Title:

Widespread southern forests during the Last Glacial Maximum, not refugia, explain genetic structure of two eastern North American hickory species

Running title:

Phylogeography of North American *Carya*

Authors and affiliations:

Jordan B. Bemmels[1,*] and Christopher W. Dick[1,2]

[1] Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA
[2] Smithsonian Tropical Research Institute, Apartado 0843–03092, Balboa, Ancon, Panama; orcid.org/0000-0001-8745-9137

[*] Corresponding author: jbemmels@umich.edu
orcid.org/0000-0001-9996-6996

Acknowledgements:

The authors thank B.J. Belcher, P. Cousineau, R. D'Andrea, J. Fiske, J. Ronson, D. Saenz and P. Tichenor for assistance with fieldwork, and A.T. Thomaz for assistance with genetic analyses. Graduate student support and research funding was provided to J.B.B. by the National Science Foundation (GRFP fellowship; DDIG 1501159), and the University of Michigan EEB Department and Rackham Graduate School.

Aim
Phylogeographic studies of temperate forest taxa often infer complex histories involving population subdivision into distinct refugia during the Last Glacial Maximum (LGM). However, temperate forests may have been broadly distributed in southeastern North America during the LGM. We investigate genome-wide genetic structure in two widespread eastern North America tree species to determine if range expansion from a contiguous area or from genetically isolated refugia better explains the postglacial history of trees and forests from this region.

Location
Eastern North America (ENA).

Taxa
Bitternut hickory (*Carya cordiformis* (Wangenh.) K.Koch) and shagbark hickory (*Carya ovata* (Mill.) K.Koch).

Methods
Genetic diversity and differentiation indices were calculated from >1,000 nuclear SNP loci genotyped in ca. 180 individuals per species sampled across ENA. Genetic structure was investigated using principle component analysis and genetic clustering algorithms. As an additional tool for inference, areas of suitable habitat during the LGM were predicted using species distribution models (SDMs).

Results
Populations across all latitudes showed similar levels of genetic diversity. Most genetic variation was weakly differentiated across ENA, with the exception of an outlier population of *Carya ovata* in Texas. Genetic structure in each species exhibited an isolation-by-distance pattern. SDMs predicted high LGM habitat suitability over much of the southeastern United States.

Main conclusions
Both hickory species likely survived the LGM in a large region of continuous habitat and recolonized northern areas in a single expanding front that encountered few migration barriers. More complex scenarios, such as forest refugia, need not be invoked to explain genetic structure. The genetically distinct Texas population of *Carya ovata* could represent a separate glacial refugium, but other explanations are possible. Relative to that of other temperate forest regions, the phylogeographic history of ENA may have been exceptionally simple, involving a northward range shift but without well defined refugia.

Keywords
eastern North America, forest refugia, isolation by distance, Last Glacial Maximum, phylogeography, range expansion, temperate trees

Introduction

Temperate forest ecosystems have long served as models for understanding how historical forces give rise to population genetic structure in terrestrial organisms (Hewitt, 1999, 2000; Petit et al., 2003; Soltis et al., 2006; Shafer et al., 2010; Qiu et al., 2011; Gavin et al., 2014; Lumibao et al., 2017). Migrational responses to Pleistocene glaciation often left large impacts on the genetic structure of temperate species, but the severity and effects of glaciation varied in different regions of the world (Hewitt, 2000; Shafer et al., 2010; Qiu et al., 2011; Lumibao et al., 2017). In Europe, where many classic phylogeographic paradigms were first established (Lumibao et al., 2017) and where glaciation during the Last Glacial Maximum (LGM, ca. 21.5 ka; Jackson et al., 2000) was particularly severe, widespread temperate taxa typically retreated to distinct glacial refugia in Mediterranean regions (Hewitt, 1999, 2000; Petit et al., 2003). Genetic diversity was progressively lost due to founder effects as populations recolonized northern areas following glacial retreat (Hewitt, 1999, 2000), except in mid-latitude areas where admixture of lineages from distinct refugia has often led to elevated genetic diversity (Petit et al., 2003).

In other temperate regions of the world, phylogeographic patterns were structured by very different geographies and glacial histories. In eastern North America (ENA), early studies tended to emphasize genetic breaks between populations separated by rivers and mountain ranges (Soltis et al., 2006; Jaramillo-Correa et al., 2009). In western North America, major refugia existed in the Pacific Northwest and Beringia, with smaller refugia on offshore islands and between continental ice sheets

(Shafer et al., 2010). In East Asia, responses to glaciation included not only latitudinal migration, but also elevational and longitudinal migration and *in situ* persistence (Qiu et al., 2011). The complexity of these classic paradigms has recently been expanded in all four northern hemisphere regions to include small, low-density cryptic refugia in areas previously thought unsuitable for habitation by temperate species (Stewart & Lister, 2001; Willis & Van Andel, 2004; McLachlan et al., 2005; Soltis et al., 2006; Provan & Bennett, 2008; Qiu et al., 2011). However, the molecular and fossil evidence supporting the existence of cryptic refugia is not universally accepted (Tzedakis et al., 2013).

Compared to the other three northern hemisphere temperate forest regions, ENA is phylogeographically unique for at least three reasons. Firstly, its geography is relatively simple, characterized by a large contiguous landmass with only a single north-south mountain range of modest height (i.e., the Appalachians), and generally gradual transitions between ecosystem types. Secondly, latitudinal temperature gradients during the LGM were particularly steep, with warm areas located in close proximity to glaciers (Tzedakis et al., 2013). Thirdly, despite numerous phylogeographic studies, well-delineated glacial refugia generally shared by most species have not conclusively been identified. Proposed refugial locations include the Gulf Coast, the Atlantic Coast, Florida, Texas, the Ozark Plateau, the Lower Mississippi River Valley, the Appalachians, and interior areas near ice sheets (Griffin & Barrett, 2004; Magni et al., 2005; Soltis et al., 2006; Jaramillo-Correa et al., 2009; Morris et al., 2010; Barnard-Kubow et al., 2015; McCarthy & Mason-Gamer, 2016; Peterson & Graves, 2016), which together sum to nearly the entire unglaciated region of eastern North America. While some species may have survived in one or more of these distinct refugia, fossil and genetic evidence

suggests that some temperate taxa were widespread over vast areas of the southeastern United States during the LGM (Bennett, 1985; Magni et al., 2005; McLachlan et al., 2005; Peterson & Graves, 2016; Lumibao et al., 2017).

Given the diversity of hypotheses that have been evoked to explain phylogeographic patterns in ENA taxa, studies assessing genome-wide patterns of genetic variation in widely distributed model species would provide valuable insight into the history of temperate forests from the region as a whole. Surprisingly, we are aware of no such studies (but Eckert et al. (2010) and Nadeau et al. (2015) have conducted studies of more narrowly distributed tree species). Here, we use genome-wide genetic variation to examine the phylogeographic history of two widespread, ENA tree species: bitternut hickory (*Carya cordiformis* (Wagenh.) K.Koch) and shagbark hickory (*Carya ovata* (Mill.) K.Koch). We construct and analyze single-nucleotide polymorphism (SNP) datasets from nearly rangewide collections of each species and build paleodistribution models in order to characterize geographic patterns of genetic diversity and differentiation across ENA. In particular, we aim to determine if genetic structure is best explained by recolonization from distinct forest refugia (and if so, where these refugia where located), or by a single expansion from a large, continuous forest region.

Methods

Study species

Carya cordiformis and *Carya ovata* are wind-pollinated, animal-dispersed trees co-distributed from southern Quebec to eastern Texas (Fig. 1). Their ranges roughly

correspond to the overall geographic distribution of temperate deciduous forests in ENA, making them excellent model taxa to study the phylogeography of eastern deciduous forests as a whole. *Carya ovata* additionally occurs in several small, disjunct populations in the Sierra Madre Oriental of northern Mexico (Little, 1971). In the northern half of temperate ENA, *C. cordiformis* occupies many habitats but occurs most frequently on mesic soils and bottomlands (Smith, 1990), whereas *C. ovata* is common on a wider variety of sites (Graney, 1990). In southern areas both species are less common and typically restricted to wet, fertile soils (Graney, 1990; Smith, 1990).

Phylogeographic knowledge is completely lacking in *C. cordiformis*. In *C. ovata*, analysis of cpDNA haplotypes has revealed no clear pattern, as some haplotypes are widespread throughout the entire range and others are more spatially restricted, including in formerly glaciated areas (Lumibao et al., 2017). *Carya* pollen is not typically distinguished to the species level, but LGM-age pollen of *Carya* has been found at low density over large areas of the southeastern USA (Prentice et al., 1991; Jackson et al., 2000). *Carya* macrofossils dating to the LGM have been found in southern Tennessee (35ºN; Jackson et al., 2000), and trace amounts of pollen as far north as southern Illinois (39ºN; Grüger, 1972). However, the presence of small amounts of pollen in northern areas is not necessarily indicative of local presence, as pollen can be reworked from layers representing different time periods, or transported by wind over long distances (Tzedakis et al., 2013).

