DR. RICHARD G.J. HODEL (Orcid ID : 0000-0002-2896-4907)

Article type : Original Article

Terrestrial species adapted to sea dispersal: differences in propagule dispersal of two Caribbean mangroves

Richard G. J. Hodel[1,2,3,6]
L. Lacey Knowles[3]
Stuart F. McDaniel[1,4]
Adam C. Payton[1]
Jordan F. Dunaway[1]
Pamela S. Soltis[2,4,5,7]
Douglas E. Soltis[1,2,4,5,7]

Affiliations
[1]Department of Biology, University of Florida, Gainesville, FL 32611, USA
[2]Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA
[3]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA
[4]The Genetics Institute, University of Florida, Gainesville, FL 32610, USA
[5]The Biodiversity Institute, University of Florida, Gainesville, FL 32611, USA

[6]Author for correspondence: richiehodel@gmail.com
[7]P.S.S. and D.E.S. are co-senior authors

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/mec.14894

This article is protected by copyright. All rights reserved

Author Manuscript

Abstract

A central goal of comparative phylogeography is to understand how species-specific traits interact with geomorphological history to govern the geographic distribution of genetic variation within species. One key biotic trait with an immense impact on the spatial patterns of intraspecific genetic differentiation is dispersal. Here we quantify how species-specific traits directly related to dispersal affect genetic variation in terrestrial organisms with adaptations for dispersal by sea, not land—the mangroves of the Caribbean. We investigate the phylogeography of white mangroves (*Laguncularia racemosa*, Combretaceae) and red mangroves (*Rhizophora mangle*, Rhizophoraceae) using chloroplast genomes and nuclear markers (thousands of RAD-Seq loci) from individuals throughout the Caribbean. Both coastal tree species have viviparous propagules that can float in salt water for months, meaning they are capable of dispersing long distances. Spatially explicit tests of the role of ocean currents on patterning genetic diversity revealed that ocean currents act as a mechanism for facilitating dispersal, but other means of moving genetic material are also important. We measured pollen- versus propagule-mediated gene flow and discovered that in white mangroves, seeds were more important for promoting genetic connectivity between populations, but in red mangroves, the opposite was true: pollen contributed more. This result challenges our concept of the importance of both proximity to ocean currents for moving mangrove seeds and the extent of long-distance pollen dispersal. This study also highlights the importance of spatially explicit quantification of both abiotic (ocean currents) and biotic (dispersal) factors contributing to gene flow to understand fully the phylogeographic histories of species.

Keywords: Caribbean, comparative phylogeography, long-distance dispersal, mangroves, RAD-Seq

Introduction

In many terrestrial taxa with populations separated by water (e.g., island systems), dispersal is typically constrained by the lack of an intrinsic mechanism of over-water dispersal. In contrast, in some species adapted to coastal habitats, dispersal by sea, not land, predominates (Kinlan & Gaines, 2003), as in many marine animal species where ocean currents facilitate larval dispersal (Galindo et al., 2006; Nathan et al., 2008). For example, unlike most tree species, sea

This article is protected by copyright. All rights reserved

Author Manuscript

dispersal via propagules characterizes different mangrove taxa, which live exclusively at the interface of the marine environment and coastlines. While ocean currents clearly are an important abiotic factor influencing genetic patterns in some mangrove species (Gillespie et al., 2011), biotic properties of the propagules of species also differ, raising the prospect that species-specific traits, like those studied in their terrestrial plant counterparts (e.g., Massatti & Knowles, 2016), could also be important in shaping the degree to which coastal communities exhibit similar genetic patterns. Here we explore the relative roles of abiotic and biotic factors with a comparative analysis of two Caribbean coastal angiosperms, red mangroves (*Rhizophora mangle*, Rhizophoraceae) and white mangroves (*Laguncularia racemosa*, Combretaceae).

Research in several subdisciplines of biology has shown that dispersal is a powerful force that can shape both the distributions of species and patterns of genetic structure within species. Early empirical tests of island biogeography, such as Simberloff and Wilson's (1969) classic fumigation experiments, showed that islands closer to continents were colonized more quickly than distant islands, highlighting the need to understand island colonization mechanisms. Historically, dispersal was not considered interesting and/or relevant to biogeographic studies—interpreted as little more than stochastic noise—and vicariance was the mechanism that drove biogeographic patterns (reviewed in Humphries & Parenti, 1999). More recently, the biogeographic research community has better acknowledged the importance of dispersal in determining species distributions (de Queiroz, 2004). Subsequent research found that abiotic factors (e.g., environmental barriers or environmental facilitation of colonization) and biotic factors (i.e., dispersal) are both important aspects of colonization (Losos & Schulter, 2009; Gillespie et al., 2011). Early phylogeographic studies accounted for the role of dispersal to a much greater extent than biogeographic studies (e.g., Avise et al. 1992, Burban & Petit 2003). Nevertheless, a polarized view of vicariance and dispersal has continued into comparative phylogeographic studies (Papadopoulou & Knowles, 2016), in which a lack of phylogeographic concordance was considered to result from species-specific differences in dispersal, and therefore uninteresting and unimportant. As the body of comparative phylogeography literature has grown to include a wide range of organisms and greater amounts of genomic data, a more nuanced view of the vicariance-dispersal continuum has emerged—one that explicitly addresses

This article is protected by copyright. All rights reserved

Author Manuscript

88 species-specific biotic factors as well as abiotic ones, and that recognizes the importance of
89 dispersal in shaping genetic structure within species.
90 Red and white mangroves are largely co-distributed in neotropical coastal estuarine
91 habitats, and both disperse via viviparous (i.e., germinated) seedlings that abscise from the parent
92 plant and float for months in the ocean before potentially finding suitable substrate (Rabinowitz,
93 1978; Allen & Krauss, 2006; Tomlinson, 2016). Both species produce a large number of seeds,
94 at a substantial cost to the parent plant (Tomlinson, 2016), but red mangroves have larger and
95 longer-living seedlings, and live closer to the water, than do white mangroves. Thus, we predict
96 that red mangroves will exhibit greater connectivity between populations through seeds than
97 white mangroves, with relatively little difference between the two species in pollen-based
98 estimates of gene flow, because even though the two species have different pollination
99 syndromes (i.e., wind versus insect), this difference is relatively small compared to the
100 differences in propagule traits between the species. Furthermore, we predict that the patterns of
101 genetic differentiation will reflect differences in the degree of isolation among populations as a
102 function of the geographic distance separating populations, augmented by oceanic currents (i.e.,
103 the seascape; Galindo et al., 2006). We characterize the phylogeographic structure of red and
104 white mangroves using both nuclear (RAD-Seq loci) and chloroplast (chloroplast genome
105 sequences) loci from sampling locations across the Caribbean to tease apart how differences in
106 dispersal in these two species affect the observed phylogeographic patterns.
107 Moreover, we apply analyses, which in addition to quantifying the association between
108 genes and geography, also provide a framework for assessing departures from Isolation-By-
109 Distance (IBD) patterns. This includes both quantifications of statistical deviations, which when
110 considered in light of the geographic distribution of populations in a spatially explicit framework,
111 can identify general trends that might correspond with impediments from currents, as well as
112 identification of how abiotic factors influence genetic variation. We investigate the effect of two
113 abiotic factors, land type (island or continent) and island size, on the degree to which the genetics
114 of a population deviated from the null expectation (i.e., IBD). The impact of land type (island
115 versus continental) and island size on factors important for biogeographic patterns (e.g.,
116 speciation rate) has been investigated in biogeographic studies (e.g., Losos, 2009), but spatially
117 explicit phylogeographic investigation is needed in systems with both island and continental

Author Manuscript

This article is protected by copyright. All rights reserved

distributions, where abiotic and/or biotic factors may influence movement of genetic material between the two.

Not only does this study add to a growing body of work on species-specific versus assemblage-wide patterns of genetic variation (e.g., Carnaval et al., 2009; Papadopoulou & Knowles, 2016; Oliveira et al., 2018; Resende-Moreira et al., 2018), but it also expands such research to terrestrial taxa whose dispersal mode differs from most studies to date—terrestrial coastal taxa adapted to oceanic dispersal. For many taxa, the only way that genetic material will exchange between islands is through rare events. These events include slow, long-distance successful rafting that occurs with low probability, infrequent bird-mediated transport, and rare storms or hurricanes that can quickly transport genetic material long distances (Gillespie et al., 2011). We explore the rarity of long-distance dispersal (LDD) in species with continental and island distributions and how the relative frequency of LDD events affects genetic patterns. Many methodologies used in this study, particularly the spatially explicit quantification of abiotic factors that influence dispersal between populations, will be widely applicable to future research in other diverse taxa, including additional plants, as well as mammals, reptiles, and insects in oceanic island systems (e.g., Juan et al., 2000; Heaney et al., 2005), as well as in terrestrial taxa (e.g., some terrestrial systems—sky islands—have many similarities to oceanic island systems; Moore et al., 2013; Salerno et al., 2015). Finally, populations in many other terrestrial systems in diverse taxa can be viewed as islands when an environmental feature limits genetic interchange between populations. Quantifying the abiotic factor(s) that isolate populations in a spatially explicit context will allow for more powerful analyses of how these factors interact with biotic factors to shape phylogeographic patterns (Riginos et al., 2016).

