

1 Spatial, climate, and ploidy factors drive genomic diversity and resilience in the
2 widespread grass *Themeda triandra*

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24 **Summary**

25 •Fragmented grassland ecosystems, and the species that shape them, are under immense pressure.
26 Restoration and management strategies should include genetic diversity and adaptive capacity to
27 improve success but these data are generally unavailable. Therefore, we use the foundational grass,
28 *Themeda triandra*, to test how spatial, environmental, and ploidy factors shape patterns of genetic
29 variation.

30

31 •We used reduced-representation genome sequencing on 487 samples from 52 locations to answer
32 fundamental questions about how the distribution of genomic diversity and ploidy polymorphism
33 supports adaptation to harsher climates. We explicitly quantified isolation-by-distance (IBD),
34 isolation-by-environment (IBE), and predicted population genomic vulnerability in 2070.

35

36 •We found that a majority (54%) of the genomic variation could be attributed to IBD, while 22% of
37 the genomic variation could be explained by four climate variables showing IBE. Results indicate
38 that heterogeneous patterns of vulnerability across populations are due to genetic variation, multiple
39 climate factors, and ploidy polymorphism, which lessened genomic vulnerability in the most
40 susceptible populations.

41

42 •These results indicate that restoration and management of *T. triandra* should incorporate knowledge
43 of genomic diversity and ploidy polymorphisms to increase the likelihood of population persistence
44 and restoration success in areas that will become hotter and more arid.

45

46 Key words

47 adaptation; genomic diversity; genomic vulnerability; landscape genomics; polyploidy; restoration;
48 *Themeda triandra* (kangaroo grass)

49

50

51 Introduction

52 Grasses (Poaceae) are one of the most ecologically important vascular plant groups, making up 25%
53 of the world's vegetation (Shantz, 1954). They provide key ecosystem services that underpin
54 environmental health (i.e. habitat and food sources for native wildlife, nutrient cycling and carbon
55 sequestration), and carry significant economic value as they include four of the five major crops in
56 terms of global production (Raven & Thomas, 2010). Grasses are essential constituents of several
57 vegetation communities including grasslands, grassy woodlands, and alpine regions. However,
58 grasslands and grassy woodlands have historically been under immense pressure from rangeland and
59 agricultural uses (Eldridge *et al.*, 2016; Hopkins & Holz 2006), leading to the fragmentation of natural
60 populations and reductions in genetic diversity (Harrison *et al.*, 2015). Today, only about 4.6% of the
61 billions of hectares of grassland ecosystems remain worldwide (IUCN 2016). In Australia, grassland
62 systems are the most poorly conserved and degraded communities (Hobbs & Yates, 2000), and are
63 likely to experience major negative long-term effects. Many regions of Australia that support
64 grasslands are becoming warmer, drier and increasingly fire prone under climate change, highlighting
65 the importance of preserving genetic diversity and evolutionary potential (Dunlop *et al.*, 2012).
66 However, most research on genetic diversity in grass species has generally been undertaken on those
67 of agricultural importance (Buckler *et al.*, 2001) such as wheat, corn, rice, and sorghum, or those that
68 are being developed for biofuels such as switchgrass (*Panicum* – Casler *et al.*, 2007; Harrison *et al.*,
69 2015) and sugarcane (*Miscanthus* – Vermerris, 2008). While research on species such as switchgrass
70 have provided valuable insights into natural patterns of genetic diversity, adaptation across gradients,
71 and the role ploidy plays between these lines of enquiry (Morris *et al.*, 2011; Lowry *et al.*, 2014,
72 2019; Grabowski *et al.*, 2014), major gaps in knowledge for other ecologically important grasses
73 persist and continue to inhibit effective conservation management.

74

75 Genetic diversity is maintained within a species by a combination of selective (such as range shifts
76 and natural selection) and neutral processes (such as gene flow, mutation, and genetic drift) (Futuyma,
77 2013). However, grasses often have complex evolutionary histories (Stebbins, 1956) influenced by
78 factors such as clonality (Fischer & Van Kleunen, 2002), polyploidy (Keeler & Bradshaw, 1998),
79 intrageneric hybridization, genome size, and different physiologies such as photosynthetic
80 mechanisms (e.g. C3 versus C4) (Edwards *et al.*, 2010). These complex and often lineage-specific life
81 histories can complicate our ability to project findings across species, meaning that the species-
82 specific data needed for practitioners to make informed management decisions is often lacking.
83 Perhaps the lack of research on ecologically important grass species and their complex life histories
84 are not mutually exclusive. Regardless, information about how genetic diversity is distributed across
85 habitats and environmental gradients, often reflecting selection and local adaptation, can help inform
86 management and restoration strategies (Hoffmann *et al.*, 2015). This is particularly pertinent given
87 grassland communities are already showing signs of climate stress, and empirical data is urgently

88 needed to support adaptive management strategies that prepare grasslands for new climate challenges
89 by maximising evolutionary potential. In addition, research that focuses on genetic diversity across
90 species ranges can help identify populations vulnerable to climate stress, allowing practitioners to
91 prioritise management that safeguards populations at risk. For example, genomic signals of selection
92 can be used to predict climate-driven population declines (Bay *et al.*, 2018). Specifically, ‘genomic
93 vulnerability’ of individual populations, defined as the mismatch between current and predicted future
94 genomic variation inferring population susceptibility to the loss of genetic diversity and/or
95 maladaptation, can help identify populations most at risk. As our ability to integrate geospatial and
96 genomic resources continues to grow, so will the ability of researchers to identify genomic
97 vulnerability in ecologically important species, providing practitioners with improved management
98 frameworks for mitigating climate change effects on ecosystems by preserving patterns of endemism
99 and maximising adaptive potential.

100

101 Grasses often display ploidy differences among populations across their natural range. Indeed,
102 polyploidy is common among vascular plants with c. 35% of species characterised as having a recent
103 history of polyploidy (Wood *et al.*, 2009). For many species, associations between ploidy and local
104 environmental conditions reflect adaptation, a pattern which has been studied extensively in crop
105 plants (Alix *et al.*, 2017). Further, it has recently been shown that niche differentiation occurs faster in
106 polyploids than diploid relatives (Baniaga *et al.*, 2019). While the causes of polyploidy are poorly
107 understood (Soltis *et al.*, 2010), whole genome duplication events have been shown to coincide with
108 historical climate change events (Cai *et al.*, 2019), and patterns of allopolyploidy have been linked to
109 changes in environment (Wagner *et al.*, 2019). The effects of polyploidy are increasingly evident,
110 with gene expression levels shown to vary from tissue to tissue in polyploids compared to their
111 diploid counterparts (Adams *et al.*, 2003), and polyploid species often having significant fitness
112 advantages (Petit & Thompson, 1997; Bretagnolle & Thompson, 2001; Ramsey, 2011; Hahn *et al.*,
113 2012; Hoffmann *et al.*, 2015; Wei *et al.*, 2019). Genome duplication may in itself be an advantage
114 because it buffers the organism against deleterious alleles (Voigt-Zielinski *et al.*, 2012; Wagner *et al.*,
115 2019), and higher rates of heterozygosity reduce risks associated with inbreeding effects (Ronfort,
116 1999). Despite the potential benefits of polyploidy, there are known disadvantages, including the
117 potential dilution of beneficial mutations (Stebbins, 1971) and disturbance of cellular functions such
118 as epigenetic regulation, mitosis, and meiosis (Comai, 2005). However, ploidy polymorphism may
119 provide an important evolutionary pathway for species to establish in previously unsuitable habitats or
120 adapt *in situ* (Grabowski *et al.*, 2014).