Many of the ca. 13 North American *Carya* species readily hybridize with one another (Fralish & Franklin, 2002). Geographically structured hybridization may impact phylogeographic inferences in tree species (Saeki et al., 2011; Thomson et al., 2015),

165 and one limitation of our analyses is that we are unable to assess patterns of
166 hybridization with other *Carya*. However, no stable hybrid zones exist in our species and
167 we consider it unlikely that occasional hybridization would systematically bias genetic
168 structure in a similar way across thousands of loci.
169
170 *DNA sampling and SNP genotyping*
171 Silica-dried leaf tissue was collected from 182 individuals of each species from
172 populations across ENA (Fig. 1; Tables S1.1-S1.2). Sampled individuals within each
173 population were separated by a minimum of 50 m (but sometimes up to dozens of km)
174 in order to minimize the chance of sampling siblings and other close relatives. Sample
175 size varied greatly among populations depending on the number of individuals meeting
176 these requirements that could be located (mean N = 7.8; Tables S1.1-S1.2). A
177 representative voucher specimen from each population was deposited in the University
178 of Michigan Herbarium (MICH; DOI: XXXXX).
179 DNA samples were extracted using Nucleospin Plant II extraction kits (*Macherey-*
180 *Nagel*; Düren, Germany), and libraries were prepared using a modified double digest
181 Restriction Associated DNA (ddRAD) sequencing protocol following Peterson et al.,
182 (2012), with restriction enzymes *EcoRI* and *MseI.* Full details of extraction methods and
183 library preparation are provided in Appendix S1 in Supporting Information. Seven
184 libraries of 72 samples each were sequenced at The Hospital for Sick Children (Toronto,
185 ON) on an *Illumina HiSeq* (Illumina; San Diego, CA) using single-end 50-bp sequencing.
186 In order to ensure adequate depth of coverage, at least one million raw reads per

187 sample were required to process a sample, and individuals not meeting this target were
188 resequenced in subsequent libraries.

189 Loci were identified and single nucleotide polymorphisms (SNPs) were
190 genotyped using STACKS v.1.44-v.1.46 (Catchen et al., 2011, 2013). Full details of SNP
191 discovery are provided in Appendix S1. After SNPs were successfully identified, one
192 SNP genotype per locus was exported from STACKS using the *populations* tool, retaining
193 only SNPs with a minimum genotyping rate of 75% (-r 0.75) and a minimum minor allele
194 frequency (MAF) of 3.3% (--min_maf 0.033), the lowest detectable MAF in at least one
195 population of each species, following Massatti & Knowles (2014). Minimum MAF is an
196 important parameter to consider because it can impact inference of genetic structure
197 (De la Cruz & Raska, 2014). We therefore explored preliminary analyses with minimum
198 MAF = 1% and 5%, but found that using the higher minimum MAF (5%) made little
199 qualitative difference in preliminary results. With the lower minimum MAF (1%), broad-
200 scale patterns were overwhelmed by local-scale signatures, likely due to rare variants
201 shared among closely related individuals (De la Cruz & Raska, 2014). These results
202 suggested that our choice of minimum MAF = 3.3% was appropriate.

203 In order to retain only putatively nuclear SNPs, we removed any SNPs from loci
204 that aligned with a maximum of two mismatches (-v 2) to the *Juglans regia*
205 (Juglandaceae) chloroplast genome (Genbank accession NC_028617.1) or the
206 *Cucurbita pepo* (Cucurbitaceae) mitochondrion genome (NC_014050.1) using BOWTIE
207 v.1.2 (Langmead et al., 2009). Extremely variable loci were also excluded as these may
208 represent locus assembly errors; we defined these loci as those with values of θ
209 (Watterson, 1975) above the 95th percentile, with θ calculated for each locus individually

210 using the *R* package 'pegas' v.0.10 (Paradis, 2010). Individual samples with unusually
211 high levels of missing data across all loci (based on visual inspection) were also
212 excluded.
213 Final SNP datasets are available for download from the Dryad Digital Repository
214 (DOI: XXXXX).
215
216 *Genetic diversity and divergence*
217 Three genetic diversity parameters were calculated overall and for each
218 population: observed and expected heterozygosity (H_o and H_e, respectively), and
219 nucleotide diversity (π). Genetic differentiation (F_{ST}) (Nei, 1987) was calculated overall
220 and pairwise between each pair of populations. H_o, H_e, and F_{ST} were calculated in the *R*
221 package 'hierfstat' v.0.04-22 (Goudet, 2005), while π was calculated using *populations*
222 in STACKS v.1.46 (Catchen et al., 2011, 2013). Genetic diversity and differentiation
223 measures are not reported for populations represented by a single individual.
224
225 *Population genetic structure*
226 In order to test for isolation by distance (IBD), Mantel tests (Mantel, 1967) were
227 performed to assess the relationship between population pairwise F_{ST} values and
228 geographic distances. Principal component analysis (PCA) was used to investigate
229 genetic relationships among individuals and populations using the *dudi.pca* function in
230 the *R* package 'adegenet' v.2.0.1 (Jombart, 2008; Jombart & Ahmed, 2011). The NC_e
231 population (Table S1.1) was excluded for *C. cordiformis* because some, but not all
232 individuals from this population formed a distinct genetic cluster, which suggests that

several closely related individuals were unintentionally sampled and the high genetic similarity between these individuals could have biased initial PCA results.

Genetic clusters were characterized using FASTSTRUCTURE v.1.0 (Raj et al., 2014), with all populations and individuals included, using the recommended procedure for detecting subtle genetic structure. Initially, the simple prior model was used and the number of clusters (*K*) was varied from 1 to 6 for each species, and *K* was selected using the *chooseK* tool in FASTSTRUCTURE. Then, FASTSTRUCTURE was rerun 100 times using the logistic prior model for the optimal value(s) of *K*, and final estimates of genetic membership of individuals in each genetic cluster were obtained as the average membership from the five runs with the highest likelihood, following Raj et al. (2014). After investigating the broadest level of structure within each dataset, we reran FASTSTRUCTURE on individual genetic clusters in order to test for substructure within clusters.

Paleodistribution modelling

Species distribution models (SDMs) were constructed in order to predict the potential distribution of each species during the Last Glacial Maximum (LGM; 21.5 ka). Complete details of SDM construction and data sources are given in Appendix S1, and occurrence records are available for download from the Dryad Digital Repository (DOI: XXXXX). Briefly, occurrence records were obtained from the US Forest Service Forest Inventory Analysis Database (O'Connell et al., 2012), while environmental variables were obtained at 2.5-arcminute resolution from the WORLDCLIM v.1.4. (Hijmans et al., 2005) and ENVIREM (Title & Bemmels, 2017) databases, for both current and LGM

256 conditions. SDMs were constructed using MAXENT v.3.4.1 (Phillips et al., 2004, 2006,
257 2017) in the *R* package 'dismo' (Hijmans et al., 2015), with models optimized according
258 to best practices, following Title & Bemmels (2017).
259
260 **Results**
261
262 *Genetic diversity and differentiation*
263 The final genetic datasets for *C. cordiformis* and *C. ovata* contained 177
264 individuals genotyped at 1,046 SNPs, and 180 individuals genotyped at 1,018 SNPs,
265 respectively. The overall genotyping rate for both species was 89%.
266 While some populations were represented by very few individuals (Tables S1.1-
267 S1.2), very small sample sizes are typically sufficient to obtain accurate population
268 genomic measures of genetic diversity and differentiation if calculated across thousands
269 of SNPs (Willing et al., 2012; Nazareno et al., 2017). Furthermore, genetic diversity
270 estimates were generally uncorrelated with population sample size (Fig. S1.1), except
271 that a negative relationship was found between H_o and sample size in *C. ovata* (R^2 =
272 0.21, p = 0.035). However, as H_o is computed on a per-individual basis, there is no
273 theoretical reason to expect that sample size might bias estimates of H_o and we suspect
274 that this correlation is spurious.
275 Genetic diversity showed little variation among populations for both species (Fig.
276 2). A significant decline in genetic diversity with increasing latitude was not observed for
277 any genetic diversity measure (H_o, H_e, π) for either species. Instead, a significant
278 increase in H_o with increasing latitude was observed in *C. cordiformis* (R^2 = 0.23, p =