Materials and Methods

Sample collection and DNA isolation

We collected leaf tissue from mature plants of each species from sampling locations in North America, Central America, South America, and Caribbean Islands (Supplemental Table 1). Vouchers were deposited in the University of Florida herbarium (FLAS) at the Florida Museum of Natural History (accession numbers: FLAS 267604, 267605, 267607, 267608, 267610-267612, 267614, 267615, 267617-267619). These two species are co-distributed throughout coastlines in

This article is protected by copyright. All rights reserved

Author Manuscript

148 the neotropics; in total, we sampled 28 populations of red mangroves and 20 populations of
149 white mangroves. At each location, we collected one leaf from 1-16 individuals that were
150 spaced at least 15 m apart to minimize collecting closely related individuals. Herbarium
151 specimens from the New York Botanical Garden (NYBG) were used in cases for sampling
152 locations that were difficult or prohibitively expensive to reach. GPS coordinates for each
153 sampling location were recorded. For each sampling location, we used between one and eight
154 individuals in genetic analyses; in locations where herbarium specimens were used, we were
155 often limited to one individual per location. In locations where more than eight individuals were
156 sampled, we randomly selected eight individuals for use in genetic analyses. Each sampled leaf
157 was placed in a labeled bag with silica gel and stored for 1-12 months at 4^{o} C; we then extracted
158 DNA from the dried leaf tissue using a standard CTAB protocol (Doyle & Doyle, 1987).
159
160 **RAD-Seq library preparation and data processing**
161 We followed the double-digest RAD-Seq protocol of Peterson et al. (2012). We
162 constructed DNA libraries for each sample by digesting approximately 200 ng genomic DNA
163 with *Eco*RI and *Mse*I and then ligating Illumina adapters and unique 8-, 9-, 10-, and 14-
164 nucleotide barcodes to the DNA fragments. The DNA libraries were PCR-amplified in 22
165 separate reactions and pooled to minimize early PCR bias. We size-selected 250-450-bp
166 fragments using a PIPPIN ELF gel and sequenced the DNA fragments using the 1X100-bp
167 setting on the Illumina HiSeq 4000 platform at the University of Florida Interdisciplinary Center
168 for Biotechnology Research (ICBR). Raw sequence data were deposited in the NCBI Sequence
169 Read Archive (SRA; accession numbers SRR7501584-SRR7501638; SRR7504176-
170 SRR7504186). We processed the raw Illumina reads using the iPyrad pipeline (Eaton, 2014;
171 http://ipyrad.readthedocs.io/). We used iPyrad to perform all necessary steps for processing
172 RAD-Seq data (sorting, filtering, clustering, consensus, clustering, formatting). As all barcodes
173 differed by at least two nucleotides, we demultiplexed the loci, allowing one mismatch in the
174 barcode and using the most stringent filtering of Illumina adapters.
175 The loci were assembled using a *de novo* approach with the following cut sites: CAATTC,
176 ATT. We added a C before the *Eco*RI cut site (AATTC became CAATTC) because our double-
177 digest RAD-Seq protocol adds a 'protector base' to prevent any undigested restriction enzymes

This article is protected by copyright. All rights reserved

Author Manuscript

from cleaving off recently incorporated adapters after the ligation step. For all other assembly parameters, we used the iPyrad defaults. We then filtered the loci for human, fungal, and microbial contamination and filtered loci by representation across individuals using an R script (Data_Filtering.R; this script and all other scripts are available at https://github.com/richiehodel/Caribbean_mangroves). We used minimal filtering of loci to avoid excluding informative loci, as both *in silico* and empirical studies indicate that high amounts of missing data do not negatively impact RAD-Seq datasets (Huang & Knowles, 2016; Hodel et al., 2017).

We obtained an average of 3,991,640 reads (minimum: 70,631, maximum: 18,253,041) for red mangrove individuals in the RAD-Seq analysis. The final red mangrove nuclear dataset consisted of an average of 28,929 RAD-Seq loci across 122 individuals (average 19,219; minimum 426; maximum 27,978). The white mangrove reads consisted of an average of 1,446,498, minimum of 119,681, and maximum of 7,267,782. The white mangrove nuclear dataset had 29,767 loci (average 14,253; minimum 360; maximum 28,799) RAD-Seq loci for 54 individuals.

Chloroplast genome sequencing and assembly

We selected 50 individuals of each species for complete chloroplast genome sequencing using a random-shearing genome skimming approach (Straub et al., 2011; Steele et al., 2012). The individuals were selected to provide wide coverage of the sampling locations; 1-3 individuals per species per sampling location were used in chloroplast genome sequencing. DNA libraries were constructed by RAPiD Genomics (Gainesville, FL, USA) and sequenced at the UF ICBR using a HiSeq 4000 with 2X100bp reads. Raw reads were *de novo* assembled into contigs using Velvet (Zerbino & Birney, 2008) with Kmer lengths ranging from 31 to 81. The contigs were then mapped to a reference chloroplast genome of *Oenothera villaricae* (NCBI accession number NC_030532.1) for white mangroves and *Populus alba* (NCBI accession number AP008956.1) for red mangroves, using Bowtie2 (Langmead & Salzberg, 2012), as implemented in Geneious (Kearse et al., 2012). These taxa were selected as references because they were the most closely related species that had publicly available chloroplast genome sequences. Raw reads and assembled chloroplast genomes were deposited in the NCBI

This article is protected by copyright. All rights reserved

Author Manuscript

Sequence Read Archive database (accession numbers SRR7779779-SRR7779767; SRR7781534-SRR7781581). The red mangrove chloroplast genome alignment (50 individuals) was 130,120 bp, and the overall pairwise sequence identity was 99.6%. The average GC content was 36.2%, and on average there were 15,707 bp of ambiguous sites per individual. The white mangrove chloroplast alignment also consisted of 50 individuals and was 135,357 bp in length. There was 99.4% pairwise sequence identity overall, and on average there were 48,930 ambiguous sites per individual.

Phylogeographic analyses

Phylogeographic analyses were performed on both the nuclear (RAD-Seq) and chloroplast genome data separately for each species. To assess phylogeographic patterns within each species, we calculated pairwise average F_{ST} using an R script (Pairwise_Fst.R) and the R packages 'hierfstat' (Goudet, 2005) and 'PopGenome' (Pfeifer et al., 2014). Pairwise F_{ST} is a valuable metric for assessing how historical factors and ongoing gene flow influence structure between populations. In these two species, historical factors (e.g., isolation between distant populations) and/or ongoing gene flow (e.g., via dispersal, pollen flow, and colonization) could impact pairwise F_{ST}.

Phylogenetic analyses with SVDQuartets and RAxML

To determine genealogical relationships among individuals within species, we used coalescent analyses in SVDQuartets (Chifman & Kubatko, 2014). This program selects the optimal topology for a quartet of taxa, and, after sampling millions of quartets, infers a phylogeny for all individuals based on choosing the quartets with the best scores and assembling them into a phylogenetic tree. For each RAD dataset, we evaluated all possible quartets and selected trees under the multispecies coalescent using QFM (Quartet Fiduccia Mattheyses) quartet assembly (Reaz et al., 2014). We used non-parametric bootstrapping (100 replicates for each dataset) to assess confidence in inferred genealogical relationships between individuals. The R script Tree_Formatting.R was used to visualize and annotate the 50% majority-rule trees from SVDQuartets using the R packages 'ape' (Paradis et al., 2004) and 'ggtree' (Yu et al., 2017). For comparative purposes, we also used RAxML (Stamatakis, 2014) to infer the

This article is protected by copyright. All rights reserved

phylogenetic relationships between individuals of both species using the RAD-Seq data. RAxML was used to infer phylogenetic relationships between chloroplast genomes for each individual in each species. In all RAxML analyses, we employed the GTRGAMMA model of evolution and ran 100 bootstrap replicates.

Isolation-By-Distance tests and Procrustes analysis

As geographic distance is often a key component in spatial patterns of genetic diversity, we conducted Mantel tests to test for IBD by comparing matrices of geographic and genetic distances with the R package 'vegan' (Oksanen et al., 2017) and a custom script (Mantel_Procrustes.R). A principal component analysis (PCA) implemented in the R package 'SNPRelate' (Zheng et al., 2012) identified clusters of individuals, using RAD data and chloroplast data separately in each species (see R script VCF_PCA.R). To further investigate the relationship between genes and geography, we implemented a Procrustes analysis, which finds an optimal transformation that maximizes the similarity between genetic variation in PCA space and sample locations in geographic space (Wang, 2010). For each species, the Procrustes analysis compared two matrices: one with the latitude and longitude of each sampling location, and one with principal components one and two, as calculated using the R packages 'gdsfmt' and 'SNPRelate' using the script VCF_PCA.R. The Procrustes analysis not only quantifies the strength of the association between genetic and geographic variation, but it also shows the amount of genetic deviance of individuals from their expected genetic position based on where they were sampled geographically. As the Procrustes analysis identifies the optimal transformation (i.e., rotation of matrices) that maximizes the similarity between genetic principal components and geographical coordinates of sampling locations, the arrows represent the degree of deviation from a signature of IBD. The direction of the arrows shows in two dimensions the strength of the deviation (e.g., how a population may genetically look much more like a neighboring population to the north than would be expected given its geographic position). For each species and each data type, we calculated t_0, the association statistic between the two matrices, and assessed its significance by running 10,000 permutations. For both species, we also measured whether the magnitude of the Procrustes deformations was correlated with certain variables, namely: latitude, longitude, direction of Procrustes errors, whether the population was

Author Manuscript

This article is protected by copyright. All rights reserved

continental or insular, and the size of the land mass containing the population. The size of the land mass was calculated by measuring the perimeter of an island or terrestrial shoreline. All correlations were tested using the R package 'vegan' in the script Correlations.R.

Ocean current analysis

We tested the relative importance of geographic distance and environmental distance (i.e., geographic distance scaled using the effects of ocean currents) using partial Mantel tests implemented in the R package 'vegan'. For each population pair, we considered the geographic distance to be the Euclidean distance between the sampling locations, and we considered environmental distance to be the Euclidean distance plus the distance added due to ocean currents preventing propagules from moving in a straight-line distance between two populations (Fig. 1). We downloaded gridded ocean current data from NOAA (https://ferret.pmel.noaa.gov/); each file contained either the northward or eastward water velocity for each marine pixel (resolution: $1/12^{o}$) in the study area over a 24-hour period. We downloaded data for 12 time points spaced over two years (February 2014-January 2016), ensuring that each month of the year was represented once to account for seasonal variation in current velocity. We averaged the northward velocities and eastward velocities for all time periods and then created a single grid layer by determining the ocean current bearing of each cell using the averaged northward and eastward velocities and the 'earthbear' function in the R package 'fossil' (see R script Ocean.R).