121

122 Understanding patterns of genetic diversity and evolutionary mechanisms for adapting to new
123 environments is key to improving the conservation of intact grasslands and the restoration of degraded
124 grassland habitats. Globally, restoration practices largely advocate the use of seed sourced from local
125 provenances, based on the assumption that local genotypes are best matched to stable local

126 environments and to avoid perceived risks associated with outbreeding (Thornhill, 1993; Edmands,
127 2006). Yet, in many cases local provenancing can lead to poor restoration outcomes (Broadhurst *et*
128 *al.*, 2008; Prober *et al.*, 2015). In highly modified landscapes the genetic integrity of many species has
129 been compromised, and local-provenancing can favour the selection of genetically depauperate and
130 maladapted seed (Jones, 2013). Also, local-provenancing gives little consideration to the persistence
131 of plantings under future climates, with growing evidence that genotypes from non-local sources may
132 outperform those sourced locally (Hoffmann *et al.*, 2015; Prober *et al.*, 2015; Breed *et al.*, 2019). In
133 addition, foundation species are especially important during the restoration process because their
134 genetic variation can shape the networks of ecological interaction influencing community assembly,
135 stability, and evolution (Gibson *et al.*, 2012; Lau *et al.*, 2016). Empirically derived restoration
136 strategies are now being widely adopted around the world to support biodiversity, evolutionary
137 potential, and restoration success, and similar approaches should also be employed for ploidy
138 polymorphism.

139

140 In this study, we assess patterns of genetic structure, genotype-ploidy-environment associations, and
141 genomic vulnerability in a foundational grassland species. *Themeda triandra*, commonly known as
142 Kangaroo Grass, has a continent wide distribution, is characterised by ploidy polymorphisms
143 (Hayman 1960) and has limited seed dispersal (Everson *et al.* 2009). The species provides critical
144 ecosystem services supporting grassland habitats throughout Australia, and is widely used in
145 grassland restorations, but is suffering major declines, shows signs of climate stress, and is in need of
146 improved restoration guidelines. Notably, several studies suggest that re-establishment of *T. triandra*
147 is an important first step for the restoration of Australia's grasslands (Adair & McDougall 1987;
148 McDonald 2000; Cole & Lunt, 2005), highlighting the importance of research geared toward
149 assessing the resilience of remnant populations, and management approaches that incorporate
150 evolutionary potential. In this context, we assess the likely drivers of genetic structure across a portion
151 of *T. triandra*'s range, predicting both isolation-by-distance (IBD) and isolation-by-environment
152 (IBE) to be key drivers due to the species' limited seed dispersal and broad climatic niche. Based on
153 estimates of gene flow and correlative measures of local adaptation, we test for genomic mismatches
154 between local gene pools and future climates to help identify populations likely to be most vulnerable
155 to new climatic challenges. Lastly, we test for associations between polyploidy and harsh climate
156 zones, to gain insights into the role of polyploidy in historical and future adaptive processes. These
157 results will provide clear pathways on how to incorporate genomic, environmental, and ploidy
158 information into improved guidance for adaptive management plans that aim to protect these
159 dwindling grassland ecosystems.

160

161 **Materials and Methods**

162 Species and sampling

163 *Themeda triandra* is a perennial C4 tussock grass, with ploidy variability, and occurs across three
164 continents (Australia, Asia, and Africa) (Dell'Acqua *et al.*, 2013; Snyman *et al.*, 2013; Linder *et al.*,
165 2018). It is Australia's most widespread species, being adapted to habitats as diverse as the semi-arid
166 interior and sub-alpine regions (Mitchell & Miller, 1990). In Australia, diploids and tetraploids are the
167 most common ploidy variants, but triploid, pentaploid, hexaploid and aneuploid individuals have also
168 been identified (Hayman, 1960). Past studies suggest that *T. triandra* originally evolved in tropical
169 Asia and migrated through coastal corridors to Australia (Hayman, 1960), with Australian lineages
170 diverging 1.37 mya (0.79 - 3.07 mya) (Dunning *et al.*, 2017). However, dating using secondary
171 calibrations, as in (Dunning *et al.*, 2017) can lead to unreliable and overly young estimates of
172 divergence (Schenk, 2016). *Themeda triandra* is widely considered a foundation species for three
173 reasons: 1) it defines particular ecosystems (Snyman *et al.*, 2013), 2) it controls the distribution and
174 abundance of associated flora and fauna (Morgan, 1998), and 3) it regulates the core ecosystem
175 processes especially through fire (Morgan & Lunt, 1999). The species is also considered to be an
176 indicator of (agro)ecosystem health (Novellie & Kraaij, 2010) and its long-term persistence provides
177 ecosystem stability, ecosystem services, resistance to plant invasions, and facilitates rehabilitation of
178 polluted and degraded habitat (Novellie & Kraaij, 2010; Dell'Acqua *et al.*, 2013). Furthermore, its
179 persistence is critical for the restoration of grasslands in Australia and is reliant on recurring fire to
180 remove old tillers and for seedling establishment (McDougall 1989). The distribution of *T. triandra* is
181 suggestive of a complex evolutionary history with high levels of genetic structuring throughout
182 Australia. Although *T. triandra* itself is not formally listed as an endangered species, it is an important
183 constituent of temperate grassland communities, which have been declared as endangered in the
184 Australian Capital Territory and New South Wales, and threatened in Victoria. The grasslands are
185 under threat due to loss and fragmentation of habitats through inadequate land management practices.
186

187 Samples were collected between 2015 and 2017 from 52 populations spanning the heterogeneous
188 climate from its eastern Australian distribution, which deliberately coincides with the densest portion
189 of its distribution. Sampling was structured to ensure different environment combinations were
190 sampled between coastal and inland (west of the Great Dividing Range, see Fig S1) sites. Sites were
191 identified using records on the Atlas of Living Australia public database (ala.org.au) and chosen using
192 the following criteria: herbaria collection or observation was after the year 2000, location data was
193 within 50 m of accuracy, and occurred on land that was publicly accessible. Between 10 and 21 leaf
194 samples were collected per location and placed directly into silica gel to rapidly desiccate leaf samples
195 for DNA preservation. Sampled plants were at least 5 m apart to ensure independence of genotypes by
196 minimising the chance of collecting clonal samples. Our collections comprised a total of 584
197 individual specimens, which were stored under laboratory conditions until required for genetic
198 analysis.
199

200 Using the work of Hayman (1960), we created a predictive map of ploidy levels for populations
201 distributed across our sampling distribution. Hayman measured ploidy levels across Australia, with
202 most of his sites overlapping our sampling distribution. We interpolated his data using nearest
203 neighbor analysis using QGIS v2.14 (Quantum GIS Development team), allowing us to extract
204 predicted ploidy level for each population location to provide us with the number of predicted
205 chromosomes (i.e. diploid = 20; tetraploid = 40; hexaploid = 60). A few individuals were equidistant
206 between two predicted ploidy levels and were assigned ploidy level between 20 and 40. This was
207 interpreted as indicating a mixed ploidy population. Ploidy predictions were verified with population-
208 level heterozygosity, see below for details.

209

210 DNA extraction and library preparation

211 For reduced-representation library preparation and sequencing, genomic DNA from each individual
212 was isolated from approximately 25 mg of silica-dried leaf tissue using the Stratec Invisorb DNA
213 Plant HTS 96 kit (Invitex, Berlin, Germany). Libraries were created similarly to Ahrens et al. (2017).
214 Briefly, extracted DNA was digested with PstI for genome complexity reduction, and ligated with a
215 uniquely barcoded sequencing adapter pair. We then amplified each sample individually by PCR to
216 avoid sample bias. We pooled samples in equimolar ratios and selected amplicons between 350 and
217 600 bp from an agarose gel. The library pool was sequenced on three Illumina NextSeq400 lanes
218 using a 75bp paired-end protocol on a high output flowcell at the Biomolecular Resources Facility at
219 the Australian National University, generating ~864 million read pairs.