279 0.021), as was a marginally non-significant increase in H_o with increasing latitude in *C.*
280 *ovata* (R^2 = 0.19, p = 0.051). Although genetic variation was fairly uniform across
281 latitudes, far northern and far southern populations sometimes showed slightly lower
282 values of H_e and π than typical of mid-latitude populations (Fig. 2), as expected for
283 range-edge populations (Jaramillo-Correa et al., 2009). Among-population genetic
284 differentiation (F_{ST}) is low in both species overall (*C. cordiformis*: 0.047; *C. ovata*: 0.038),
285 and among most pairs of populations (Tables S1.3-S1.4).
286
287 *Spatial genetic structure*
288 Spatial genetic structure in both species is very weak and dominated by a pattern
289 of isolation by distance (IBD). Mantel tests of IBD were statistically significant in both
290 species (*C. cordiformis*: r = 0.36, p = 0.0017; *C. ovata*: r = 0.47, p = 8.1 x 10^{-5}; Fig. 3).
291 Principal component analysis (PCA) also revealed an IBD-like pattern, without
292 clearly defined, distinct genetic clusters (Fig. 4). One exception to this pattern is that in
293 *C. ovata*, the Texas population (TX; bright pink dots, Fig. 4b) forms a separate cluster
294 that does not overlap with any other populations. However, the relative position of TX
295 along PC axes is still closest to that of geographically proximate western and southern
296 populations, consistent with an IBD pattern.
297 Lack of strong genetic structure was also suggested by FASTSTRUCTURE
298 results. Under the model with simple priors, the optimal number of genetic clusters was
299 *K* = 1 for both species. Under the model with logistic priors, which is more useful for
300 detecting subtle structure (Raj et al., 2014), optimal *K* ranged from 1-6. However, the
301 logistic priors model is prone to overfitting (Raj et al., 2014) and *K* > 2 did not produce

302 results that were biologically interpretable. We therefore note that *K* = 1 or 2 is likely the
303 optimal model complexity to explain genetic structure. In both species with *K* = 2,
304 genetic structure was weak and similar to an IBD-like pattern (Figs. 5, S1.2). In *C.*
305 *cordiformis*, a gradual north-south transition between clusters was evident. In *C. ovata*,
306 the transition between clusters was primarily from east to west. No substructure was
307 evident within any genetic cluster for any species, except that within the western cluster
308 for *C. ovata*, optimal *K* = 2 and the Texas population (TX) forms a distinct subcluster
309 relative to the other four populations (AR, IA, ON, WI; data not shown). However, the
310 grouping of these four populations into a distinct subcluster may be only a statistical
311 artifact reflecting the substantial additional membership of each these four western
312 populations in the main eastern cluster.
313
314 *Paleodistribution modelling*
315 The same four climatic variables were coincidentally retained in the SDMs for
316 both species: maximum temperature of the coldest month, potential evapotranspiration
317 of the warmest quarter, mean annual precipitation, and climatic moisture index (Table
318 S1.5). For both species, models were able to predict the current species distribution (Fig.
319 1) very well along the northern and western range edges, but performed more poorly at
320 delineating the southern range edge (Fig. 5). This poorer performance may reflect the
321 fact that both species are rare in the southern portion of their ranges, where presence
322 and absence may be determined more by soil type and topography (Graney, 1990;
323 Smith, 1990) than by broad-scale climatic differences among sites. For both species, a
324 large, continuous area of high LGM habitat suitability is predicted to have extended over

much of the southeastern US, from central Texas to exposed continental shelf off the coast of North Carolina, and south to northern Florida (Fig. 5).

Discussion

Carya cordiformis and *Carya ovata* likely survived the LGM in a large, contiguous forest region covering much of the southeastern United States, and recolonized northern areas in a single expanding front. This scenario is supported by our genetic results showing weak genetic structure and an isolation-by-distance (IBD) pattern, and by our paleodistribution models and the fossil record. Genetic differentiation is continuous across ENA, except that a Texas population of *C. ovata* is more genetically distinct than other populations. The cause of this distinctiveness remains unclear. Insights from both species are likely applicable to understanding the phylogeographic history of temperate forests from ENA as a whole, and suggest that ENA may lack the complex refugial dynamics characteristic of other temperate forest regions of the world.

Weak genetic structure

In both species, genetic structure is weak and geographic patterns of genetic variation are primarily characterized by IBD, rather than sharp genetic breaks among regions. Although some geographic structure was detected with FASTSTRUCTURE, IBD is known to bias tests of hierarchical genetic structure (Frantz et al., 2009; Meirmans, 2012). In particular, such tests are susceptible to incorrect inference of multiple genetic clusters when populations are geographically subsampled from within a single larger

348 cluster subject to IBD (Frantz et al., 2009; Meirmans, 2012). We note that a rangewide
349 IBD pattern is present in our datasets (Fig. 3), and genetic structure is weak in both
350 species (optimal K = 1 to 2), with only gradual geographic transitions between inferred
351 genetic clusters (Fig. 1). Similarly, substantial overlap among populations exists along
352 the first and second principal component axes (Fig. 4), with the exception of the Texas
353 population of *C. ovata* (see below). Rather than indicating the true presence of
354 biologically meaningful genetic clusters, our FASTSTRUCTURE results are likely a
355 statistical artifact of underlying IBD and suggest that over the majority of the species
356 range, genetic differentiation is continuous.

357 In addition to lack of phylogeographic breaks, there are no identifiable regions of
358 elevated genetic diversity (Fig. 2). Similar to many other temperate tree species from
359 ENA (Lumibao et al., 2017), *C. cordiformis* and *C. ovata* do not exhibit declines in
360 genetic diversity with increasing latitude, and population genetic diversity (H_e, π) is
361 relatively uniform across the species range. The absence of regions of elevated
362 diversity suggests either historical recolonization in a single, slowly expanding migration
363 front experiencing little loss of diversity during migration, or else very high gene flow
364 among populations (Jaramillo-Correa et al., 2009). Both of these situations are likely
365 applicable to our study species. In particular, low population genetic differentiation (*C.*
366 *cordiformis*, F_{ST} = 0.047; *C. ovata*, F_{ST} = 0.038) provides further evidence that gene flow
367 among populations is likely high. Low population differentiation is commonly observed in
368 widespread, wind-pollinated forest trees, due to their large population sizes and
369 capacity for long-distance pollen-mediated gene flow (Hamrick et al., 1992; Savolainen
370 et al., 2007; Alberto et al., 2013).

371 Despite generally uniform genetic diversity across populations in terms of H_e and
372 π, observed heterozygosity (H_o) increases with increasing latitude in both species (Fig.
373 2). Higher genetic diversity in northern regions might reflect the effects of larger
374 populations in the north that are less subject to genetic drift (Griffin & Barrett, 2004), but
375 genetic drift would be expected to simultaneously affect H_e and π (not only H_o). A more
376 likely explanation is that decreased H_o in the south reflects an increase in homozygotes
377 due to inbreeding in generally smaller, more isolated southern populations, but that this
378 effect has not yet led to a loss of overall genetic diversity at the population level (H_e, π).
379 We also note that far northern and far southern populations of both species
380 sometimes exhibit slightly lower population genetic diversity (H_e, π) than mid-latitude
381 populations (Fig. 2). In this case, we do suspect that this pattern reflects reduced gene
382 flow between core and peripheral populations and loss of diversity due to genetic drift in
383 generally smaller, more isolated peripheral populations (Hampe & Petit, 2005; Jaramillo-
384 Correa et al., 2009). However, in some European taxa, elevated mid-latitude genetic
385 diversity is believed to instead reflect historical secondary contact and admixture of
386 lineages from distinct southern refugia (Petit et al., 2003). Nonetheless, we find no
387 evidence of distinct refugia or mid-latitude admixture among lineages, making
388 secondary contact a less likely explanation for the patterns we observe.
389
390 *Temperate forests in ENA during the LGM*
391 The phylogeographic history of both *C. cordiformis* and *C. ovata* is best
392 characterized by simple latitudinal range shifts. Our genetic data and species
393 distribution models suggest that both species inhabited large areas of the southeastern

394 United States during the Last Glacial Maximum and gradually expanded northward to
395 occupy their current distribution as glaciers retreated. We find no evidence of highly
396 genetically distinct geographic regions or of strong phylogeographic breaks that would
397 suggest population fragmentation into multiple refugia (except possibly range-edge *C.*
398 *ovata* from Texas; see below). We also find no evidence that postglacial recolonization
399 of northern areas has occurred via multiple routes or been impeded by major landscape
400 barriers. LGM survival over large areas of the southeastern United States and gradual
401 northward recolonization is also compatible with the fossil record for the genus *Carya*
402 (Prentice et al., 1991; Jackson et al., 2000).