The straight-line distance between each pair of populations was measured, and we then identified every grid cell in the averaged layer that intersected the line. If the bearing in a pixel were identical to the bearing of the line, the pixel would be given a score of zero. If the bearing of the pixel were different from the bearing of the line, it would be given a score between zero and 180, where 180 implies that the ocean current bearing of the pixel is perpendicular to the line connecting the populations. We calculated the bearing of the line for both directions, but only retained the direction that minimized the sum of the absolute deviations from the bearings in the pixels. We then averaged the deviations across all pixels intersected by the line to obtain the scaled environmental distance, which would be greater than the Euclidean distance. In this way, we used the averaged ocean current layer as an environmental resistance layer and measured its

This article is protected by copyright. All rights reserved

Author Manuscript

importance for explaining geographic patterns of genetic variation. Due to the coarse resolution of the gridded layers, some sampling locations appeared to be in marine (as opposed to terrestrial) pixels. Therefore, for this analysis, some GPS coordinates were shifted slightly to ensure that each sampling location was located on a terrestrial pixel. The data acquisition and processing were completed using the script Ocean.R. We followed Massatti et al. (2017) and used partial Mantel tests to measure the correlation between distances penalized by ocean current and genetic distances (i.e., pairwise F_{ST}), controlling for Euclidean geographic distance separating populations.

Pollen versus seed analysis

The maternally inherited chloroplast genome and the biparentally inherited nuclear genome have different evolutionary histories and as such will exhibit different amounts of genetic differentiation among sampling locations. In both red and white mangroves the chloroplast is presumably maternally inherited, given well-documented evidence of maternal transmission in multiple closely related taxa (i.e., several representatives from families in the same order as each mangrove species; Zhang, 2003). When the rate of seed migration is smaller than that of pollen migration, population genetic theory predicts that greater subpopulation structure will be detected in (maternally inherited) chloroplast markers than in (biparentally inherited) nuclear markers (Petit et al., 2004). The maternal contribution to gene flow can be measured using chloroplast markers, and the paternal contribution to gene flow can be calculated by subtracting the maternal contribution to gene flow from the biparental contribution to gene flow. Thus, the ratio of seed migration to pollen migration (r) can be calculated using the following equation: $r = (A\text{-}2C)/C$, where $A = (1/F_{STnuclear}) - 1$, and $C = (1/F_{STchloroplast}) - 1$ (Hamilton & Miller, 2002). We used F_{ST} to calculate the ratio of seed migration to pollen migration for each species in a pairwise fashion for all pairs of sampling locations.

Results

Geographic structuring of genetic variation

In both mangrove species we found greater differentiation among sampling locations in chloroplast DNA versus nuclear DNA (Supplemental Tables 2 and 3). Pairwise F_{ST} estimated

This article is protected by copyright. All rights reserved

Author Manuscript

for red mangroves using RAD loci was usually low, ranging from 0 to 0.679 (average F_{ST} = 0.209; Supplemental Table 2), and pairwise F_{ST} using chloroplast DNA ranged from 0.138 to 1.0 (average F_{ST} = 0.561; Supplemental Table 3). Sampling locations on the margins of the study region (e.g., Anguilla, Senegal, New Port Richey) usually had higher values of pairwise F_{ST} (Supplemental Table 2). There is a large range in the pairwise genetic differentiation estimated by chloroplast DNA for white mangroves (F_{ST} ranges from 0.075 to 1.0 with average F_{ST} = 0.506; Supplemental Table 3), and the pairwise F_{ST} values for RAD loci vary from low differentiation (F_{ST} = 0.0 between several Florida and Grand Bahamas populations; Supplemental Table 2) to high differentiation (F_{ST} = 0.595 between a Florida population (Melbourne) and Antigua; Supplemental Table 2). The average pairwise F_{ST} using RAD loci in white mangroves was 0.285. The ratio of seed migration to pollen migration (r), calculated using the F_{ST} values for both species for both genomes, showed greater pollen gene flow than seed gene flow in red mangroves (ratio of pollen:seed = 7.87). In white mangroves, the ratio of pollen:seed gene flow is 0.16, indicating that the lower genetic differentiation among populations most likely is attributable to propagule, rather than pollen, dispersal.

The SVDQuartets trees showed that individuals and sampling locations were often clustered by geography when using nuclear loci (i.e., RAD-Seq data; Fig. 2). Notably, several major clades in each species clustered geographically. In red mangroves, the following clades containing more than one sampling location had 100% bootstrap support: Florida, Florida+Cuba+Grand Bahama, Florida+Cuba+Grand Bahama+Belize+Costa Rica (Caribbean)+Colombia+Nicaragua+Mexico+Honduras, and the previous clade+Costa Rica (Pacific)+Panama+Aruba+Venezuela+Cayman Islands+Long Island+Jamaica+Hispaniola (Fig. 2). These relationships were also observed with high support when using RAxML (Supplemental Fig. 1). Similarly, in white mangroves, multiple clades with 100% bootstrap support were congruent with geography: Florida+Grand Bahama, Antigua+Aruba+Grenada+Puerto Rico, and the previous two clades+Costa Rica+Belize+Jamaica+Cayman Islands (Fig. 2). As in red mangroves, the SVDQuartets and RAxML trees for white mangroves based on RAD-Seq data were largely congruent. The RAxML trees inferred using chloroplast genomes in both species had fewer highly supported clades than trees generated from RAD-Seq data, although there were fewer tips in the chloroplast

This article is protected by copyright. All rights reserved

Author Manuscript

trees (Supplemental Fig. 2). Additionally, the phylogenetic relationships were less congruent with the geographic relationships of sampling locations than in the nuclear trees. In red mangroves, all Florida samples formed a highly supported clade, as did some individuals from Aruba, Antigua, Grenada, and Jamaica—although not all Jamaica or Grenada individuals were represented in the clade. In white mangroves, one notable highly supported clade included samples from Belize, Colombia, Honduras, Hispaniola, Mexico, and Puerto Rico—although not all samples from Colombia and Puerto Rico were in the clade. In general, the phylogenetic relationships in the chloroplast trees of white mangroves are less congruent with geography than those of red mangroves.

Dispersal limitations as a function of geographic distance

Mantel tests were significant for both species when comparing geographic distance matrices with genetic matrices (Table 1); IBD explained at least a portion of genetic variance. Mantel tests are discussed in more detail in the subsequent section, where the results of the partial Mantel tests are summarized. For red mangroves, the Procrustes analysis based on RAD loci indicated that nuclear genetic data were significantly correlated with the geography of the sampling locations (t_o = 0.561; P<0.01; with Pacific samples included, t_0 = 0.602; Fig. 3; Supplemental Fig. 3). Similarly, in white mangroves, the Procrustes analysis based on RAD loci revealed that nuclear genetic data were significantly correlated with geography (t_o = 0.684; P<0.01; Table 2; Fig. 4). For red mangroves, the Procrustes analysis also showed that there was not a significant relationship between geographic and genetic distance when using chloroplast data, although the relationship was barely non-significant (t_o = 0.284; P=0.0575). For white mangroves, the Procrustes analysis using chloroplast DNA also found that genes were not significantly correlated with geography (t_o = 0.0808; P=0.845). In white mangroves, the magnitude of deformations from the Procrustes analysis was significantly correlated with both latitude and longitude, but not with three other factors associated with sampling locations (island vs. continental location, island size, direction of Procrustes deformations; Table 3). In contrast, all five factors associated with sampling locations were significantly correlated with the magnitude of Procrustes errors in red mangroves (Table 3).

Author Manuscript

This article is protected by copyright. All rights reserved

Dispersal mediated by ocean current

In both red and white mangroves, Mantel tests were significant when comparing geographic distance and genetic distance (F_{ST}), and when comparing ocean current penalized distance and genetic distance (Table 1). In white mangroves, the *r*-value was higher in the Mantel test for geography ($r = 0.694$) than in ocean current penalized distance ($r = 0.275$; Table 1). There was a similar trend in red mangroves ($r = 0.426$ for geographic distance; $r = 0.126$ for ocean current penalized distance), and both *r*-values in red mangroves were lower than the corresponding value in white mangroves. Additionally, partial Mantel tests were significant ($r = 0.249$ for white mangroves and $r = 0.242$ for red mangroves; Table 1) and revealed that the ocean current penalty matrices explained some of the variation in spatial genetic patterns when controlling for covariance associated with geographic distance.

Discussion

This study highlights how we can quantify key biotic traits of species to evaluate their interaction with abiotic factors, and how this interaction shapes the spatial distribution of genetic variation within species. Specifically, we investigated how differences in dispersal ability are affected by ocean currents, and how this interaction affects phylogeographic patterns. We predicted that red mangroves would exhibit greater connectivity between populations through seeds than white mangroves, and that the patterns of genetic differentiation would reflect differences in the degree of isolation among populations as a function of the geographic distance separating populations, augmented by oceanic currents. Here we showed that comparative phylogeographic patterns observed in the two mangrove species, combined with spatially explicit analyses to assess abiotic drivers of phylogeographic patterns (i.e., ocean currents), revealed that species-specific dispersal traits such as propagule movement were important for patterning spatial genetic diversity—sometimes in unexpected ways—and that they were not the only important factor. Several key results supported the importance of ocean currents transporting propagules, but there was also evidence that other means of moving genetic material were important. Phylogenetic, population genetic differentiation, and IBD analyses indicated both concordant and discordant phylogeographic patterns when we compared these two mangrove species adapted for ocean dispersal. Here we showed that ocean currents act as a mechanism for

Author Manuscript

This article is protected by copyright. All rights reserved

facilitating dispersal using spatially explicit tests of the role of ocean currents on patterning genetic diversity. Additionally, we measured relative amounts of pollen versus propagule gene flow, which indicated critical differences between the two species regarding the importance of propagule movement. Contrary to expectations, white mangroves exhibited greater connectivity between populations through seeds than red mangroves. Importantly, methods in this study can be applied to many other species with spatial patterns of genetic variation impacted by both biotic and abiotic factors; below we discuss the implications of our results, focusing on how our findings impact understanding of how species-specific biotic traits and abiotic factors interact to shape phylogeographic patterns.