220

221 For long-reads via the MinION sequencer (Oxford Nanopore Technologies, UK), we used the open
222 access high molecular weight DNA extraction protocol developed by Jones & Borevitz (2019).
223 Briefly, 30 g of fresh leaf material from a known diploid individual was processed with 150 mL
224 nuclei isolation buffer using a high-powered blender. The homogenate was filtered repeatedly using a
225 funnel, through sequentially 2, 4 and 8 layers of Miracloth. Next, 100% Triton X-100 was added for
226 nuclei isolation and the mixture centrifuged to create a pellet of nuclei. The pellet was washed twice
227 with a pre-chilled nuclei buffer. DNA extraction from the nuclei was initiated by adding fresh lysis
228 buffer with 3% Sodium dodecyl sulfate (SDS) at 50°C. Binding buffer was added to use Sera-Mag
229 beads to remove the lysis buffer from the DNA solution, washing with 70% ethanol 3 times until the
230 beads were clean. The beads were removed by adding 220 uL of ultra-pure H₂O and resuspending the
231 beads with attached DNA. The supernatant was removed and subsequently size selected for fragments
232 longer than 30 kb using a PippinHT (Sage Science, Beverly MA). MinION library preparation and
233 sequencing was performed as per the manufacturer's instructions and specifications, and resulted in
234 412,906 reads (Fig S2). Median read length was 27,156 bases, and the longest read length was
235 144,466 bases, with an overall average read-quality of 10 (Fig S2).

236

237 SNP calling

238 We checked the quality of the raw short-read sequencing reads with FastQC (v0.10.1, [Andrews,
239 2012]). Then, we demultiplexed the raw reads associated with each sample's unique combinatorial
240 barcode using AXE v0.2.6 (Murray & Borevitz, 2018). During this step we were unable to assign
241 19% of the reads. We trimmed each sequence to 64 basepairs while removing the barcodes and
242 ensured quality of the reads using trimmomatic v 0.38 (Bolger *et al.*, 2014). Quality was assessed
243 using a sliding window of 4 basepairs (the number of bases used to average quality) and a quality
244 score of 15 (the average quality required among the sliding window), and if the average quality
245 dropped below 15, the sequences were cut. Then we indexed the long-reads (Fig S2 for distribution of
246 length and number of reads sequenced) using the BWA software and the *index* argument. We aligned
247 the short-reads to the long-reads for more accurate SNP calling compared to a *de novo* pipeline. Short-
248 reads were aligned using BWA-mem (v0.7.17-r1198, [Li *et al.*, 2013]), as paired reads, with 82.5% of
249 reads successfully mapped. Samtools v 1.9 (Li *et al.*, 2009) was used to transform the SAM files to
250 BAM files for use within STACKS v 2.41 (Catchen *et al.*, 2013). The argument *gstacks* and
251 *populations* were used in that order on the BAM files to create a VCF file, minimum thresholds
252 (minor allele frequency = 0.01; one random SNP per read was retained) were set here for further
253 cleaning in R (R core development team 2019). The mean coverage per sample was 15.8× with a
254 standard deviation of 20×, this resulted in many samples being dropped (see below for details). Lastly,
255 VCFtools v 0.1.16 (Danecek *et al.*, 2011) was used to create a 012 file for further cleaning of the snp
256 matrix in R.

257

258 The missing data threshold was set to 50% per locus and individual which resulted in an average of
259 30% missing data from the whole SNP dataframe. Minor allele frequency was set to 0.05 to avoid
260 identifying patterns of population structure that may be due to locally shared alleles (De la Cruz &
261 Raska, 2014). Then we removed SNPs in high linkage disequilibrium (>50% similar). We also
262 removed possible clones in Genodive v 2.0b27 (Meirmans & Van Tienderen, 2004) using the *assign*
263 *clones* function, removing nine individuals. After conservative SNP filtering, we were left with 487
264 individuals from 52 populations.

265

266 Analysis

267 Genodive was used to estimate population summary statistics for the total number of alleles observed
268 across loci, total heterozygosity, and the inbreeding coefficient (G_{IS} ; Nei, 1987). We expected that the
269 degree of heterozygosity within populations would reflect ploidy status (i.e. higher heterozygosity
270 would imply polyploids) as described by Soltis & Soltis (2000). Consequently, we validated predicted
271 ploidy level among populations from Hayman's map (see above for details) by comparing those
272 predictions to population-level heterozygosity. G_{IS} is the same as F_{IS} for a single locus with two
273 alleles (Chakraborty & Leimar 1987), and is calculated by the ratio of observed heterozygosity within
274 subpopulations to the expected heterozygosity and ranges from -1 (complete outbreeding) to 1
275 (complete inbreeding). Genodive was also used for an analysis of molecular variance (AMOVA)

276 using the Excoffier method (Excoffier et al. 1995). Global F_{ST} with 95% confidence intervals was
277 calculated using the *fstat* argument and the population pairwise F_{ST} was calculated using the
278 *pairwise.fst* argument in the *hierfstat* package in R (Goudet, 2005).

279

280 *Themeda triandra* has a broad geographic distribution spanning a variety of environmental gradients,
281 therefore we wanted to estimate the amount of genetic variation that could be attributed to isolation-
282 by-distance (IBD) and -environment (IBE). First, we downloaded the 19 bioclim variables from
283 worldclim.org (Fick & Hijmans, 2017), and extracted all of the climate variables for each of the
284 sample locations in R using the package *raster* (Hijmans & van Etten 2012). A Principle Components
285 Analysis (PCA) was performed to determine potential correlations between the 19 climate variables
286 and produce an environmental dataset consisting of least correlated variables (Fig S3). We chose to
287 retain variables from six of the loose clusters (temperature mean diurnal range (T_{RANGE}), maximum
288 temperature of the warmest month (T_{MAX}), precipitation seasonality (P_{SEAS}), mean annual temperature
289 (T_{MA}), mean annual precipitation (P_{MA}), and precipitation of the driest month (P_{DM})).

290

291 We used sNMF (Frichot *et al.*, 2014) in the LEA package in R (Frichot & François, 2015) to
292 investigate the observed patterns of population structure that include contributions from both
293 geography (IBD) and environment (IBE). sNMF estimates ancestry coefficients based on sparse non-
294 negative matrix factorisation and least-squares optimisation. The sparse non-negative matrix
295 factorisation is robust to departures from traditional population genetic model assumptions, making
296 this algorithm ideal to use with polyploid species such as *T. triandra*. We performed sNMF with the
297 following attributes: $k = 1-10$, 10 replications per k -value (number of ancestral clusters), and 1,000
298 iterations. Entropy scores for each k -value were compared to choose the optimal number of clusters
299 using the recommendations in the sNMF instruction manual. A consensus for the optimal k -value was
300 created by averaging the results over the 10 replicate runs using CLUMPP v1.1.2 (Jakobsson &
301 Rosenberg, 2007) and drawn using DISTRUCT v1.1 (Rosenberg, 2003).

302

303 We used Moran's Eigenvector Maps (MEM) to test if IBD was a major determinant of the species'
304 genetic diversity, as described in previous work (Dray *et al.*, 2006; Legendre & Legendre, 2012) but
305 called PCNM in the first papers. Briefly, MEM calculates a matrix of pairwise Euclidean distances **D**
306 among the sampling sites, then transform the **D** matrix into a similarity matrix to produce the MEM.
307 Eigenvalues are produced corresponding to orthogonal vectors of similarity. To ascertain spatial
308 patterns of genetic diversity we used the R package memgene (Galpern *et al.*, 2014). Memgene
309 identifies spatial neighbourhoods in genetic distance data that adopts a regression framework where
310 the predictors are generated using MEMs, this multivariate technique was developed for spatial
311 ecological analyses but is recommended for genetic applications. Memgene identifies variables
312 (eigenvalues) that represent significant spatial genetic patterns at multiple spatial scales. Each variable

313 explains a proportion of the total variance explained by spatial patterns. For this study, we show two
314 variables because it explains most of the variation described by IBD.

315

316 Using the environmental data layers we employ a generalized dissimilarity model (GDM) to identify
317 the importance of specific climate variables responsible for shaping observed patterns of genetic
318 structure within our dataset. Analyses were performed using the *gdm* package v 1.3.7 in R (Manion *et*
319 *al.*, 2018) and a pairwise F_{ST} matrix (based on all SNP loci) to estimate allelic turnover through
320 climatic space (deviations in allele frequency associated with environment type). Where GDM holds
321 all variables in the model constant to identify the partial genomic distance associated with the climate
322 factor (Ferrier *et al.*, 2007), whereby accounting for spatial patterns caused by demographic processes
323 (Fitzpatrick & Keller, 2015). After running the GDM analysis, only four of the climates remained
324 (T_{MAX} , P_{SEAS} , T_{MA} , and P_{MA}), as the other two climate factors were removed by a
325 backward elimination procedure. The GDM output includes the deviance explained by the climate
326 and spatial variables, and a spline plot for each climate and spatial variable. Spline plots were
327 predicted across the study area and beyond for every 2.5km grid cell. These predicted grids were
328 mapped using *ggplot* in R (Wickham, 2011) to describe the relative IBE.