403 While we do not detect any signatures of complex refugial dynamics or multiple
404 postglacial recolonization routes, an alternative scenario we must consider is that one or
405 both species might have experienced a more complex phylogeographic history, the
406 signatures of which have been subsequently obscured by extensive contemporary gene
407 flow (e.g., He et al., 2013). Low population genetic differentiation and an IBD pattern
408 suggest that gene flow among populations is indeed fairly high. However, both species
409 are slow growing and long lived, with peak reproduction occurring in *C. cordiformis* from
410 ages 50 to 125 (Smith, 1990), and in *C. ovata* from ages 60 to 200 (Graney, 1990).
411 Relatively few generations have therefore passed since the LGM, meaning that there
412 has likely been insufficient time for gene flow to erode genetic signatures of postglacial
413 expansion from multiple refugia.

414 Given that *C. cordiformis* and *C. ovata* are common, widespread tree species
415 with a geographic distribution roughly matching that of temperate forests in ENA, we
416 consider them to be model taxa for understanding the phylogeographic history of

417 temperate forests from this region as a whole. Both species occupy a variety of habitat
418 sites, but do show relevant differences in their ecology (Graney, 1990; Smith, 1990). In
419 particular, *C. ovata* is a habitat generalist, and phylogeographic patterns for this species
420 are likely to be broadly representative of those for ENA forest taxa in general. In
421 contrast, *C. cordiformis* is a more mesic, bottomland species in many parts of its range,
422 and thus would be expected to be particularly susceptible to fragmentation into refugia
423 and therefore exhibit strong phylogeographic structure. However, low genetic structure
424 and lack of any evidence of distinct refugia in *C. cordiformis* provide particularly strong
425 evidence that temperate forests in ENA during the LGM were not highly fragmented.
426 Several other widespread woody plant species from ENA are also believed to
427 have expanded from a large area covering much of the southeastern United States,
428 including *Acer rubrum* (McLachlan et al., 2005), *Dirca palustris* (Peterson & Graves,
429 2016), *Fagus grandifolia* (Bennett, 1985; McLachlan et al., 2005), and *Quercus rubra*
430 (Magni et al., 2005). The presence of climatic conditions able to support temperate trees
431 over large areas of the southeastern United States during the LGM is also well
432 supported by pollen records (Prentice et al., 1991; Jackson et al., 2000; Williams, 2002).
433 Despite these sources of phylogeographic concordance, more complex scenarios
434 involving divisions into distinct refugia or multiple recolonization routes have been
435 proposed for many other plant species, especially those that are less geographically
436 widespread (e.g., Griffin & Barrett, 2004; Gonzales et al., 2008; Eckert et al., 2010;
437 Barnard-Kubow et al., 2015; Nadeau et al., 2015; Zinck & Rajora, 2016). In addition,
438 many forest communities with no modern analogue were present in ENA during the
439 LGM (Jackson et al., 2000), suggesting that not all taxa responded to glaciation in the

440 same way. It is therefore unclear whether any general migrational responses to
441 glaciation are applicable to ENA forests, or whether most taxa exhibited species-specific,
442 idiosyncratic responses.

443 One possible explanation for different responses among species is that suitable
444 habitat for species adapted to a narrower range of climatic or edaphic conditions could
445 have been more geographically fragmented during the LGM than for more widespread
446 species. In particular, ENA taxa with a strictly southern, warm-temperate distribution
447 may have become fragmented into distinct far-southern refugia in Florida, Texas, or
448 along the Gulf or Atlantic Coasts (e.g., Gonzales et al., 2008; Eckert et al., 2010). In
449 contrast, more widespread species such as *C. cordiformis* and *C. ovata* could have
450 survived in more expansive inland areas of cool-temperate conditions that extended
451 farther north (e.g., Fig. 5). As model taxa, *C. cordiformis* and *C. ovata* may be most
452 appropriate for understanding the phylogeographic history of temperate forests as a
453 whole, and may be less representative of taxa with widely differing habitat requirements
454 and narrower ecological amplitudes.

455 Nonetheless, our results suggest that temperate forests from ENA may have
456 experienced a relatively simple phylogeographic history. Previous phylogeographic
457 studies have established that major rivers and the Appalachian Mountains are important
458 phylogeographic barriers for some ENA taxa, but these findings are not universal and
459 are typically found in small animals with limited dispersal ability (Soltis et al., 2006). In
460 contrast, rivers are unlikely to present substantial dispersal barriers to large trees, and
461 the phylogeographic impact (or lack thereof) of the Appalachians on tree populations
462 remains unclear (Jaramillo-Correa et al., 2009). Overall, there are few topographic or

463 climatic barriers likely to lead to complex phylogeographic histories for ENA forest trees,
464 especially in comparison to other temperate regions of the world (Hewitt, 2000; Shafer
465 et al., 2010; Qiu et al., 2011).
466
467 *Genetic distinctiveness of Texas shagbark hickory*
468 Although an IBD pattern characterizes genetic structure throughout most of ENA
469 in both species, the Texas population (TX) of *C. ovata* presents an exception to this
470 general pattern. TX forms its own PCA genetic cluster that does not overlap with any
471 other population (pink dots; Fig. 4b), and it is the only population with membership
472 almost exclusively in the western cluster identified by FASTSTRUCTURE.
473 There are several possible interpretations of these results. One possibility is that
474 TX may be derived from a separate glacial refugium. Glacial refugia have previously
475 been inferred in Texas and northern Mexico for several southern *Pinus* and *Prunus*
476 species (Schmidtling & Hipkins, 1998; Schmidtling, 2003; Shaw & Small, 2005;
477 Jaramillo-Correa et al., 2009; Eckert et al., 2010). Alternatively, ancestors of the TX
478 population may have experienced gene flow with *C. ovata* populations in the mountains
479 of northern Mexico (Little, 1971). Although we have not included any high-elevation
480 Mexican populations in our genetic analyses or paleodistribution models, we cannot
481 exclude the possibility that these populations may have migrated to lower elevations
482 and come into contact with other populations during the LGM or at another time during
483 the Pleistocene.
484 However, we do not necessarily need to invoke a separate refugium or contact
485 with Mexican populations to explain the genetic distinctiveness of the range-edge TX

population, because our results could also merely reflect low gene flow between TX and other populations. There is likely very little gene flow between TX and populations we sampled further to the east due to the absence of *C. ovata* in the Lower Mississippi River Valley (Fig. 1). In addition, the Ozark and Ouachita Mountains could conceivably reduce gene flow with populations sampled to the north. Denser population sampling across the southwestern portion of the species range could help fill in geographic gaps in our PC analyses, in order to determine whether TX is truly a distinct gene pool, or represents the extreme end of a rangewide IBD pattern, but is subject to greater genetic isolation than other populations.

Given the uncertain origin of the Texas population of *C. ovata*, we suggest that any conservation efforts in this species should ensure inclusion of populations from Texas and surrounding regions. Genetically distinct southern populations of temperate species have often been identified as high conservation priority (Petit et al., 2003; Hampe & Petit, 2005; Médail & Diadema, 2009). However, across most of the range of *C. cordiformis* and *C. ovata*, we see little reason to prioritize conservation of southern populations, due to their lack of genetic distinctiveness and lack of elevated genetic diversity. On the other hand, most temperate tree species exhibit geographically structured climatically adaptive genetic variation (Savolainen et al., 2007; Aitken & Bemmels, 2016) unlikely to be captured by the putatively neutral SNP markers we employed. Conserving populations from a variety of climates across the species range would therefore likely maximize conservation of adaptively relevant genetic diversity.

Conclusions

509 Genome-wide patterns of genetic variation, predictions of paleodistibution
510 models, and the fossil record all suggest that *C. cordiformis* and *C. ovata* recolonized
511 postglacial ENM from a single, continuous region of temperate forest that likely covered
512 much of the southeastern United States during the LGM. We generally find no evidence
513 of distinct glacial refugia or strong phylogeographic breaks. However, as an exception to
514 this overall pattern, it is unclear whether the higher genetic distinctiveness of a Texas
515 population of *C. ovata* relative to other populations reflects the effects of a separate
516 Texas refugium, historical contact with Mexican populations, or contemporary patterns
517 of gene flow. The two *Carya* species we have studied represent excellent model taxa for
518 understanding the phylogeographic history of eastern deciduous forests as a whole.
519 Whereas complex refugial dynamics must be invoked to explain genetic structure in
520 other temperate regions of the world, our results suggest that the phylogeographic
521 history of temperate forests from ENA can largely be explained by simple latitudinal
522 range shifts, without division into distinct refugia.
523

524 **References**

525

526 Aitken, S.N. & Bemmels, J.B. (2016) Time to get moving: assisted gene flow of forest
527 trees. *Evolutionary Applications*, **9**, 271–290.