Concordance and discord between species adapted for ocean dispersal

Several types of evidence supported similar phylogeographic patterns in the two mangrove species investigated. Measures of population differentiation revealed a wide range of interpopulation genetic differentiation in both species; typically, more proximate populations had lower differentiation (Supplemental Tables 2 and 3). Additionally, Mantel tests for IBD showed that Euclidean distance between populations was a significant predictor of genetic distance (F_{ST}) between populations in the nuclear genome for each species (Table 1). Phylogenetic analyses of each species corroborated this interpretation, as individuals from the same population or region often grouped together (Fig. 2, Supplemental Fig. 1, Supplemental Fig. 2). However, there were key differences between the species: we detected greater differentiation between sampling locations in both nuclear and chloroplast data in white mangroves than in red mangroves (Supplemental Tables 2 and 3), suggesting that some mechanism makes genetic exchange easier between populations of red mangroves than white mangroves. Several results indicated that LDD via water might not be as important as has been assumed. Specifically, IBD and Procrustes analyses revealed important insights about the patterns of genetic diversity in each of the species (Figs. 3 and 4, Tables 1 and 2). Genetic differentiation in the nuclear genome of both species was partially determined by geography—indicating that successful LDD events in these species were not the only process shaping spatial patterns of genetic diversity for the species.

Ocean currents as a mechanism for dispersal

This article is protected by copyright. All rights reserved

Author Manuscript

LDD via ocean currents is often considered the only important mechanism that moves genetic material between distant populations of mangroves (Tomlinson, 2016). Our results show that ocean currents may not be as important as assumed, but they do have a role in transporting propagules frequently enough to impact genetic diversity between populations (Table 3). Ocean currents had an effect on spatial genetic patterns in both species, as shown in the ocean current analysis (Fig. 1, Table 1). However, ocean currents were not the only significant predictor of genetic patterns: Mantel and partial Mantel tests revealed that geographic distance was also important (Table 1). This implies that LDD via ocean currents is an important driver of genetic patterns in red and white mangroves, but that other processes, such as genetic drift, which led to IBD patterns, need to be considered. Historical factors, such as past environmental or physical barriers between populations, or changes in ocean currents and/or wind patterns, could have also affected estimates of pairwise F_{ST}, and accordingly could impact IBD and other downstream analyses.

Key distinctions between island and continental populations exist in these two species that can disperse between the two land types. The magnitude of Procrustes deformations in each species was correlated with environmental factors, such as island size, longitude, latitude, and direction of deformation (Table 3). Many mangrove populations, especially small populations, could have been recently founded, or could be readily extirpated in a major storm event—making it important to consider a variety of historical processes that could have led to the current patterns of genetic variation among populations. In the Procrustes analysis of red mangroves, the deviations from expected patterns of variation under IBD are in the direction of the Cayman Islands, indicating that individuals from geographically distant populations are nonetheless very similar genetically to individuals sampled in the Cayman Islands; one potential hypothesis explaining this pattern is recent migration (Fig. 3). In addition to ocean currents, other abiotic factors influenced observed genetic patterns—in red mangroves, island size was correlated with the Procrustes deformations that assessed discordance between genes and geography; islands with less coastline are less likely to be a landing spot for drifting propagules (Table 3). This pattern was not detected in white mangroves, meaning that propagule movement in white mangroves may be sufficient to counteract the effect of island size (Table 3). In general, smaller islands had larger deformations, meaning that in small islands, genetic differentiation was more

This article is protected by copyright. All rights reserved

Author Manuscript

different than expected based on geography, as compared to large islands. Propagule dispersal was less important than expected in red mangroves, so successful colonization or immigration via propagules may be very rare and subject to stochastic effects regarding propagule origin. As island size alone was not able to explain the Procrustes deformations in both species, this result highlights the importance of considering both species-specific biotic traits and abiotic factors in comparative phylogeography studies.

The importance of propagule dispersal relative to pollen

Biotic factors other than propagule dispersal are important for determining genetic patterns. We collected genetic data from both the nuclear and chloroplast genomes so we could assess the relative importance of gene flow via propagules and gene flow via other processes (i.e., pollen movement). In white mangroves, propagule movement is important—seeds are almost six times as important for moving genes as pollen is. However, in red mangroves, propagule movement is not nearly as important; the contribution of pollen to the movement of genes is over eight times greater than that of propagules. Contrary to our predictions, propagule movement in red mangroves was less than in white mangroves. The result for red mangroves strongly contradicts our expectation—the large, long-lived red mangrove propagules were predicted to contribute heavily to spatial patterns of genetic diversity. In white mangroves, we expected that propagules would contribute more to the genetics of the species than pollen, although the relative importance of propagules to pollen in white mangroves as compared to red mangroves is surprising.

Our results indicate that ocean currents may not be the major driving force of dispersal in red mangroves, as currently assumed (Nettle & Dodd, 2007; Takayama et al., 2014; Wee et al., 2015; Hodel et al., 2016; reviewed in Tomlinson, 2016). For a typical wind-pollinated plant species, red mangrove pollen movement was perhaps not unexpected. In empirical studies, the rate of pollen movement is often at least one order of magnitude larger than the rate of seed movement (Petit et al., 2004). However, one would expect viviparous mangrove species, that purportedly have the ability to disperse long distances using ocean currents, to have a lower pollen:seed ratio. White mangroves seem more in line with expectations for a mangrove species: a low pollen:seed ratio for a ‘typical’ plant, but expected because of its dispersal mechanism. As

This article is protected by copyright. All rights reserved

Author Manuscript

noted previously, pairwise F_{ST} can account for both historical factors and ongoing gene flow, so the inferred amounts of pollen- and seed-mediated gene flow may not be solely due to contemporary gene flow.

In summary, the pollen:seed ratio in red mangroves is surprisingly high for a mangrove species, whereas that of white mangroves more closely matches expectations. A previous study of black mangroves (*Avicennia germinans*; Acanthaceae), another mangrove species distributed throughout the Caribbean, found a pollen:seed ratio of 5.1 (Nettle & Dodd, 2007). Black mangrove propagules are intermediate in size and longevity between those of white and red mangroves, and black mangrove parent plants occur at an intermediate distance from the water compared to the two focal species of this study. The results for black mangroves imply that smaller propagules may be more valuable for LDD in mangrove systems, as the pollen:seed ratio for black mangroves is smaller than that of red mangroves. The Nettle and Dodd (2007) study used few markers—only two polymorphic chloroplast SSRs—so their chloroplast F_{ST} estimates would be expected to have large variance.

Many studies of mangrove genetic diversity (e.g., studies that used microsatellites) use ocean currents to explain the geographic distribution of genetic variation (Nettle & Dodd, 2007; Takayama et al., 2014; Wee et al., 2015; Hodel et al., 2016). This approach is logical, as mangrove species have various types of vivipary, and propagules can float in salt water for months, often establishing in suitable substrate many kilometers away (Rabinowitz, 1979). However, propagules are not the only means of transmitting genetic material—pollen can be critically important in patterning genetic diversity as well. Wind pollination, which typically moves pollen greater distances than transport via insects, characterizes red mangroves, in contrast to white mangroves, which are insect-pollinated (Tomlinson, 2016). Both species are self-compatible, and red mangroves have been documented to produce fruit from self-pollination at approximately one tenth of the frequency of wind pollination (Nadia & Machado, 2014). White mangrove flowers have been observed to self-pollinate when not visited by insects (Landry, 2012). Although both of these pollination syndromes can move genetic material via pollen, the majority of gene flow in mangrove species is assumed to occur via propagules, but until now, this had not been tested with genetic data in a spatially explicit context. Although differences in pollination syndrome could have impacted the results, our analyses demonstrated

This article is protected by copyright. All rights reserved

Author Manuscript

that propagule movement is more important than pollen in determining spatial patterns of genetic variation in white mangroves, but that the opposite was true in red mangroves.

Applications to other species

Differences in dispersal ability between taxa can leave subtly different genetic signatures that require high-resolution data to tease apart. Historically, comparative phylogeography studies typically used single-locus DNA sequences (i.e., mitochondrial or chloroplast DNA) or nuclear microsatellite loci (Avise, 2000; Soltis et al., 2006). The number of markers used frequently numbered fewer than 20 microsatellites or fewer than 10 linked regions on the chloroplast or mitochondrial genomes. Adequately testing dispersal requires sufficient resolution in different genomes (i.e., both nuclear and organellar). Recent studies have embraced using many more (i.e., thousands) loci, such as phylogeographic studies using RAD-Seq data (reviewed in Andrews et al., 2016). However, many comparative phylogeographic studies do not sample loci from both the nuclear and organellar genomes (Riddle, 2016). Even though organellar genomes are effectively single loci, the differences in their inheritance compared to the nuclear genome make including organellar data valuable for phylogeographic inference (Petit et al., 2004). For instance, in an animal species with biased sex ratios and/or differences in mobility between the sexes, mitochondrial and nuclear data would provide different inferences of dispersal. Similarly, in a plant species with vast differences in the amount of dispersal possible via seeds and pollen, the chloroplast and nuclear genomes would show different genomic signatures regarding dispersal.

This study highlighted the importance of ocean currents and species-specific dispersal traits and should inform how future studies consider the interplay of biotic and abiotic traits. Specifically, our study has implications beyond these two mangroves—the results directly apply to species that disperse over oceans and indirectly apply to other species that disperse across some type of barrier. Our results show that for coastal species that use water to facilitate dispersal, it is important to consider all of the mechanisms that spatially pattern genetic diversity. Studies of other water-dispersed plants, such as *Hibiscus tiliaceus* (Takayama, 2006), used chloroplast DNA and detected moderate genetic structure (F_{ST} ~ 0.25) between populations not immediately connected by water. However, the choice of markers (i.e., maternally inherited

This article is protected by copyright. All rights reserved

Author Manuscript

chloroplast DNA) made it impossible to tease apart the effect of pollen on the patterning of genetic diversity across geographic space. As in red mangroves, just because a species expends a lot of energy producing propagules that can disperse long distances, there is no guarantee that the propagules will successfully travel via ocean currents to establish new populations or migrate to other distant existing populations. Future studies of water-dispersed plants should use both nuclear and uniparentally inherited organellar markers to assess the relative impacts of seeds and pollen on genetic patterns in the species of interest. Collecting both adequate nuclear and organellar genetic data is a prudent strategy in almost all systems.

