2013), could have been overwhelmed by undetectable outcross events and potentially
underestimated outcrossing at this northern range limit.

However, a greater investment of resources at the tree-level inevitably limited our ability to 470 genotype progeny arrays from a larger number of maternal trees (n = 2-5 trees per collection 471 site). Our sampling design may have provided robust tree-level estimates, but our estimates 472 473 scaling up to the population level should be interpreted with caution. Sampling few maternal 474 trees can bias these estimates towards tree-specific outcrossing rates that may not be representative of the entire collection site. Although our estimates (based on n = 23 maternal 475 trees) are consistent with a substantial reduction in outcrossing compared to estimates from 476 tropical conspecifics (based on n = 22 maternal trees; Nettel-Hernanz et al., 2013), and are not 477 478 consistent with a systematic adaptive shift in mating system towards the northern range limit, 479 further supported by evidence of density-dependent variation at the range margin, more intensive sampling of progeny arrays at each collection site is needed to obtain more definitive estimates 480 of population-level mating system variation. Further research into mating systems at range 481 margins, or areas with reduced genetic variation, should consider an investment in both more 482 progeny arrays and large numbers of offspring per progeny array to generate reliable population-483 484 level outcrossing estimates.

485

486 *4.4 Conclusions* 

This research suggests that ecological structure influences the mating system of the neotropical 487 black mangrove, A. germinans, at varying spatial scales towards its expanding northern 488 distributional limit on the Atlantic coast of Florida. First, subtropical Florida A. germinans 489 exhibited significant reductions in outcrossing compared to tropical conspecifics, consistent with 490 reductions in pollinator diversity and mangrove abundance with latitude. Second, the transition 491 from mangrove to salt marsh dominance along Atlantic Florida may create a more open pollen-492 493 dispersal neighbourhood that is conducive to elevated A. germinans outcrossing, until conspecific abundances become too low towards the range limit. Third, greater inter-individual 494 isolation at the range margin resulted in drastic reductions in tree-level outcrossing, consistent 495 496 with density-dependent plastic shifts in mating system that we presume will continue to shift towards greater outcrossing as these mangroves continue to proliferate with forecast climate 497 trends. Further research needs to evaluate the effect of A. germinans mating system variation on 498 the survival and fitness of offspring and on the extent of population-level local adaptation at 499 expanding distributional limits. 500

501

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## **Highlights**

- Range expansion of foundation species may lead to shifts in their mating systems.
- Subtropical Florida Avicennia germinans exhibited predominant self-fertilization. •
- Outcrossing did not systematically decline towards the Florida range limit. •
- Outcrossing increased with conspecific cover at the Florida range margin. •
- Ecological structure may impact mangrove mating systems at varying spatial scales. •

## **Declaration of interests**

 $\boxtimes$  The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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