528 Alberto, F.J., Aitken, S.N., Alía, R., González-Martínez, S.C., Hänninen, H., Kremer, A.,
529 Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R., & Savolainen, O. (2013)
530 Potential for evolutionary responses to climate change - evidence from tree
531 populations. *Global Change Biology*, **19**, 1645–1661.

532 Barnard-Kubow, K.B., Debban, C.L., & Galloway, L.F. (2015) Multiple glacial refugia
533 lead to genetic structuring and the potential for reproductive isolation in a
534 herbaceous plant. *American Journal of Botany*, **102**, 1842–1853.

535 Bennett, K.D. (1985) The spread of *Fagus grandifolia* across eastern North America
536 during the last 18 000 years. *Journal of Biogeography*, **12**, 147–164.

537 Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., & Cresko, W.A. (2013)
538 STACKS: an analysis tool set for population genomics. *Molecular Ecology*, **22**, 3124–
539 3140.

540 Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J.H. (2011)
541 STACKS: building and genotyping loci de novo from short-read sequences. *G3:*
542 *Genes, Genomes, Genetics*, **1**, 171–182.

543 Eckert, A.J., Van Heerwaarden, J., Wegrzyn, J.L., Nelson, C.D., Ross-Ibarra, J.,
544 González-Martínez, S.C., & Neale, D.B. (2010) Patterns of population structure and
545 environmental associations to aridity across the range of loblolly pine (*Pinus taeda*
546 L., Pinaceae). *Genetics*, **185**, 969–982.

547 Fralish, J.S. & Franklin, S.B. (2002) *Taxonomy and Ecology of Woody Plants in North*
548 *American Forests.* John Wiley & Sons, New York.
549 Frantz, A.C., Cellina, S., Krier, A., Schley, L., & Burke, T. (2009) Using spatial Bayesian
550 methods to determine the genetic structure of a continuously distributed population:
551 Clusters or isolation by distance? *Journal of Applied Ecology*, **46**, 493–505.
552 Gavin, D.G., Fitzpatrick, M.C., Gugger, P.F., et al. (2014) Climate refugia: joint inference
553 from fossil records, species distribution models and phylogeography. *New*
554 *Phytologist*, **204**, 37–54.
555 Gonzales, E., Hamrick, J.L., & Chang, S.M. (2008) Identification of glacial refugia in
556 south-eastern North America by phylogeographical analyses of a forest
557 understorey plant, *Trillium cuneatum*. *Journal of Biogeography*, **35**, 844–852.
558 Goudet, J. (2005) HIERFSTAT, a package for R to compute and test hierarchical F-
559 statistics. *Molecular Ecology Notes*, **5**, 184–186.
560 Graney, D.L. (1990) *Carya ovata* (Mill.) K. Koch. *Silvics of North America: 2. Hardwoods.*
561 *Agricultural Handbook 654* (ed. by R.M. Burns and B.H. Honkala), US Department
562 of Agriculture, Forest Service, Washington, DC.
563 Griffin, S.R. & Barrett, S.C.H. (2004) Post-glacial history of *Trillium grandiflorum*
564 (Melianthiaceae) in eastern North America: inferences from phylogeography.
565 *American Journal of Botany*, **91**, 465–473.
566 Grüger, E. (1972) Late quaternary vegetation development in south-central Illinois.
567 *Quaternary Research*, **2**, 217–220.
568 Hampe, A. & Petit, R.J. (2005) Conserving biodiversity under climate change: the rear
569 edge matters. *Ecology Letters*, **8**, 461–467.

570 Hamrick, J.L., Godt, J.W., & Sherman-Broyles, S.L. (1992) Factors influencing levels of
571 genetic diversity in woody plant species. *New Forests*, **6**, 95–124.
572 He, Q., Edwards, D.L., & Knowles, L.L. (2013) Integrative testing of how environments
573 from the past to the present shape genetic structure across landscapes. *Evolution*,
574 **67**, 3386–3402.
575 Hewitt, G. (1999) Post-glacial re-colonization of European biota. *Biological Journal of*
576 *the Linnean Society*, **68**, 87–112.
577 Hewitt, G. (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.
578 Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., & Jarvis, A. (2005) Very high
579 resolution interpolated climate surfaces for global land areas. *International Journal*
580 *of Climatology*, **25**, 1965–1978.
581 Hijmans, R.J., Phillips, S., Leathwick, J., & Elith, J. (2015) Package "dismo". R package.
582 http://cran.r-project.org/web/packages/dismo/index.html.
583 Jackson, S.T., Webb, R.S., Anderson, K.H., Overpeck, J.T., Webb III, T., Williams, J.W.,
584 & Hansen, B.C.S. (2000) Vegetation and environment in Eastern North America
585 during the Last Glacial Maximum. *Quaternary Science Reviews*, **19**, 489–508.
586 Jaramillo-Correa, J.P., Beaulieu, J., Khasa, D.P., & Bosquet, J. (2009) Inferring the past
587 from the present phylogeography structure of North American forest trees: seeing
588 the forest for the genes. *Canadian Journal of Forest Research*, **39**, 286–307.
589 Jombart, T. (2008) ADEGENET: a R package for the multivariate analysis of genetic
590 markers. *Bioinformatics*, **24**, 1403–1405.
591 Jombart, T. & Ahmed, I. (2011) ADEGENET 1.3-1: new tools for the analysis of genome-
592 wide SNP data. *Bioinformatics*, **27**, 3070–3071.

593 De la Cruz, O. & Raska, P. (2014) Population structure at different minor allele
594 frequency levels. *BMC proceedings*, **8**, S55.
595 Langmead, B., Trapnell, C., Pop, M., & Salzberg, S.L. (2009) Ultrafast and memory-
596 efficient alignment of short DNA sequences to the human genome. *Genome*
597 *Biology*, **10**, R25.
598 Little, E.L. (1971) *Atlas of United States trees, volume 1. Conifers and important*
599 *hardwoods.* USDA Miscellaneous Publication 1146, Washington, DC.
600 Lumibao, C.Y., Hoban, S.M., & McLachlan, J. (2017) Ice ages leave genetic diversity
601 "hotspots" in Europe but not in Eastern North America. *Ecology Letters*, in press.
602 Magni, C.R., Ducousso, A., Caron, H., Petit, R.J., & Kremer, A. (2005) Chloroplast DNA
603 variation of *Quercus rubra* L. in North America and comparison with other
604 Fagaceae. *Molecular Ecology*, **14**, 513–524.
605 Mantel, N. (1967) The detection of disease clustering and a generalized regression
606 approach. *Cancer Research*, **27**, 209–220.
607 Massatti, R. & Knowles, L.L. (2014) Microhabitat differences impact phylogeographic
608 concordance of codistributed species: genomic evidence in montane sedges
609 (*Carex* L.) from the Rocky Mountains. *Evolution*, **68**, 2833–2846.
610 McCarthy, D.M. & Mason-Gamer, R.J. (2016) Chloroplast DNA-based phylogeography
611 of *Tilia americana* (Malvaceae). *Systematic Botany*, **41**, 865–880.
612 McLachlan, J.S., Clark, J.S., & Manos, P.S. (2005) Molecular indicators of tree
613 migration capacity under rapid climate change. *Ecology*, **86**, 2088–2098.
614 Médail, F. & Diadema, K. (2009) Glacial refugia influence plant diversity patterns in the
615 Mediterranean Basin. *Journal of Biogeography*, **36**, 1333–1345.

616 Meirmans, P.G. (2012) The trouble with isolation by distance. *Molecular Ecology*, **21**,
617 2839–2846.
618 Morris, A.B., Graham, C.H., Soltis, D.E., & Soltis, P.S. (2010) Reassessment of
619 phylogeographical structure in an eastern North American tree using Monmonier's
620 algorithm and ecological niche modelling. *Journal of Biogeography*, **37**, 1657–1667.
621 Nadeau, S., Godbout, J., Lamothe, M., Gros-Louis, M.-C., Isabel, N., & Ritland, K.
622 (2015) Contrasting patterns of genetic diversity across the ranges of *Pinus*
623 *monticola* and *P. strobus*: a comparison between eastern and western North
624 American postglacial colonization histories. *American Journal of Botany*, **102**,
625 1342–1355.
626 Nazareno, A.G., Bemmels, J.B., Dick, C.W., & Lohmann, L.G. (2017) Minimum sample
627 sizes for population genomics: an empirical study from an Amazonian plant species.
628 *Molecular Ecology Resources*, in press.
629 Nei, M. (1987) *Molecular Evolutionary Genetics.* Columbia University Press, New York.
630 O'Connell, B.M., LaPoint, E.B., Turner, J.A., Ridley, T., Boyer, D., Wilson, A.M.,
631 Waddell, K.L., & Conkling, B.L. (2012) *The Forest Inventory and Analysis*
632 *Database: database description and users manual version 5.1.4 for Phase 2. Gen.*
633 *Tech. Rep.,* US Department of Agriculture, Forest Service, Washington, DC.
634 Paradis, E. (2010) Pegas: an R package for population genetics with an integrated-
635 modular approach. *Bioinformatics*, **26**, 419–420.
636 Peterson, B.J. & Graves, W.R. (2016) Chloroplast phylogeography of *Dirca palustris* L.
637 indicates populations near the glacial boundary at the Last Glacial Maximum in
638 eastern North America. *Journal of Biogeography*, **43**, 314–327.