Conclusions

We investigated how differences in a biotic factor between two species, dispersal—which differed between the species due to propagule size, longevity, and the proximity of the parent plant to water—affected geographic patterns of genetic variation. Additionally, this study used explicit modeling of ocean currents, which assessed how much an abiotic factor impacted genetic differentiation between populations by moving propagules. However, propagule movement in the ocean is important in both species, and it is actually more important for moving genetic material in white mangroves. Pollen movement, facilitated by wind, may be the driving force behind transporting genetic material between populations of red mangroves. The results of this study will impact how shared phylogeographic patterns are investigated in taxa other than mangroves and coastal species. This study illustrates how it is important to fully investigate subtle differences between species using multiple types of data in a spatially explicit context. Without either the chloroplast or nuclear data, we still could have detected differences in genetic differentiation between species, but we would have been unable to determine the relative importance of propagule versus pollen gene flow, which was critical to interpreting the results of this study. Furthermore, without explicitly investigating how ocean currents related to genetic differentiation, we would have been unable to explain the geographic patterns of genetic variation beyond an IBD scenario, and perhaps may have discounted the importance of LDD in these species. Identifying subtle genetic differences between species requires a carefully designed study and understanding the species-specific biotic factors and abiotic environmental factors that are important for the study system and that led to observed patterns. Future studies

This article is protected by copyright. All rights reserved

Author Manuscript

598 comparing species with similar phylogeographic patterns should use data from both nuclear and
599 organellar genomes and incorporate relevant biotic and abiotic drivers of intraspecific genetic
600 variation, such as life-history traits (e.g., dispersal, as in this study) or environmental data (e.g.,
601 spatially explicit ocean current data). Increasingly, it will be possible to investigate thoroughly
602 the biotic and abiotic influences on genetic diversity, as more resources such as genomic data,
603 digitized specimen records, and environmental data layers become available and can be
604 integrated (Soltis & Soltis, 2017).
605 Finally, investigating the phylogeography of these two mangrove species also has
606 practical applications. Coastal species are often more vulnerable to the effects of climate change
607 than plants occupying inland habitats (Christensen, 2000; Barbier et al., 2011; Tomlinson, 2016).
608 Mangroves provide crucial ecosystem services: mitigating damage due to storm surges,
609 providing habitat for animal species, and filtering water (Ewel et al., 1998; Rönnbäck, 1999;
610 Walters et al., 2008; Barbier et al., 2011). Anthropogenic climate change, overdevelopment of
611 coastal areas, and increased shipping are negatively impacting mangroves (Kristensen et al.,
612 2008). Conservation genetics theory has shown the importance of characterizing genotypes
613 present in natural populations to combat deleterious forces such as inbreeding depression,
614 outbreeding depression, decline in genetic diversity, and loss of genetic adaptive potential
615 (Moritz 1994; Crandall et al., 2000; Frankham, 2005). Our improved understanding of the
616 phylogeographic structure of mangroves will enable the efficient protection of these crucial
617 coastal tree species throughout the Caribbean. Based on the results of this study, if it is not
618 possible to use local propagules in restoration/reintroduction, locations that were identified as
619 source populations should be used as propagule sources because there is a history of successful
620 migration and colonization from those locations.
621
622 **Acknowledgements**
623 We thank the following people for help collecting leaf tissue: Dr. Andrew Crowl, Dr.
624 Pablo Allen, Kelly Speer, Dr. Riccardo Baldini, D'Shan Maycock, and Lori-Ann Harris. We
625 thank the following people for help with lab work: Veronica Moiño and Wade Chen. We thank
626 the following agencies for allowing us to collect leaf tissue and for facilitating field collections:
627 Bahamas Department of Agriculture, Jamaica National Environment and Planning Agency,

This article is protected by copyright. All rights reserved

Author Manuscript

Jamaica Urban Development Corporation, Cayman Islands Department of Environment, Aruba Department of Agriculture, Husbandry and Fisheries. We thank the NYBG for allowing us to sample from herbarium specimens.

References

Allen JA, Krauss KW (2006) Influence of propagule flotation longevity and light availability on establishment of introduced mangrove species in Hawai'i. *Pacific Science*, **60**, 367–376.

Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA (2016) Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics*, **17**, 81-92.

Avise JC, Alisauskas RT, Nelson WS, Ankney CD (1992) Matriarchal population genetic structure in an avian species with female natal philopatry. *Evolution*, **46**, 1084-1096.

Avise JC (2000) *Phylogeography: The history and formation of species*, Harvard University Press, Cambridge, Massachusetts.

Burban C, Petit RJ (2003) Phylogeography of maritime pine inferred with organelle markers having contrasted inheritance. *Molecular Ecology*, **12**, 1487-1495.

Barbier EB, Hacker SD, Kennedy C *et al.* (2011) The value of estuarine and coastal ecosystem services. *Ecological Monographs*, **81**, 169–193.

Carnaval AC, Hickerson MJ, Haddad CFB, Rodrigues MT, Moritz C (2009) Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. *Science*, **323**, 785–9.

Chifman J, Kubatko L (2014) Quartet inference from SNP data under the coalescent model. *Bioinformatics*, **30**, 3317–24.

Christensen NL (2000) *North American terrestrial vegetation*, Cambridge University Press, Cambridge.

This article is protected by copyright. All rights reserved

Author Manuscript

651 Crandall KA, Bininda-Emonds ORPO, Mace GM, Wayne RK (2000) Considering evolution
652 processes in conservation biology. *Trends in Ecology & Evolution*, **15**, 290–295.

653 de Queiroz A (2005) The resurrection of oceanic dispersal in historical biogeography. *Trends in*
654 *Ecology & Evolution*, **20**, 68–73.

655 Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf
656 tissue. *Phytochemical Bulletin*, **19**, 11–15.

657 Eaton DAR (2014) PyRAD: assembly of de novo RADseq loci for phylogenetic analyses.
658 *Bioinformatics*, **30**, 1844–1849.

659 Ewel KC, Twilley RR, Ong JE (1998) Different kinds of mangrove forests provide different
660 goods and services. *Global Ecology & Biogeography Letters*, **7**, 83–94.

661 Frankham R (2005) Stress and adaptation in conservation genetics. *Journal of Evolutionary*
662 *Biology*, **18**, 750–755.

663 Galindo HM, Olson DB, Palumbi SR (2006) Seascape genetics: A coupled oceanographic-
664 genetic model predicts population structure of Caribbean corals. *Current Biology*, **16**, 1622–
665 1626.

666 Gillespie RG, Baldwin BG, Waters JM *et al.* (2011) Long-distance dispersal: a framework for
667 hypothesis testing. *Trends in Ecology & Evolution*, **27**, 47–56.

668 Goudet J (2005) hierfstat, a package for R to compute and test hierarchical F-statistics.
669 *Molecular Ecology Notes*, **5**, 184–186.

670 Hamilton MB, Miller JR (2002) Comparing relative rates of pollen and seed gene flow in the
671 island model using nuclear and organelle measures of population structure. *Genetics*, **162**,
672 1897–1909.

Author Manuscript

This article is protected by copyright. All rights reserved

673 Heaney LR, Walsh JS, Townsend Peterson A (2005) The roles of geological history and
674 colonization abilities in genetic differentiation between mammalian populations in the
675 Philippine archipelago. *Journal of Biogeography*, **32**, 229–247.

676 Hodel RGJ, Cortez MB, Soltis PS, Soltis DE (2016) Comparative phylogeography of black
677 mangroves (*Avicennia germinans*) and red mangroves (*Rhizophora mangle*) in Florida:
678 Testing the maritime discontinuity in coastal plants. *American Journal of Botany*, **103**, 730–
679 739.

680 Hodel RGJ, Chen S, Payton AC *et al.* (2017) Adding loci improves phylogeographic resolution
681 in red mangroves despite increased missing data: Comparing microsatellites and RAD-Seq
682 and investigating loci filtering. *Scientific Reports*, **7**.

683 Huang H, Knowles LL (2016) Unforeseen consequences of excluding missing data from next-
684 generation sequences: Simulation study of RAD sequences. *Systematic Biology*, **65**, 357–65.

685 Humphries CJ, Parenti LR (1999) *Cladistic biogeography: Interpreting patterns of plant and*
686 *animal distributions*. Oxford University Press, Oxford.

687 Juan C, Emerson BC, OromíP , Hewitt GM (2000) Colonization and diversification: towards a
688 phylogeographic synthesis for the Canary Islands. *Trends in Ecology & Evolution*, **15**, 104–
689 109.

690 Kearse M, Moir R, Wilson A *et al.* (2012) Geneious Basic: an integrated and extendable desktop
691 software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**,
692 1647–9.

693 Kinlan BP, Gaines SD (2003) Propagule dispersal in marine and terrestrial environments: A
694 community perspective. *Ecology*, **84**, 2007–2020.

695 Kristensen E, Bouillion S, Dittmar T, Marchand C (2008) Organic carbon dynamics in mangrove
696 ecosystems: A review. *Aquatic Botany*, **89**, 201–219.

Author Manuscript

This article is protected by copyright. All rights reserved

Landry CL (2013) Pollinator-mediated competition between two co-flowering Neotropical mangrove species, *Avicennia germinans* (Avicenniaceae) and *Laguncularia racemosa* (Combretaceae). *Annals of Botany*, **111**, 207–14.

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nature Methods*, **9**, 357–359.

Losos JB, Ricklefs RE (2009) Adaptation and diversification on islands. *Nature*, **457**, 830–836.

Losos JB, Schluter D (2000) Analysis of an evolutionary species–area relationship. *Nature*, **408**, 847–850.