329

330 We calculated ‘genomic vulnerability’ for the sampling area following Bay *et al.* (2018), which
331 consists of three main components: exposure, sensitivity, and adaptive capacity (Dawson *et al.*, 2011).
332 Genomic vulnerability is the amount of genomic change required to track environmental change over
333 time and is interpreted as expected population decline. To do this, we substituted predictive maps in
334 2070 using the CCSM4 model with the representative concentration pathway 8.5 (worldclim.org),
335 which is a prediction based on the anthropogenic carbon dioxide output not deviating from its current
336 trajectory. These maps were also downloaded from *worldclim* and developed in the same way as
337 described above. Lastly, we subtracted the projected genomic differentiation from the current
338 genomic differentiation to get a difference between the two. We estimate genomic vulnerability twice,
339 with and without predicted ploidy levels to understand how ploidy may affect population decline,
340 particularly in the most vulnerable areas.

341

342 Results

343 We estimated patterns of population structure among 487 samples from 52 sample locations for *T.*
344 *triandra* using a dataset consisting of 3,443 polymorphic SNPs with a minor allele frequency (MAF)
345 of 0.05 and an average of 30% missing data. AMOVA indicated that a significant proportion of the
346 genetic variance (10%) could be attributed to difference among sample sites ($P = 0.001$; $F_{ST} = 0.22$),
347 while the majority of the variance (79.3%) was attributed to differences between individuals ($P <$
348 0.01 ; $F_{IT} = 0.31$). Large and significant positive inbreeding coefficients (G_{IS}) were observed for many
349 sites, indicating an excess of homozygotes, while three populations had negative inbreeding
350 coefficients indicating homozygote deficits (Table 1). Levels of genetic diversity (number of alleles

351 and heterozygosity) was variable among populations, with a mean number of alleles of 1.082 (95%
352 CI 1.078-1.086; range 1.109 - 1.366) and a mean heterozygosity within populations (H_S) of 0.074
353 (range 0.06 - 0.12; Table 1). Heterozygosity estimates reflect patterns that are consistent with the
354 hypothesis that greater ploidy levels are present in the hotter regions of our sampling distribution (Fig
355 1). However, this linear model, although significant ($r^2 = 0.086$; $P = 0.035$), explains only a small
356 proportion of the variation. This pattern is likely driven by the three populations in the hottest region.
357 Heterozygosity and predicted chromosome number were in agreement for these three populations, the
358 populations with the highest T_{MAX} (QLD, PR, SWC). Some populations with high heterozygosity
359 were predicted to be diploids (UL, GOR, NAM), but these populations were nearly equidistant to
360 tetraploid and diploid populations and are likely tetraploid populations (Fig 1).

361

362 General patterns of population structure show a clear delineation between southern and northern
363 populations (Fig 2) with an optimal k -value of 3 (Fig S4). The third k -value is found in two
364 populations, and partially assigned in two other populations. These populations containing the third
365 ancestral cluster were generally found in the central area of the sampling region. Notably, there are
366 portions of populations, particularly in the south central portion of the sampling region, that have been
367 assigned to the northern ancestral cluster. While there are a few individuals in the north assigned to
368 the southern ancestral cluster.

369

370 Isolation-by-distance (IBD) was found to be significant in *T. triandra*. In fact, IBD accounts for 54%
371 of the total genomic variation (Fig 3). Two axes are shown in separate figures, and together they
372 explained 95% of the variation explained by IBD alone. The first axis shows a strong split between
373 the northern and southern sections of the sampling area (Fig 3a), similar to the population structure
374 identified in the sNMF results. A second pattern of IBD occurs in the northern part of the sampling
375 region and is between the inland and coastal populations, while the most westerly population is
376 slightly more similar to the northern sampling region (Fig 3b).

377

378 In addition to spatially driven genomic variation, isolation-by-environment (IBE) explains a
379 significant amount of variation. While we chose six independent climate variables to explore IBE,
380 only four were found to be significant (T_{MA} , T_{MAX} , P_{MA} , P_{SEAS} ; maps for climate variables in Fig S5).
381 The GDM analysis was able to identify that 31.3% of the variation was attributable to these climate
382 and spatial variables (Fig 4), and 22.0% of the variation was attributable directly to climate. When
383 performing the same analysis with the inclusion of ploidy level, the variation explained rose by only
384 0.4%, but under this model, the T_{MAX} variable explained less variation (red lines in Fig 4) while all
385 other variables remained similar. In the current climate, the differences between the two models were
386 negligible (Fig 5a & c). However, when forecasting the differences in 2070, the outputs suggest a
387 heterogeneous population decline by 0 and 25% (Fig 5b) with the highest proportion of change
388 occurring inland of the eastern coast. Critically, the inclusion of ploidy polymorphism showed

389 genomic vulnerability dropping by 5% in the most vulnerable areas (Fig 5b & d; ploidy map provided
390 in Fig S5), in this output, we find that genomic vulnerability occurs where the land transitions from
391 the alpine region to the inland region. The lowest probability of change (population decline or gene
392 pool turnover) is in the mountainous ecosystems in the southeastern portion of the sampling region.
393

394 Discussion

395 Our study indicates contemporary structuring of genomic diversity in *Themeda triandra* is being
396 driven largely by a combination of spatial and climate factors. These patterns are indicative of a
397 species with limited propagule dispersal and restricted gene flow. The apparent lack of connectivity
398 among remnant populations suggests gene flow is unlikely to help local populations adapt to future
399 climate challenges. Instead, their adaptive potential will rely on trait plasticity and standing genetic
400 variation that allows for adaptation *in situ*. Strong associations between gene pools and climate may
401 reflect patterns of local adaptation, and heterogeneity in climatic conditions at both local and regional
402 scales, suggests that the impacts of climate change on remnant populations are likely to be uneven.
403 This is supported by assessments of mismatches between current and predicted future genomic
404 variation, creating heterogeneous patterns of ‘genomic vulnerability’ across populations. We also
405 demonstrate polyploidy associations with harsh climate zones, suggesting polyploidy is potentially
406 linked to historical adaptation processes and may assist populations in overcoming future climate
407 challenges. This study highlights the need for adaptive management strategies that incorporate
408 evolutionary potential, including seed sourcing and population mixing strategies that can help
409 overcome genomic vulnerability and maladaptation under future climates.

410

411 Isolation-by-distance

412 The majority of genomic variation found in *T. triandra* could be explained by geographic isolation.
413 This is likely to be due to low levels of gene flow and seed dispersal between populations contributing
414 to strong genetic structuring, as found in South African populations (Everson *et al.*, 2009). However,
415 this structure could also be driven by a partially apomictic reproductive system in *T. triandra* (Brown
416 & Emery, 1957; Birari, 1980), with clonal reproduction inflating signals of population-level genetic
417 uniqueness. We found some evidence of clonal *T. triandra* genotypes, but these individuals were
418 removed during the data filtering phase prior to analyses. While our data are unable to confirm the
419 relationship between clonality and polyploidy due to low replication, our data suggests that
420 polyploidy occurs infrequently at milder temperatures, while being dominant among populations
421 occurring in the highest temperature environments. These findings are consistent with Hayman (1960)
422 who argues that the diploid landrace is likely absent in the harsher climates, suggesting the presence
423 of positive selection for polyploid landraces in the hot and dry inland environments.

424

425 Perhaps the most germane work of this nature is that of the grass species *Panicum virgatum*. Similar
426 to *T. triandra*, *P. virgatum*'s ploidy level increases with distance from the coast, with higher ploidy

427 levels found in more arid inland environments (Zhang *et al.*, 2011; Lowry *et al.*, 2014; Grabowski *et al.*, 2014). As demonstrated in *P. virgatum*, we provide evidence for polyploidy evolution through
428 multiple, isolated events rather than the establishment and expansion of polyploids from one
429 duplication event. For example, some populations of predicted polyploids are more closely related to
430 diploid populations rather than other tetraploid populations. This suggests genome doubling can occur
431 spontaneously within populations and is both induced and maintained by selection under certain
432 environmental scenarios. Indeed, it has been shown that polyploids can have an increased fitness
433 advantage under heat- and water- stressed conditions (Rey *et al.*, 2017).