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., & Hoekstra, H.E. (2012) Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE*, **7**, .

Petit, R.J., Aguinagalde, I., de Beaulieu, J.-L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Müller-Starck, G., Demesure-Musch, B., Palmé, A., Martín, J.P., Rendell, S., & Vendramin, G.G. (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. *Science*, **300**, 1563–1565.

Phillips, S.J., Anderson, R.P., Dudík, M., Schapire, R.E., & Blair, M.E. (2017) Opening the black box: an open-source release of MAXENT. *Ecography*, **40**, 887–893.

Phillips, S.J., Anderson, R.P., & Schapire, R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.

Phillips, S.J., Dudík, M., & Schapire, R.E. (2004) A maximum entropy approach to species distribution modeling. *Proceedings of the Twenty-First International Conference on Machine Learning*, **2004**, 655–662.

Prentice, C., Bartlein, P.J., & Webb III, T. (1991) Vegetation and climate change in eastern North America since the Last Glacial Maximum. *Ecology*, **72**, 2038–2056.

Provan, J. & Bennett, K.D. (2008) Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology and Evolution*, **23**, 564–571.

Qiu, Y.X., Fu, C.X., & Comes, H.P. (2011) Plant molecular phylogeography in China and adjacent regions: tracing the genetic imprints of Quaternary climate and environmental change in the world's most diverse temperate flora. *Molecular Phylogenetics and Evolution*, **59**, 225–244.

662 Raj, A., Stephens, M., & Pritchard, J.K. (2014) FASTSTRUCTURE: variational inference
663 of population structure in large SNP data sets. *Genetics*, **197**, 573–589.
664 Saeki, I., Dick, C.W., Barnes, B. V., & Murakami, N. (2011) Comparative
665 phylogeography of red maple (*Acer rubrum* L.) and silver maple (*Acer saccharinum*
666 L.): impacts of habitat specialization, hybridization and glacial history. *Journal of*
667 *Biogeography*, **38**, 992–1005.
668 Savolainen, O., Pyhäjärvi, T., & Knürr, T. (2007) Gene flow and local adaptation in trees.
669 *Annual Review of Ecology, Evolution, and Systematics*, **38**, 595–619.
670 Schmidtling, R.C. (2003) The southern pines during the Pleistocene. *Acta Horticulturae*,
671 **615**, 203–209.
672 Schmidtling, R.C. & Hipkins, V. (1998) Genetic diversity in longleaf pine (*Pinus*
673 *palustris*): influence of historical and prehistorical events. *Canadian Journal of*
674 *Forest Research*, **28**, 1135–1145.
675 Shafer, A.B.A., Cullingham, C.I., Côté, S.D., & Coltman, D.W. (2010) Of glaciers and
676 refugia: a decade of study sheds new light on the phylogeography of northwestern
677 North America. *Molecular Ecology*, **19**, 4589–4621.
678 Shaw, J. & Small, R.L. (2005) Chloroplast DNA phylogeny and phylogeography of the
679 North American plums (*Prunus* subgenus *Prunus* section *Prunocerasus*,
680 Rosaceae). **92**, 2011–2030.
681 Smith, H.C. (1990) *Carya cordiformis* (Wangenh.) K. Koch. *Silvics of North America: 2.*
682 *Hardwoods. Agricultural Handbook 654* (ed. by R.M. Burns and B.H. Honkala), US
683 Department of Agriculture, Forest Service, Washington, DC.
684 Soltis, D.E., Morris, A.B., McLachlan, J.S., Manos, P.S., & Soltis, P.S. (2006)

Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology*, **15**, 4261–4293.

Stewart, J.R. & Lister, A.M. (2001) Cryptic northern refugia and the origins of the modern biota. *Trends in Ecology and Evolution*, **16**, 608–613.

Thomson, A.M., Dick, C.W., & Dayanandan, S. (2015) A similar phylogeographical structure among sympatric North American birches (*Betula*) is better explained by introgression than by shared biogeographical history. *Journal of Biogeography*, **42**, 339–350.

Title, P.O. & Bemmels, J.B. (2017) ENVIREM: An expanded set of bioclimatic and topographic variables increases flexibility and improves performance of ecological niche modeling. *Ecography*, in press.

Tzedakis, P.C., Emerson, B.C., & Hewitt, G.M. (2013) Cryptic or mystic? Glacial tree refugia in northern Europe. *Trends in Ecology and Evolution*, **28**, 696–704.

Watterson, G.A. (1975) On the number of segregating sites in genetical models without recombination. *Theoretical Population Biology*, **7**, 256–276.

Williams, J.W. (2002) Variations in tree cover in North America since the last glacial maximum. *Global and Planetary Change*, **35**, 1–23.

Willing, E.M., Dreyer, C., & van Oosterhout, C. (2012) Estimates of genetic differentiation measured by F_{ST} do not necessarily require large sample sizes when using many SNP markers. *PLoS ONE*, **7**, 1–7.

Willis, K.J. & Van Andel, T.H. (2004) Trees or no trees? The environments of central and eastern Europe during the Last Glaciation. *Quaternary Science Reviews*, **23**, 2369–2387.

Zinck, J.W.R. & Rajora, O.P. (2016) Post-glacial phylogeography and evolution of a wide-ranging highly-exploited keystone forest tree, eastern white pine (*Pinus strobus*) in North America: single refugium, multiple routes. *BMC Evolutionary Biology*, **16**, 56.