Massatti R, Knowles LL (2016) Contrasting support for alternative models of genomic variation based on microhabitat preference: species-specific effects of climate change in alpine sedges. *Molecular Ecology*, **25**, 3974–3986.

Massatti R, Doherty KD, Wood TE (2017) Resolving neutral and deterministic contributions to genomic structure in *Syntrichia ruralis* (Bryophyta, Pottiaceae) informs propagule sourcing for dryland restoration. *Conservation Genetics*, **19**, 85–97.

Moore W, Meyer WM, Eble JA *et al.* (2013) Introduction to the Arizona sky island Arthropod project (ASAP): Systematics, biogeography, ecology, and population genetics of Arthropods of the Madrean sky islands. *Proceedings RMRS*, **2013**, 144–168.

Moritz C (1994) Defining Evolutionarily-Significant-Units for conservation. *Trends in Ecology & Evolution*, **9**, 373–375.

Nadia TL, Machado IC (2014) Wind pollination and propagule formation in *Rhizophora mangle* L. (Rhizophoraceae): resource or pollination limitation? *Anais da Academia Brasileira de Ciências*, **86**, 229–38.

Nathan R, Schurr FM, Spiegel O *et al.* (2008) Mechanisms of long-distance seed dispersal. *Trends in Ecology & Evolution*, **23**, 638–647.

This article is protected by copyright. All rights reserved

Author Manuscript

721 Nettel A, Dodd RS (2007) Drifting propagules and receding swamps: genetic footprints of
722 mangrove recolonization and dispersal along tropical coasts. *Evolution*, **61**, 958–71.

723 Oksanen JF, Blanchet G, Friendly M *et al.* (2017) vegan: Community ecology package. R
724 package version 2.4-4.

725 Oliveira EF, Martinez PA, São-Pedro VA *et al.* (2018) Climatic suitability, isolation by distance
726 and river resistance explain genetic variation in a Brazilian whiptail lizard. *Heredity*, **120**,
727 251–265.

728 Papadopoulou A, Knowles LL (2016) Toward a paradigm shift in comparative phylogeography
729 driven by trait-based hypotheses. *Proceedings of the National Academy of Sciences of the*
730 *United States of America*, **113**, 8018–24.

731 Paradis E, Claude J, Strimmer K (2004) ape: analyses of phylogenetics and evolution in R
732 language. *Bioinformatics*, **20**, 289–290.

733 Peterson BK, Weber JN, Kay EH *et al.* (2012) Double digest RADseq: An inexpensive method
734 for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE*, **7**,
735 e37135.

736 Petit RJ, Duminil J, Fineschi S *et al.* (2004) Comparative organization of chloroplast,
737 mitochondrial and nuclear diversity in plant populations. *Molecular ecology*, **14**, 689–701.

738 Pfeifer B, Wittelsbürger U, Ramos-Onsins SE, Lercher MJ (2014) PopGenome: An efficient
739 Swiss army knife for population genomic analyses in R. *Molecular Biology and Evolution*,
740 **31**, 1929–1936.

741 Rabinowitz D (1978) Dispersal properties of mangrove propagules. *Biotropica*, **10**, 47–57.

742 Reaz R, Bayzid MS, Rahman MS *et al.* (2014) Accurate phylogenetic tree reconstruction from
743 quartets: A heuristic approach. *PLoS ONE*, **9**, e104008.

Author Manuscript

This article is protected by copyright. All rights reserved

744 Riddle BR (2016) Comparative phylogeography clarifies the complexity and problems of
745 continental distribution that drove A. R. Wallace to favor islands. *Proceedings of the*
746 *National Academy of Sciences of the United States of America*, **113**, 7970–7.

747 Riginos C, Crandall ED, Liggins L, Bongaerts P, Treml EA (2016) Navigating the currents of
748 seascape genomics: how spatial analyses can augment population genomic studies. *Current*
749 *Zoology*, **62**, 581–601.

750 Resende-Moreira LC, Knowles LL, Thomas AT, Prado JR, Souto AP, Lemos-Filho JP, Lovato
751 MB (2018) Relictual Amazonian savannas: genetic tests of past connections versus a history
752 of isolation from central Cerrado. *J. Biogeography, in review.*

753 Rönnbäck P (1999) The ecological basis for economic value of seafood production supported by
754 mangrove ecosystems. *Ecological Economics*, **29**, 235–252.

755 Salerno PE, Señaris JC, Rojas-Runjaic FJM, Cannatella DC (2015) Recent evolutionary history
756 of Lost World endemics: Population genetics, species delimitation, and phylogeography of
757 sky-island treefrogs. *Molecular Phylogenetics and Evolution*, **82**, 314–323.

758 Santiago-Valentín E, Olmstead RG (2004) Historical biogeography of Caribbean plants:
759 Introduction to current knowledge and possibilities from a phylogenetic perspective. *Taxon*,
760 **53**, 299–319.

761 Simberloff DS, Wilson EO (1969) Experimental zoogeography of islands: The colonization of
762 empty islands. *Ecology*, **50**, 278–296.

763 Soltis DE, Morris AB, McLachlan JS, Manos PS, Soltis PS (2006) Comparative phylogeography
764 of unglaciated eastern North America. *Molecular Ecology*, **15**, 4261–93.

765 Soltis DE, Soltis PS (2017) Mobilizing and integrating big data in studies of spatial
766 and phylogenetic patterns of biodiversity. *Plant Diversity*, **38**, 264–270.

Author Manuscript

This article is protected by copyright. All rights reserved

767 Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of
768 large phylogenies. *Bioinformatics*, **30**, 1312–3.

769 Steele PR, Hertweck KL, Mayfield D *et al.* (2012) Quality and quantity of data recovered from
770 massively parallel sequencing: Examples in Asparagales and Poaceae. *American Journal of*
771 *Botany*, **99**, 330–348.

772 Straub SC, Fishbein M, Livshultz T *et al.* (2011) Building a model: developing genomic
773 resources for common milkweed (*Asclepias syriaca*) with low coverage genome
774 sequencing. *BMC Genomics*, **12**, 211.

775 Takayama K, Kajita T, Murata J, Tateishi Y (2006) Phylogeography and genetic structure of
776 *Hibiscus tiliaceus*—speciation of a pantropical plant with sea-drifted seeds. *Molecular*
777 *Ecology*, **15**, 2871–2881.

778 Takayama K, Tamura M, Tateishi Y, Webb EL, Kajita T. (2013). Strong genetic structure over
779 the American continents and transoceanic dispersal in the mangrove genus *Rhizophora*
780 (Rhizophoraceae) revealed by broad-scale nuclear and chloroplast DNA analysis. *American*
781 *Journal of Botany*, **100**, 1191–1201.

782 Tomlinson PB (2016) *The Botany of Mangroves*. Cambridge University Press, Cambridge.

783 Walters BB, Rönnbäck P, Kovacs JM et al. (2008) Ethnobiology, socio-economics and
784 management of mangrove forests: A review. *Aquatic Botany*, **89**, 220–236.

785 Wang C, Szpiech ZA, Degnan JH et al. (2010) Comparing spatial maps of human population
786 genetic variation using Procrustes analysis. *Statistical Applications in Genetics and*
787 *Molecular Biology*, **9**, 13.

788 Wee AKS, Takayama K, Chua JL *et al.* (2015) Genetic differentiation and phylogeography of
789 partially sympatric species complex *Rhizophora mucronata* Lam. and *R. stylosa* Griff. using
790 SSR markers. *BMC Evolutionary Biology*, **15**, 57.

This article is protected by copyright. All rights reserved

Author Manuscript

Yu G, Smith DK, Zhu H, Guan, Y, Lam TTY (2017) ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution*, **8**, 28–36.

Zerbino DR, Birney E (2008) Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. *Genome Research*, **18**, 821–829.

Zhang Q, Liu Y, Sodmergen (2003) Examination of the cytoplasmic DNA in male reproductive cells to determine the potential for cytoplasmic inheritance in 295 Angiosperm species. *Plant and Cell Physiology*, **44**, 941–951.

Zheng X, Levine D, Shen J *et al.* (2012) A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, **28**, 3326–3328.

Data Accessibility

- DNA Sequences: Raw reads from the RAD-Seq libraries (accession numbers SRR7501584-SRR7501638; SRR7504176-SRR7504186) and chloroplast genomes (accession numbers SRR7779779-SRR7779767; SRR7781534-SRR7781581) are deposited in the NCBI SRA.
- Supplemental files, including DNA matrices, sampling locations, and ocean current files are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.53mp5ng
- Scripts are publicly available on Github; repository location: https://github.com/richiehodel/Caribbean_mangroves

Author Contributions

R.G.J.H., P.S.S., and D.E.S. designed the research, R.G.J.H., A.C.P., and J.F.D. performed the research, R.G.J.H., L.K.K., S.F.M., P.S.S., and D.E.S. contributed reagents and analytical tools, R.G.J.H., A.C.P., and J.F.D. analyzed data, and R.G.J.H., L.K.K., S.F.M., P.S.S., and D.E.S. wrote the paper.

Author Manuscript

This article is protected by copyright. All rights reserved

Table 1. Mantel tests test for significance between genetic and geographic distance, genetic and ocean current penalized distance, and a partial Mantel test that tests the impact of ocean current penalized distance while controlling for covariance from geographic distance (* = significant when $\alpha = 0.05$, ** = significant when $\alpha = 0.01$, *** = significant when $\alpha = 0.001$).

	Laguncularia racemosa		Rhizophora mangle	
	r	P	r	P
Mantel (Geography)	0.694	0.001**	0.426	0.01*
Mantel (Ocean)	0.275	0.011*	0.126	0.046*
Partial Mantel	0.249	0.021*	0.242	0.001**

Author Manuscript

This article is protected by copyright. All rights reserved

Table 2. The results of the Procrustes analyses in each species for nuclear and chloroplast data. For each species and each marker type, the t_0 value and P value are shown (*** = significant when $\alpha = 0.001$).