434

436 Isolation-by-environment and genomic vulnerability

437 Along with geography, climate factors describe a large percentage of genomic variation found in *T.*
438 *triandra*. We found strong associations between gene pools and environments (particularly with T_{MAX}
439 and P_{SEAS}), possibly reflecting adaptation to climate. While quantitative tests are needed to validate
440 these findings (e.g. common garden experiments – Sork, 2017), our results are consistent with the idea
441 that signals of adaptation are ubiquitous throughout genomes (Kern & Hahn, 2018). Maximum
442 temperature of the warmest month or week (T_{MAX}) has been found to be an important driving force of
443 selection in other Australian plants (Steane *et al.*, 2017a,b; Jordan *et al.*, 2017; Ahrens *et al.*, 2019).
444 Interestingly, evidence suggests that climatic factors can have different impacts on patterns of genetic
445 diversity and adaptation in different grass species. For example, *T. triandra* and *Andropogon gerardii*
446 are both dominant C4 grass species, with temperature and precipitation factors being key selective
447 forces driving diversity in *T. triandra*, while lower precipitation suppresses genetic diversity in *A.*
448 *gerardii* (Avolio *et al.*, 2013). Despite these differences, polyploidy appears dominant in harsher
449 regions in both species indicating there are ploidy based adaptive responses to climate, enabling the
450 expansion of species into habitats unsuitable or less suitable for diploids. The line of adaptation
451 demarcation is stronger for *T. triandra*, where persistence in the semi-arid landscape appears entirely
452 dependent on polyploids, compared to *A. gerardii*, where ploidy mixing occurs in harsher parts of its
453 climate range (Keeler, 1990).

454

455 Our analyses of genomic vulnerability across the study area suggest that some populations of *T.*
456 *triandra* will be more adversely impacted by climate change than others. For example, the most inland
457 populations of our sampling are most vulnerable where we estimate that populations will need to
458 change by over 20%, this region includes both diploid and polyploid populations. The least vulnerable
459 populations are located in the southern and mountainous regions where we would expect populations
460 to change by 0 to 5%. The future mismatch of predicted gene pools in some regions suggests that a
461 change of as much as 25% will be necessary for adaptation to the new challenges. Our predictions are
462 based only on correlative analyses, and caution should be taken when interpreting these findings given
463 the uncertainty associated with the genetic mechanisms (i.e. epistatic interactions (Juenger *et al.*,
464 2005), pleiotropy (Solovieff *et al.*, 2013), chromosomal rearrangements (Juenger *et al.*, 2005;

465 Yeaman, 2013), and polyploidy (Van de Peer *et al.*, 2017)) and ecological interactions likely to dictate
466 future adaptive responses (Fordyce, 2006). Indeed, our findings further highlight the need for
467 quantitative experiments (i.e. common garden) to validate these findings by testing the physiological
468 limits and safety margins of individual populations.

469

470 Not surprisingly, the genomic vulnerability of several populations was buffered by as much as 5% by
471 the presence of polyploids, and this is likely to be an underestimation due to under-predicting which
472 populations are polyploids. Polyploidy is known to provide fitness advantages in many plant species
473 persisting in hot and arid environments, including *T. triandra* populations (Godfree *et al.* 2017). The
474 increased heterozygosity associated with polyploidy may have the effect of slowing the loss of genetic
475 variation and providing more variants for selection to act upon (Comai, 2005). Elevated fitness may
476 also be influenced by duplicated genes and genomes, each set capable of independent selection and
477 evolving new functions (Soltis & Soltis, 2000) by retaining multiple gene copies and acquiring a new
478 function in one copy (Wendel, 2000). Further, increased performance could be due to differential
479 levels of expression between ploidy landraces (e.g. Cromie *et al.*, 2017; Wang *et al.*, 2018; Liqin *et*
480 *al.*, 2019), and be partially dependent on different epigenetic patterns (Nagymihály *et al.*, 2017).

481 However, quantitative measures are needed to determine how differential expression between diploid
482 and tetraploid landraces may affect their ability to persist in their optimal climates. We argue that
483 these types of processes are likely occurring in *T. triandra* landraces, allowing polyploids to persist
484 and outperform their diploid counterparts in hotter and drier climates.

485

486 Management and restoration implications

487 We are at a critical juncture in history where management and restoration of grassland ecosystems is
488 necessary to preserve these ecosystems and their services. However, the interplay of habitat
489 fragmentation and rapid climate change poses a significant challenge for the conservation and
490 restoration of functionally important plant species. Prioritising investments requires an understanding
491 of species biology and ecology to apply frameworks for identifying the species and populations most
492 at risk. *Themeda triandra*, the most widely distributed species in Australia, is at a critical inflection
493 point due to its use as a food crop (Pascoe, 2018), for native pasture (Fourie *et al.*, 1985), as a
494 foundational species (Snyman *et al.*, 2013), for selective breeding (e.g. *Lolium/Festuca* – Yamada *et*
495 *al.*, 2005), and in the restoration of degraded lands (Cole & Lunt, 2005; Snyman *et al.*, 2013). Our
496 results provide a critical first step and baseline information to support these new interests, future
497 studies and the development of empirically based management strategies that target grassland and
498 open woodland ecosystems. In Australia, research efforts have mostly focused on *Eucalyptus* species,
499 finding that eucalypt populations are often connected by high levels of gene flow and adapted to local
500 climates (e.g. Steane *et al.*, 2015; Jordan *et al.*, 2017; Supple *et al.*, 2018; Ahrens *et al.*, 2019). In one
501 of the first landscape-scale genomic studies in Australia for an understory species, we show that the
502 iconic grass *T. triandra* has very different patterns of connectivity and adaptation compared with its

503 *Eucalyptus* counterparts. Limited dispersal potential and high levels of genetic structuring among
504 remnant populations of *T. triandra* suggests that their adaptability is likely to depend largely on trait
505 plasticity and standing genetic variation that allows for adaptation *in situ*. We provide evidence of
506 genetic and ploidy variation correlated with climate, suggesting that standing genetic variation may be
507 retained within some *T. triandra* populations enabling adaptation to warmer and drier environments
508 emerging under climate change. Indeed, our findings suggest the impacts of climate change may be
509 heterogeneous across the distribution of *T. triandra*. This emphasises the importance of accounting for
510 intraspecific variation, including ploidy, when predicting species responses to new climate challenges.
511 Variability in physiological response to thermal stresses between populations has been established for
512 many plant species (Moran *et al.*, 2016), which may contribute to uneven population responses to
513 thermal stress (Miller *et al.*, 2019). These findings have implications for predicting population
514 responses to climate change, and highlight the importance of interventions (assisted migrations of pre-
515 adapted genotypes) to enhance the resilience of populations showing signs of climate stress given the
516 existence of relatively tolerant populations across the species range.

517

518 Conclusion

519 Successful establishment of *T. triandra* on three continents from its Asian centre-of-origin is likely
520 due to its ability to swiftly meet the challenges of new environmental conditions through mechanisms
521 unique to the species. Genomic analysis of a species can elucidate broad patterns of structure and
522 provide information about how those patterns are distributed across the landscape. While spatial
523 structure was the major component of the species' standing genetic diversity, environmental
524 heterogeneity was also a major component driving patterns of diversity, and patterns of neutral
525 genetic diversity have been shown to be affected by natural selection (Phung *et al.*, 2016). Thus, these
526 findings illustrate that the standing genetic variation can provide a basis for adaptation to changing
527 climates and should be incorporated into restoration projects. We were also able to investigate long
528 standing ploidy questions within a landscape genomics context. Notably, we were able to quantify
529 how ploidy might buffer the species from the most severe climate effects in the future. We found that
530 ploidy, along with standing genetic diversity, could be an important part of the puzzle that increases
531 the probability of grassland ecosystem persistence during a period of dramatic change. Our data
532 suggest that we risk underestimating the adaptive capacity of a species if we do not correct for ploidy
533 polymorphisms and we propose that they should be an integral part of management strategies moving
534 forward. Management of multi-ploidy foundational species should focus on a combination of
535 attributes, including genetic variation, intraspecific ploidy polymorphisms, and trait characteristics to
536 develop populations that are resilient to future climate scenarios ensuring ecosystem health, function,
537 and long-term restoration success.