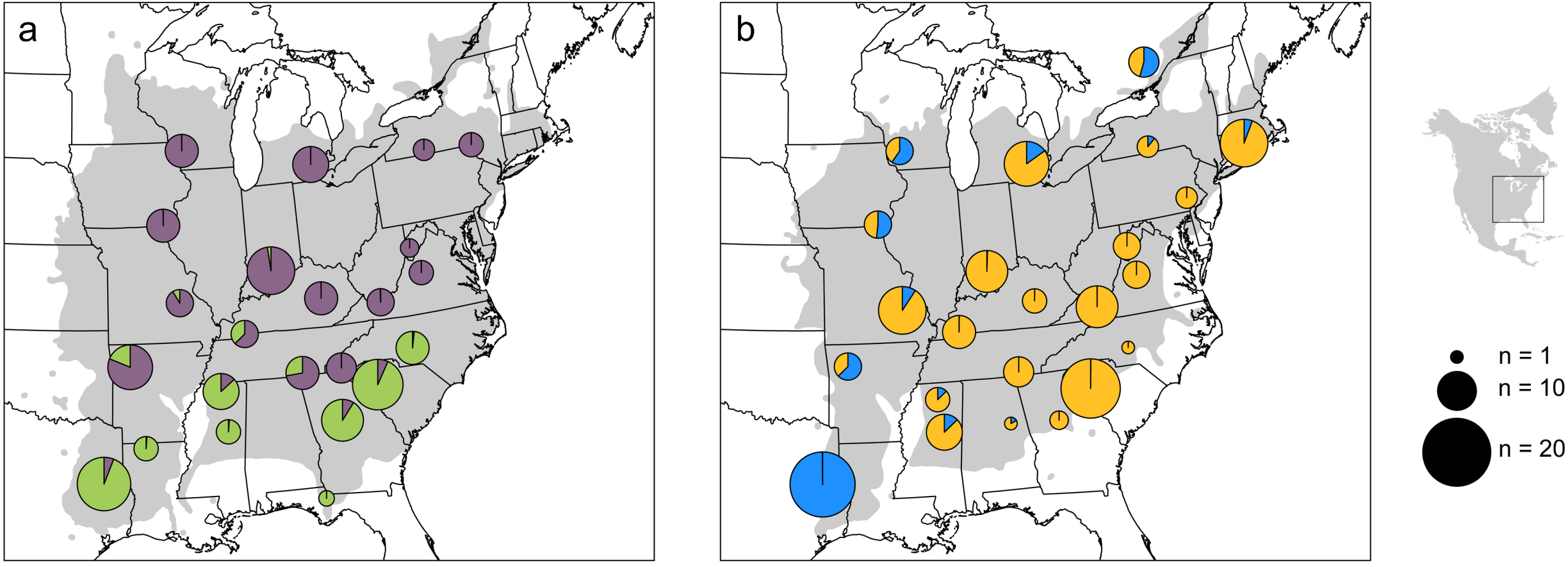
Biosketches

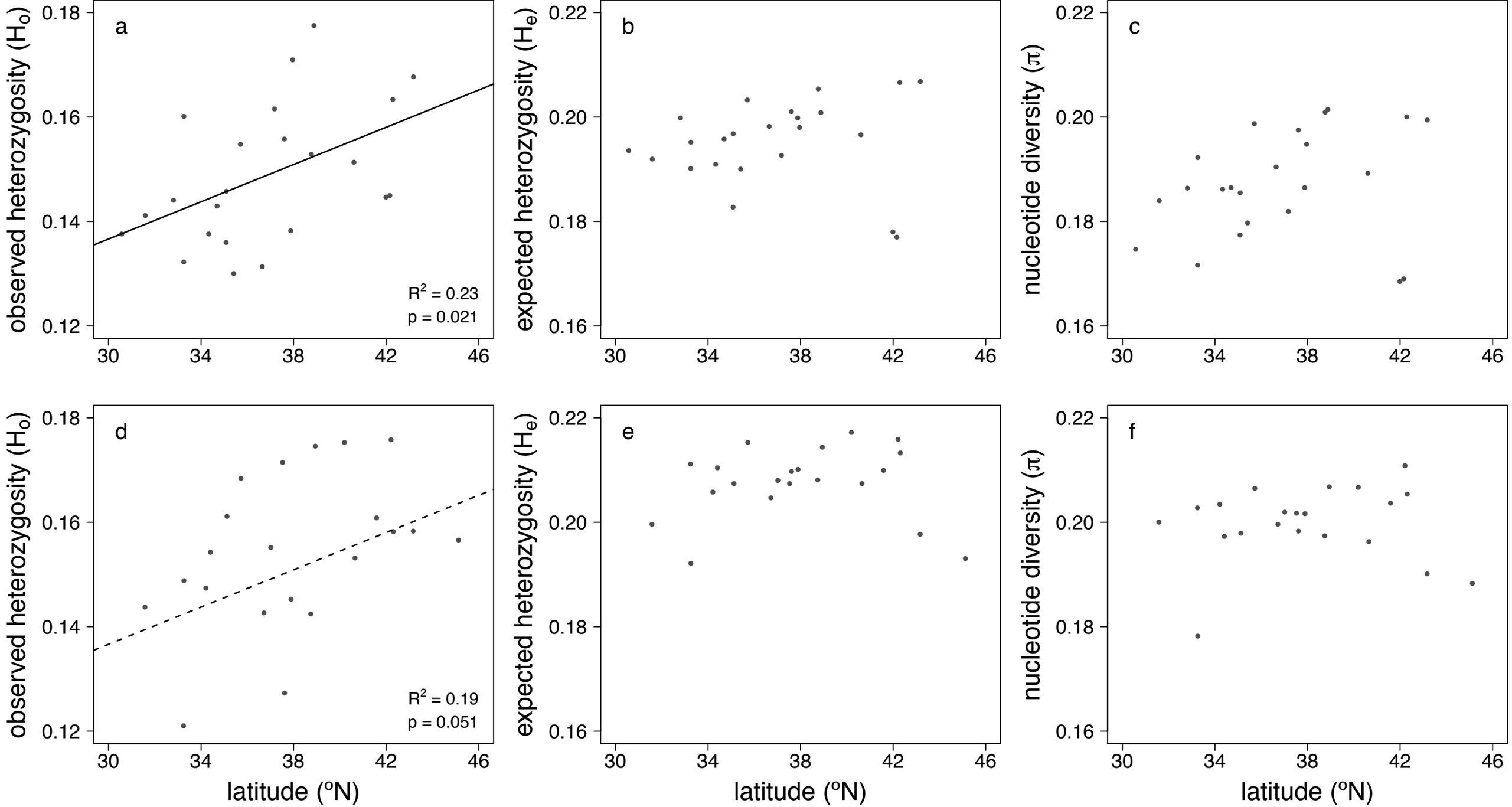
Jordan Bemmels is interested in biogeography of temperate and tropical trees and this work is part of his PhD thesis. Christopher Dick is his thesis advisor, and is broadly interested in tropical tree biogeography and evolution.

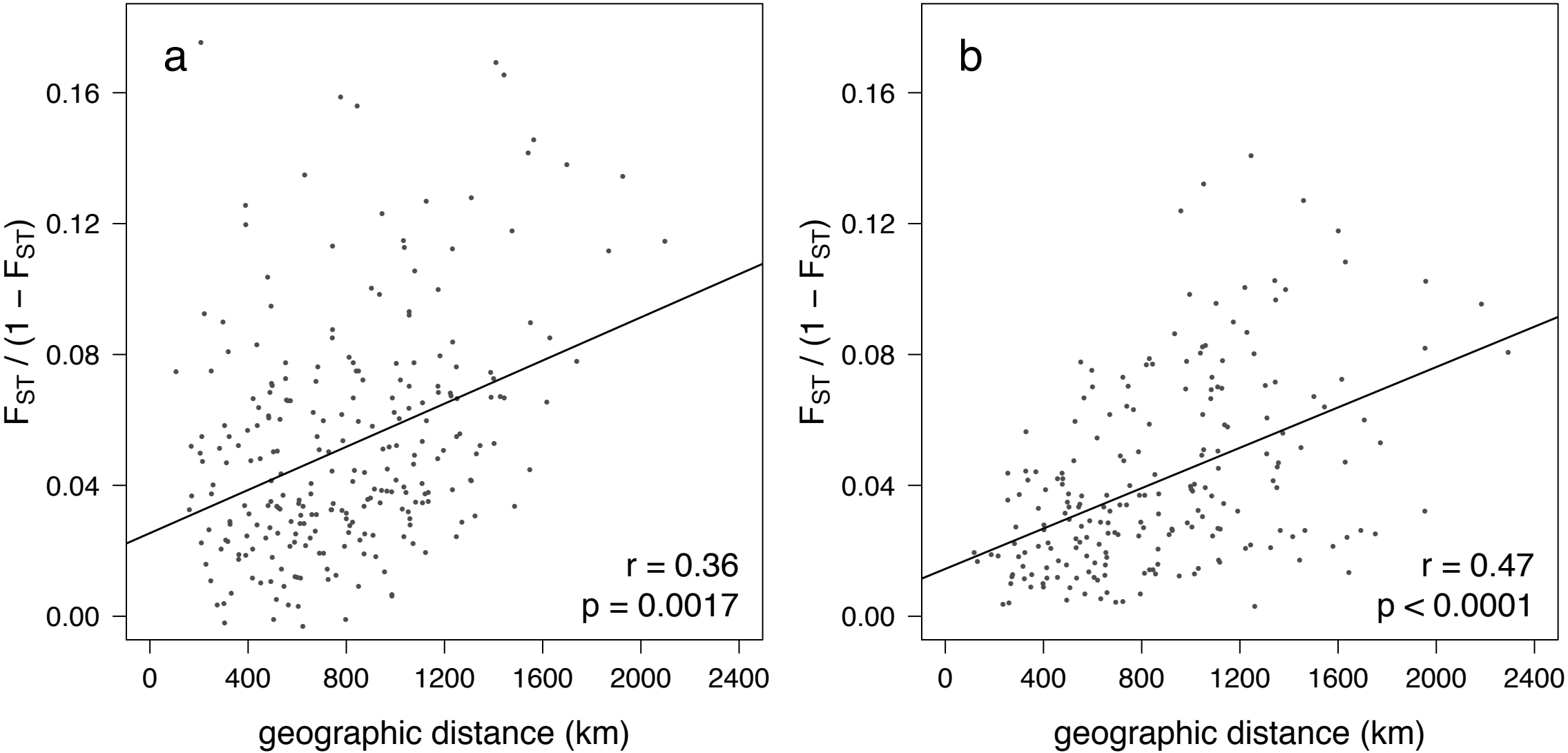
Author contributions: J.B.B. and C.W.D. conceived the project; J.B.B. performed fieldwork and labwork, and analyzed the data; J.B.B. wrote the manuscript with input from C.W.D.