	Laguncularia racemosa		Rhizophora mangle	
	nuclear	chloroplast	nuclear	chloroplast
t_0	0.684	0.808	0.561	0.284
P	<0.001***	0.845	<0.001***	0.058

Author Manuscript

This article is protected by copyright. All rights reserved

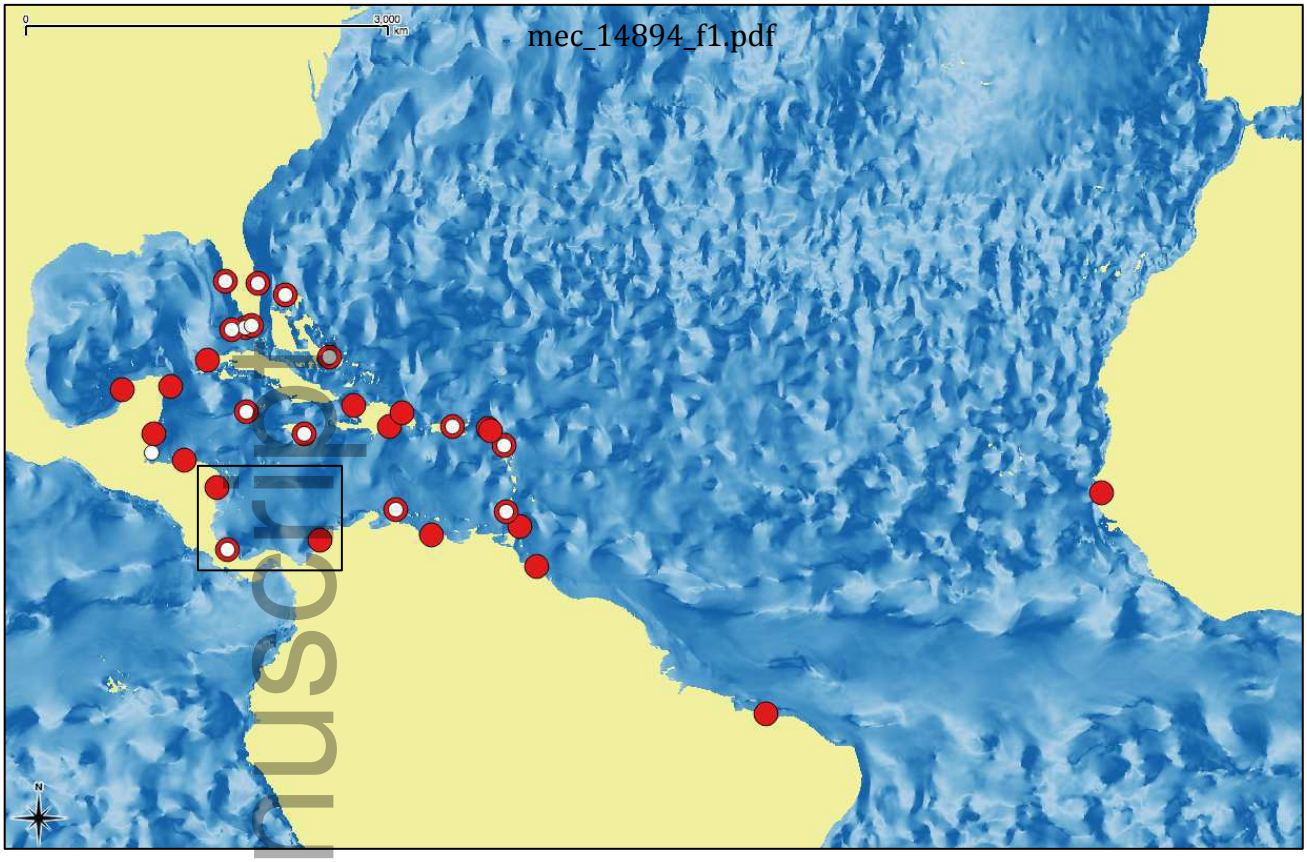
Table 3. The results of correlation tests between the magnitude of Procrustes deformations and factors associated with sampling locations. For each species, five factors were tested for correlation against the Procrustes deformations from the RAD-Seq data (* = significant when $\alpha = 0.05$, ** = significant when $\alpha = 0.01$, *** = significant when $\alpha = 0.001$).

	Laguncularia racemosa		Rhizophora mangle	
	F-value	P	F-value	P
Island vs. continental	0.18	0.68	4.23	0.042*
Island size	0.23	0.8	3.75	0.026*
Direction	0.44	0.51	18.68	<0.0001***
Latitude	10.92	0.001**	6.77	0.010*
Longitude	14.94	<0.0001***	73.2	<0.0001***

Author Manuscript

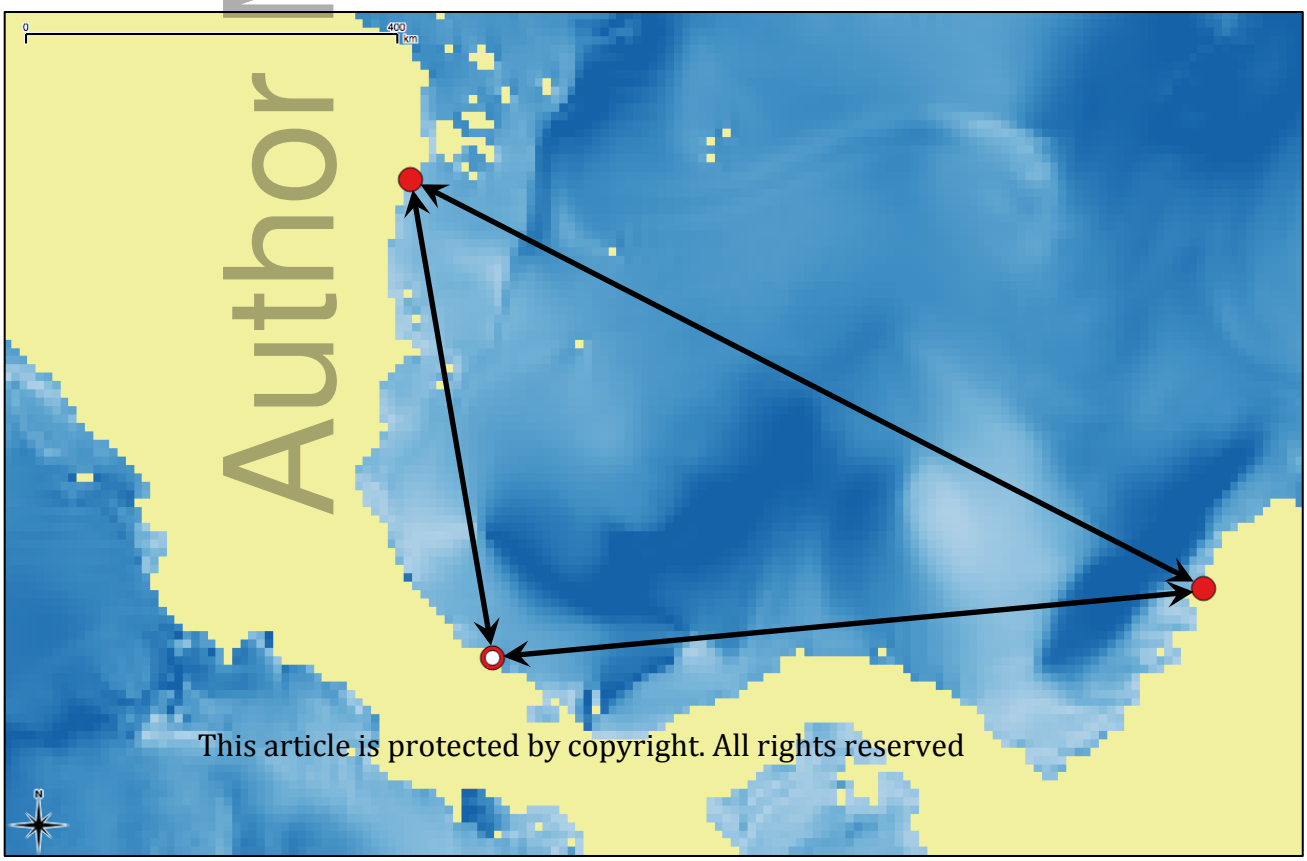
This article is protected by copyright. All rights reserved