538

539

540 Data accessibility

541 Our data will be deposited on Dryad. Including the full SNP data set and population metadata.

542

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549

550 Author Contribution

551 Design of the research was by CA and EJ; collection was performed by CA and EJ along with
552 volunteers; lab work was performed by NA; data analysis was performed by CA; and writing the
553 manuscript was performed by CA, EJ, and AM and all authors contributed to editing the manuscript.

554

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- 842
843

844

845 Figure Legends

846 **Figure 1.** Within population heterozygosity (H_s) versus maximum temperature of the warmest month.
847 Colors indicate diploid (blue), mixed populations (green; equidistant between tetraploid and diploid
848 populations), and tetraploid (red) based on Hayman's (1960) work. Ellipsoid outlines populations that
849 have high heterozygosity and may be tetraploids.

850

851 **Figure 2.** Sparse non-negative matrix factorization (sNMF) for all individuals, points on the map
852 indicate population location, map colors represent T_{MAX} (maximum temperature of the warmest
853 month). Barplot indicates identified genetic ancestral clusters for each individual (bar) with an
854 optimal k -value of three. Inset shows the Australia-wide distribution of *T. triandra* as a heat map and
855 location of the study area.

856

857 **Figure 3.** Identification of the spatial component of genetic variation using Moran's Eigenvector
858 Maps. Two distinct spatial patterns accounted for most of the 54% of genetic variation explained
859 through isolation by distance. The first MEM variable (a) explained a greater proportion of the
860 variation than the second variable (b). Circles of similar size and colour represent individuals with
861 similar scores on this axis.

862

863 **Figure 4.** Generalised dissimilarity modelling (GDM). (a) Non-linear relationship between climate
864 distance and genomic distance, where points are site pairs. (b) Relationship between predicted
865 genomic distance and observed genomic distance, where points are site pairs. (c) The geographic
866 spline showing the relationship between predicted genomic change and geographic distance. (d–g)
867 Predicted splines showing the estimated relationship between genomic distance and individual climate
868 variables: (d) mean annual precipitation (T_{MA}), (e) maximum temperature of the warmest month
869 (T_{MAX}), (f) mean annual precipitation (P_{MA}), and (g) precipitation seasonality (P_{SEAS}); inset is the
870 amount of variation explained by predicted ploidy polymorphisms (red lines are the model that
871 includes ploidy). Variation explained for the climate-only + spatial model is 31.3% (22% attributed to
872 climate), and with climate, ploidy, and spatial is 31.7% (23% attributed to climate).

873

874 **Figure 5.** Predicted spatial variation in genomic composition based on the outputs from the general
875 dissimilarity models (GDM). Maps include the (a) climate-only GDM and (b) the predicted genomic
876 vulnerability based on comparing the current GDM and the predicted GDM for 2070. Whereas, the (c)
877 climate + ploidy GDM, and (d) and the predicted genomic vulnerability are shown for direct
878 comparison to the climate-only model. A 5% reduction in genomic vulnerability is indicated in the
879 most severely affected areas when including ploidy level in the GDM. The greater the difference
880 (dark orange), the more genomic change is needed to adjust to future climate conditions.

881

882

883 Tables

884 **Table 1.** Locations and genetic diversity indices for sampled populations. T_{MAX} = maximum
 885 temperature of the warmest month; P_{SEAS} = precipitation seasonality; H_s = heterozygosity
 886 within populations; G_{IS} = inbreeding coefficient; A_N = number of alleles; C_P = predicted
 887 chromosome number.

Pop	X	Y	T_{MAX} (°C)	P_{SEAS} (mm)	A_N	H_s	C_P	G_{IS}
MTG	138.754	-34.977	26.7	52	1.357	0.088	40	0.059
BBNP	153.028	-30.420	28.1	43	1.215	0.066	20	0.181
BCR	153.054	-28.646	28.2	45	1.241	0.071	20	0.182
BL	151.737	-29.867	25.6	33	1.233	0.068	20	0.2
BLAPT	152.807	-31.395	26.9	34	1.232	0.079	20	0.317
BLARD	150.444	-35.196	25.3	24	1.21	0.066	20	0.154
BNR	151.997	-29.113	25.2	32	1.169	0.064	20	0.156
BRA	151.996	-32.631	27.2	25	1.212	0.064	20	0.179
BU	151.076	-30.189	29.5	31	1.164	0.064	20	0.207
BYR	153.620	-28.652	28.1	32	1.203	0.064	20	0.143
CB	150.674	-30.885	30.9	34	1.164	0.062	30	0.137
BCG	143.316	-37.612	26.1	23	1.142	0.043	20	0.085
DCD	150.728	-34.013	28.1	29	1.294	0.083	40	0.121
DCR	149.982	-36.356	24.8	24	1.271	0.08	30	0.062
DW	151.997	-29.114	25.2	32	1.203	0.068	20	0.165
RWCK	146.817	-36.583	28.1	32	1.185	0.062	22	0.366
BUR	145.026	-37.834	26.0	17	1.136	0.06	20	0.328
ANG	144.153	-38.335	23.9	22	1.187	0.07	20	0.138
EUN	152.888	-30.811	27.7	39	1.234	0.067	20	0.184
GHK	149.863	-36.979	24.0	18	1.259	0.073	20	0.236
GOR	150.588	-35.009	25.2	23	1.397	0.102	20	-0.04
GRES	151.219	-32.546	30.1	34	1.231	0.068	20	0.171
QLD	149.878	-27.926	33.5	29	1.309	0.12	40	0.034
JG	152.008	-30.514	25.3	38	1.187	0.065	20	0.196
KCK	152.579	-31.795	27.5	37	1.238	0.068	20	0.177
KOZ	148.402	-35.889	22.4	29	1.255	0.075	20	0.28
KUN	152.844	-31.196	27.3	37	1.20	0.069	20	0.205
L	152.292	-28.411	27.9	37	1.195	0.073	20	0.17
LO	149.998	-33.167	26.4	20	1.203	0.082	20	0.318
MGR	149.077	-36.244	25.0	19	1.254	0.07	20	0.051
ML	152.473	-28.380	26.9	40	1.285	0.083	20	0.218
MNP	150.373	-35.457	24.1	13	1.36	0.089	20	0.127
Mong	149.944	-35.426	25.5	16	1.193	0.064	20	0.252
MS	150.881	-29.988	29.8	31	1.161	0.067	40	0.165
MSF	149.055	-34.825	28.1	13	1.293	0.084	20	0.334
NAB	152.370	-32.086	27.7	35	1.217	0.063	20	0.226
NAM	152.976	-30.639	28.0	41	1.109	0.109	20	---
OPC	153.037	-29.820	28.5	40	1.109	0.064	20	0.116
PR	150.186	-31.418	31.7	34	1.366	0.115	40	-0.135
MSCP	140.631	-37.145	28.1	44	1.242	0.072	20	0.257
SIW	153.146	-30.192	27.4	40	1.147	0.062	20	0.17
SOM	151.286	-33.404	26.1	31	1.248	0.065	20	0.187
SPNR	149.747	-37.557	22.6	12	1.247	0.07	20	0.157
STCK	149.314	-35.360	26.7	13	1.257	0.075	20	0.245
SWC	149.707	-31.400	31.3	31	1.18	0.105	41	-0.753
NSW	148.142	-36.542	26.6	17	1.245	0.067	20	0.151
TOO	152.391	-28.453	28.7	39	1.221	0.068	20	0.183
UL	152.073	-30.537	25.2	39	1.234	0.099	20	-0.326
WOL	150.806	-34.434	25.6	31	1.229	0.065	20	0.183
WYE	151.491	-33.175	26.6	30	1.235	0.065	20	0.195
YNGNP	150.693	-33.057	28.2	38	1.223	0.063	26	0.196
YNR	151.076	-30.189	29.5	31	1.213	0.076	20	0.319
Overall					1.842	0.074		0.134

888

889

890 Supplementary information

891 **Table S1.** F_{ST} pairwise table and input for GDM analysis. (tsv file)

892 **Figure S1.** Elevation of the study area.