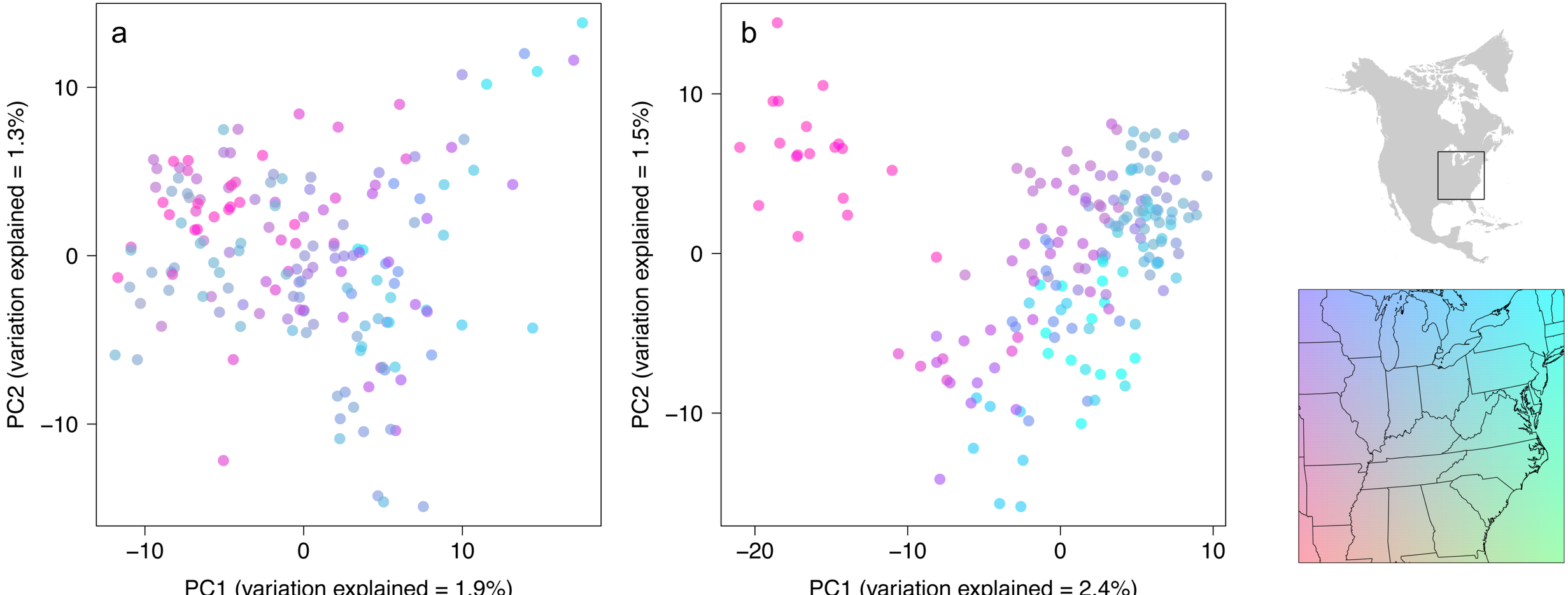
723 **Figure 1.** Membership of (a) *Carya cordiformis* and (b) *Carya ovata* populations in
724 genetic clusters (different colours on pie charts; *K* = 2) identified using FASTSTRUCTURE
725 (Raj et al., 2014). The geographic distribution of each species is shown in grey (Little,
726 1971), and the sample size (n) of each population is proportional to the size of the pie
727 chart. Note that optimal *K* = 1-2, but *K* = 1 is not shown as all individuals of each
728 species would belong to the same genetic cluster.
729
730 **Figure 2.** Genetic diversity vs. latitude for populations of *Carya cordiformis* (a-c) and
731 *Carya ovata* (d-f). Statistically significant relationships are portrayed as solid lines, and
732 marginally significant relationships as dashed lines.
733
734 **Figure 3.** Mantel test showing isolation by distance in (a) *Carya cordiformis* and (b)
735 *Carya ovata*. Each dot represents a pair of populations and the y-axis is a measure of
736 genetic differentiation between populations.
737
738 **Figure 4.** Clustering of individuals along the first and second principal component (PC)
739 axes of genetic variation in (a) *Carya cordiformis* and (b) *Carya ovata.* Each dot
740 represents a single individual, and colours correspond to the geographic location of the
741 individual (shown in the inset map of eastern North America). Note that the Texas
742 population (TX) of *C. ovata* discussed in the manuscript is represented by the pink dots
743 in the upper left corner of (b).
744

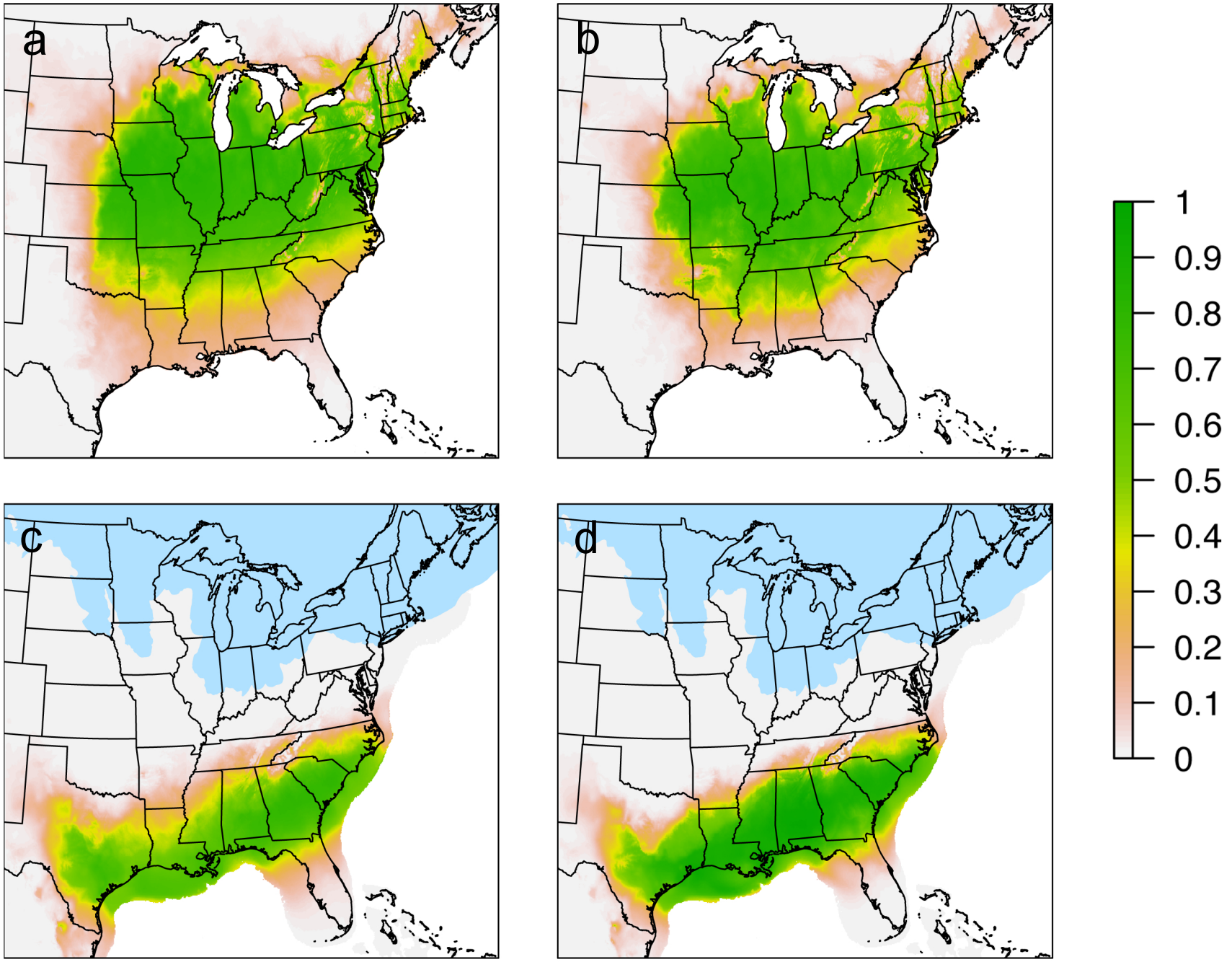
Figure 5. Species distribution models showing predicted habitat suitability (grey to green colour scale) for (a, c) *Carya cordiformis* and (b, d) *Carya ovata*, in both (a-b) the current time period and (c-d) the Last Glacial Maximum (ca. 21.5 ka). Glaciated areas are shown in blue.


a
b
n = 1
n = 10
n = 20


a
b
c
d
e
f
observed heterozygosity (H_o)
expected heterozygosity (H_e)
nucleotide diversity (π)
latitude (ºN)
R^2 = 0.23
p = 0.021
R^2 = 0.19
p = 0.051
0.12
0.14
0.16
0.18
0.20
0.22
30
34
38
42
46


a
b
0.16
0.12
0.08
0.04
0.00
$F_{ST} / (1 - F_{ST})$
0
400
800
1200
1600
2000
2400
geographic distance (km)
r = 0.36
p = 0.0017
r = 0.47
p < 0.0001


a
b
PC2 (variation explained = 1.3%)
PC1 (variation explained = 1.9%)
PC2 (variation explained = 1.5%)
PC1 (variation explained = 2.4%)
10
0
−10
−20


a
b
c
d
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0