Manuscript
Author

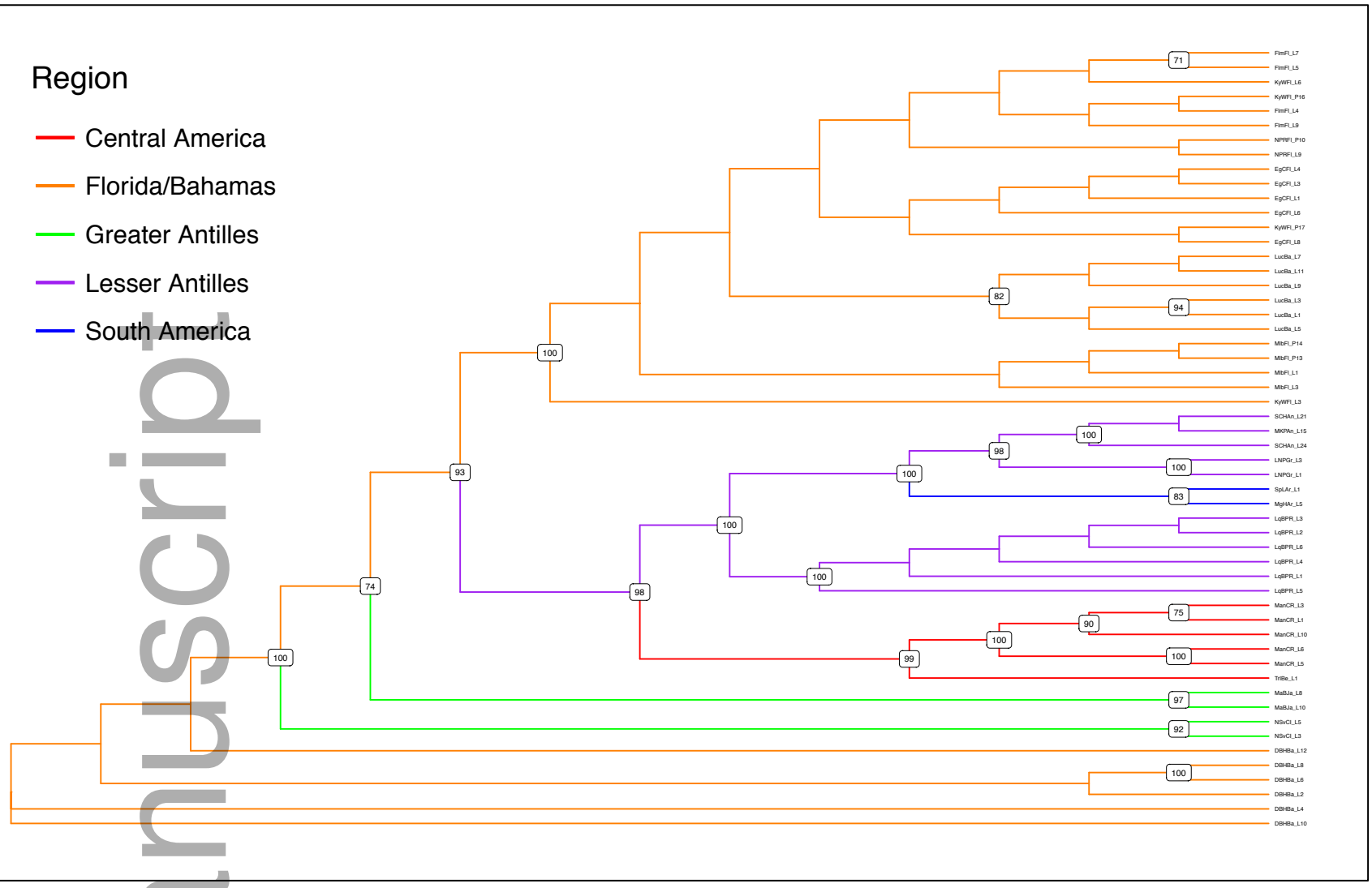


Ocean current bearing (degrees)

0 60 120 180 240 300 360

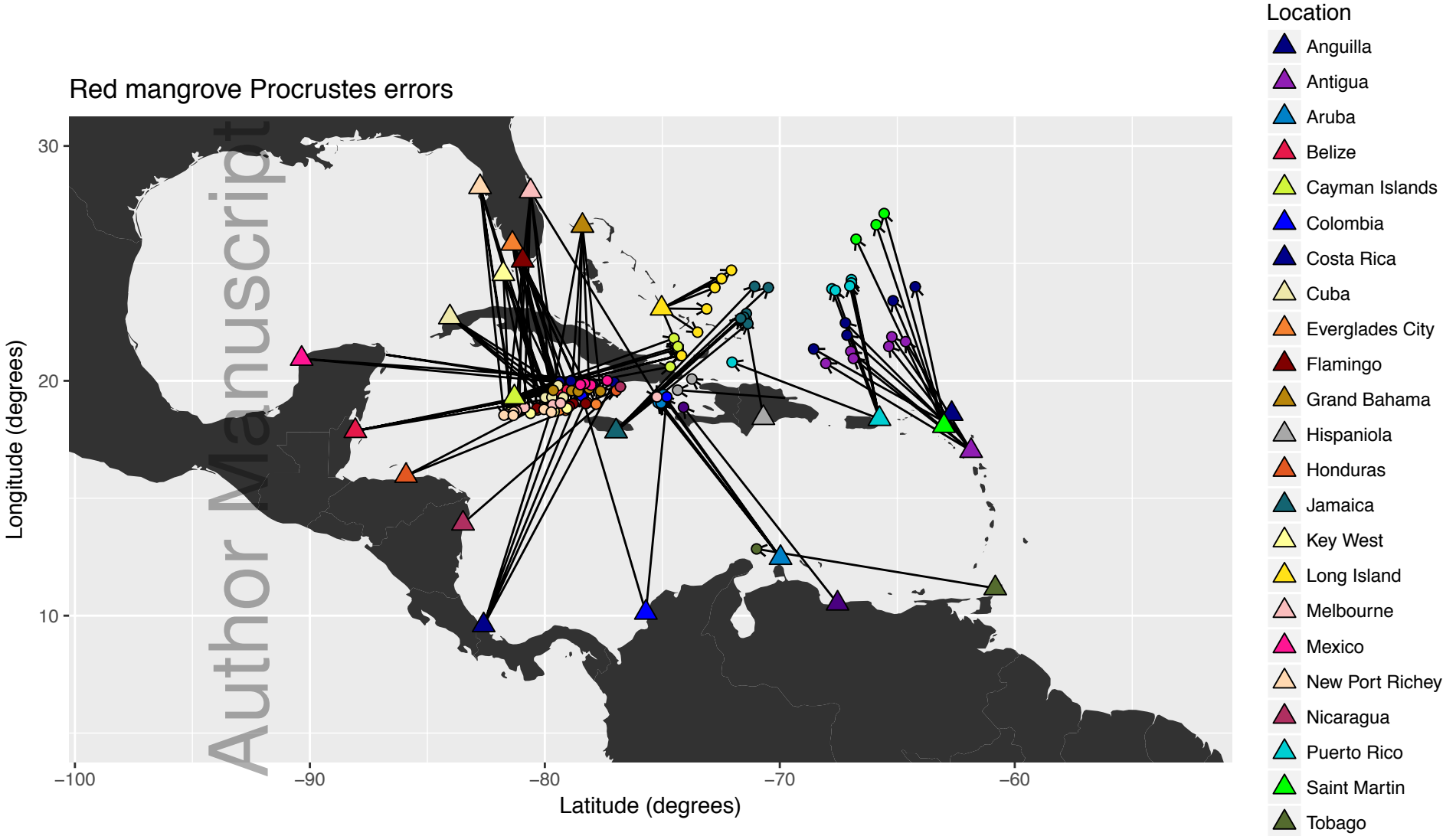


This article is protected by copyright. All rights reserved

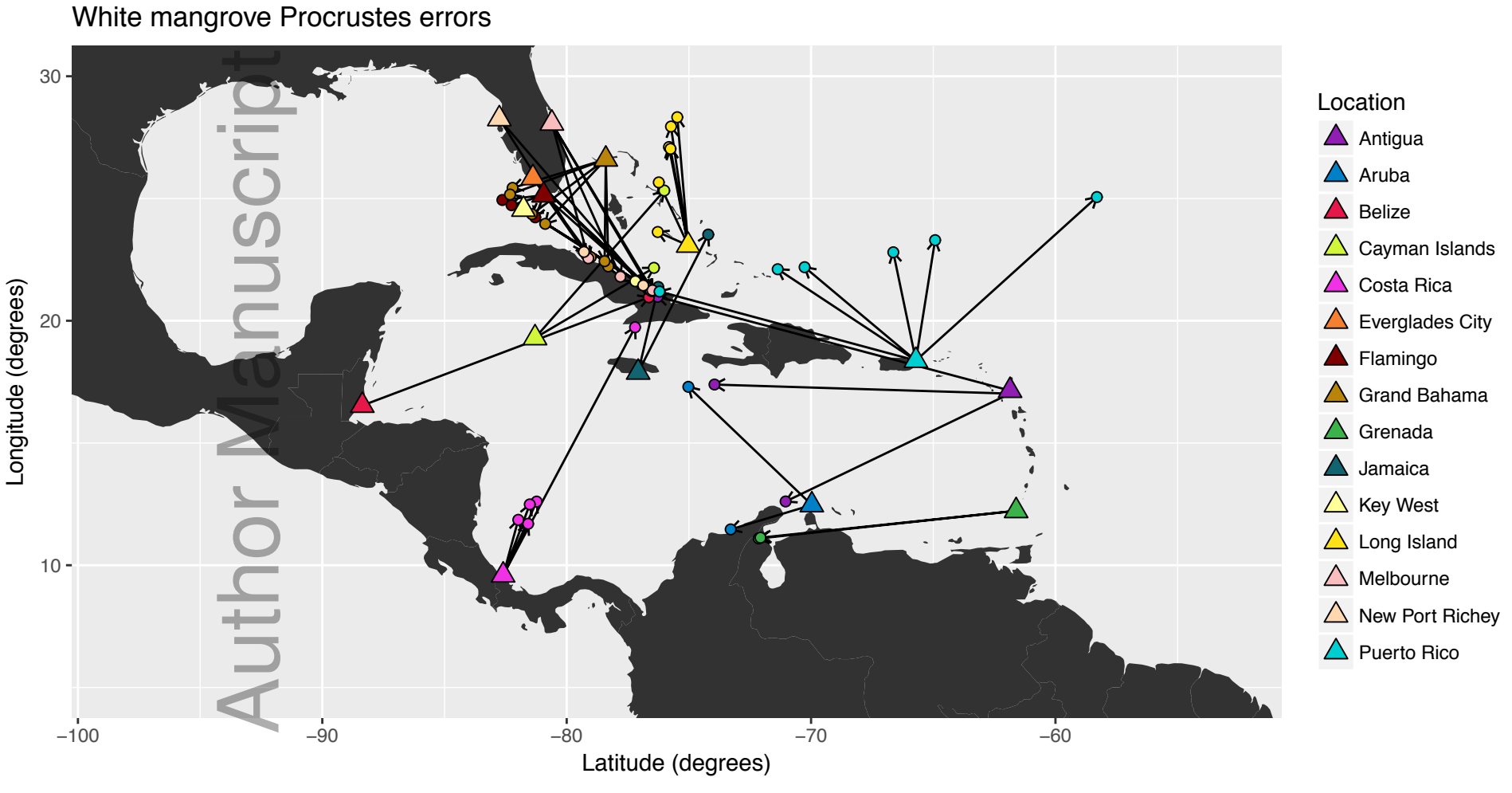


Manuscript
Author M

This article is protected by copyright. All rights reserved



This article is protected by copyright. All rights reserved



This article is protected by copyright. All rights reserved