893 **Figure S2.** Histogram of MinION long-read read-lengths and average read quality.

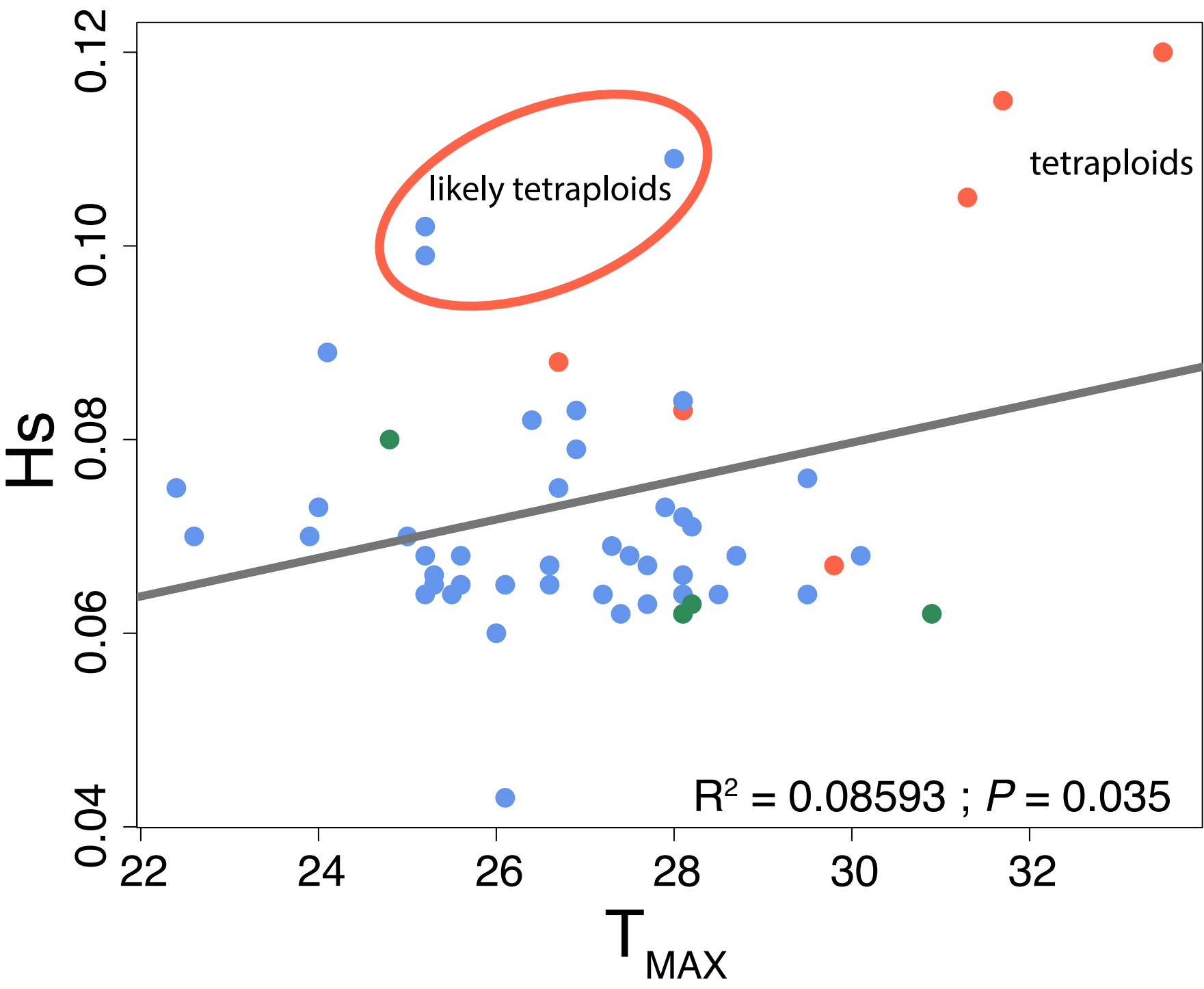
894 **Figure S3.** Principal components analysis for all 19 bioclim variables.

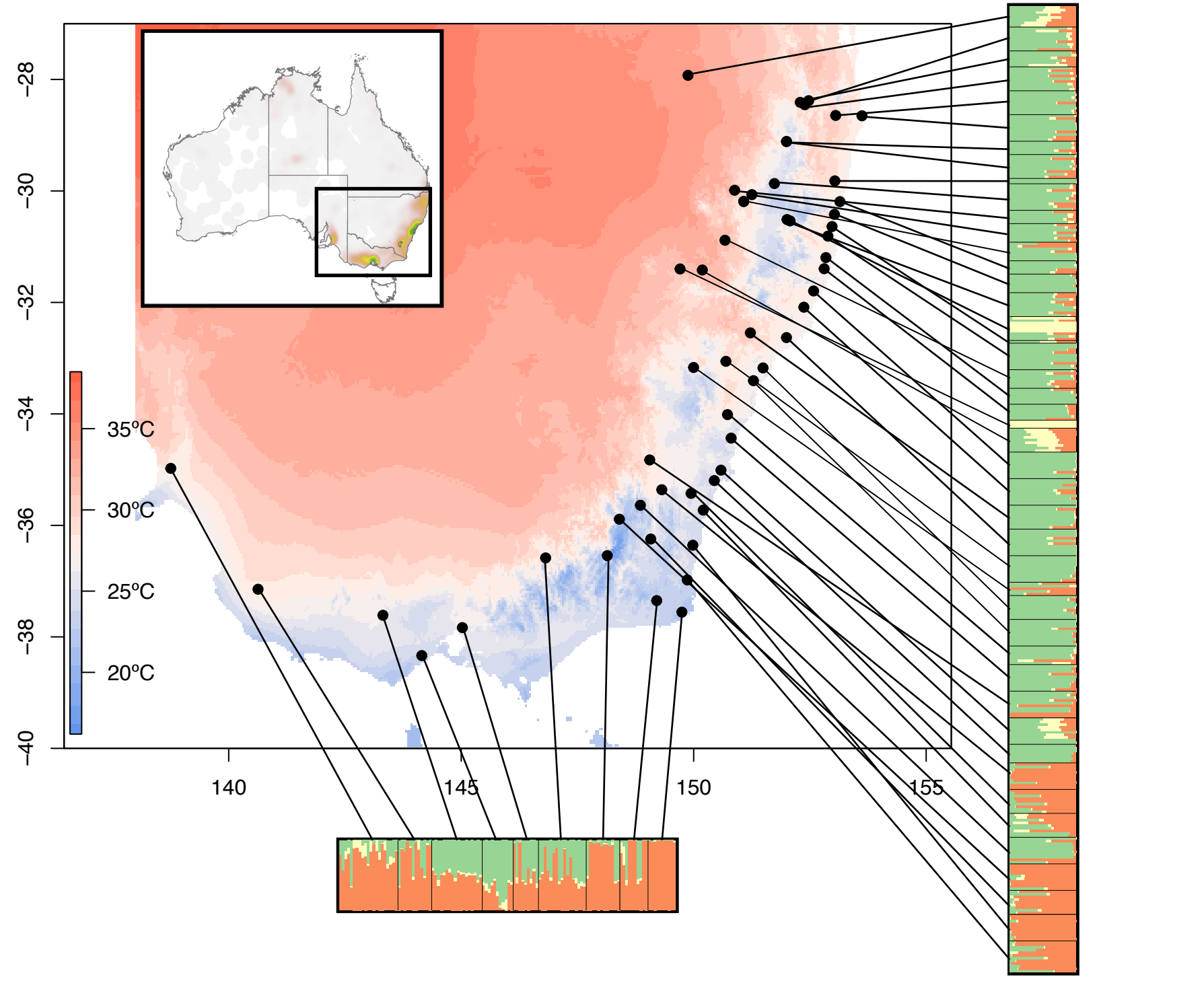
895 **Figure S4.** Cross entropy plot to determine the k -value for sNMF results.

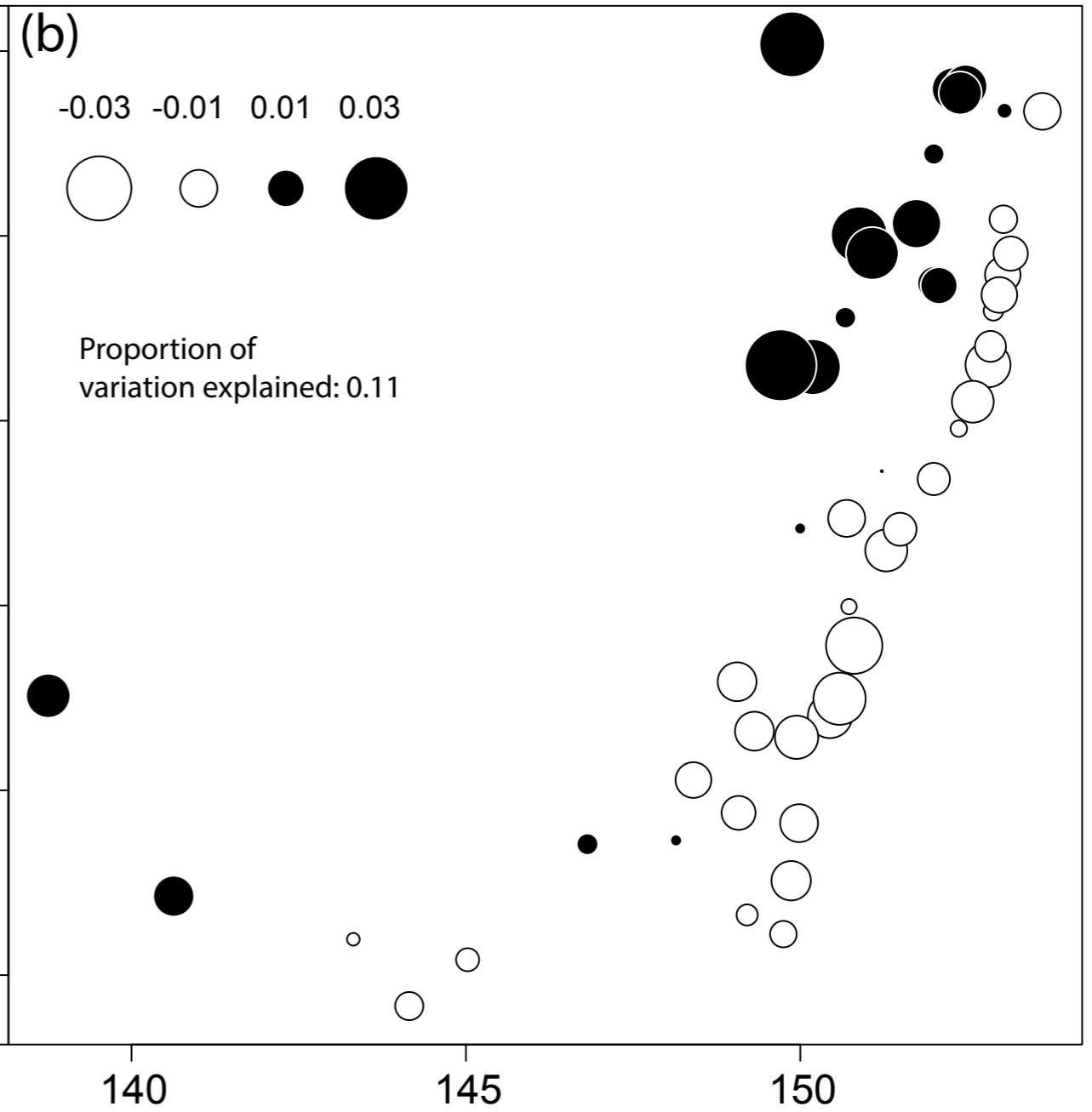
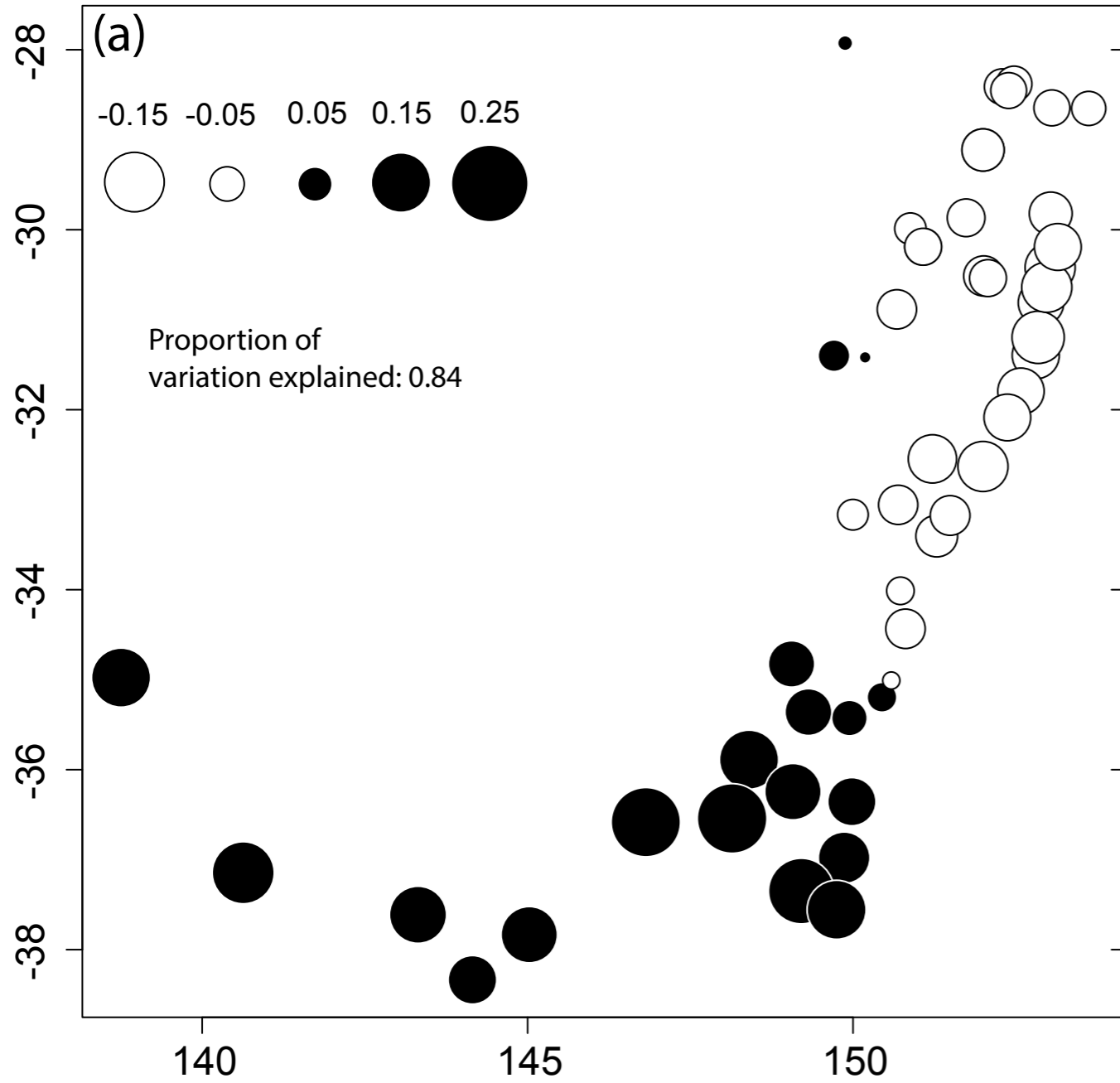
896 **Figure S5.** Maps for all four climate variables and ploidy distribution.

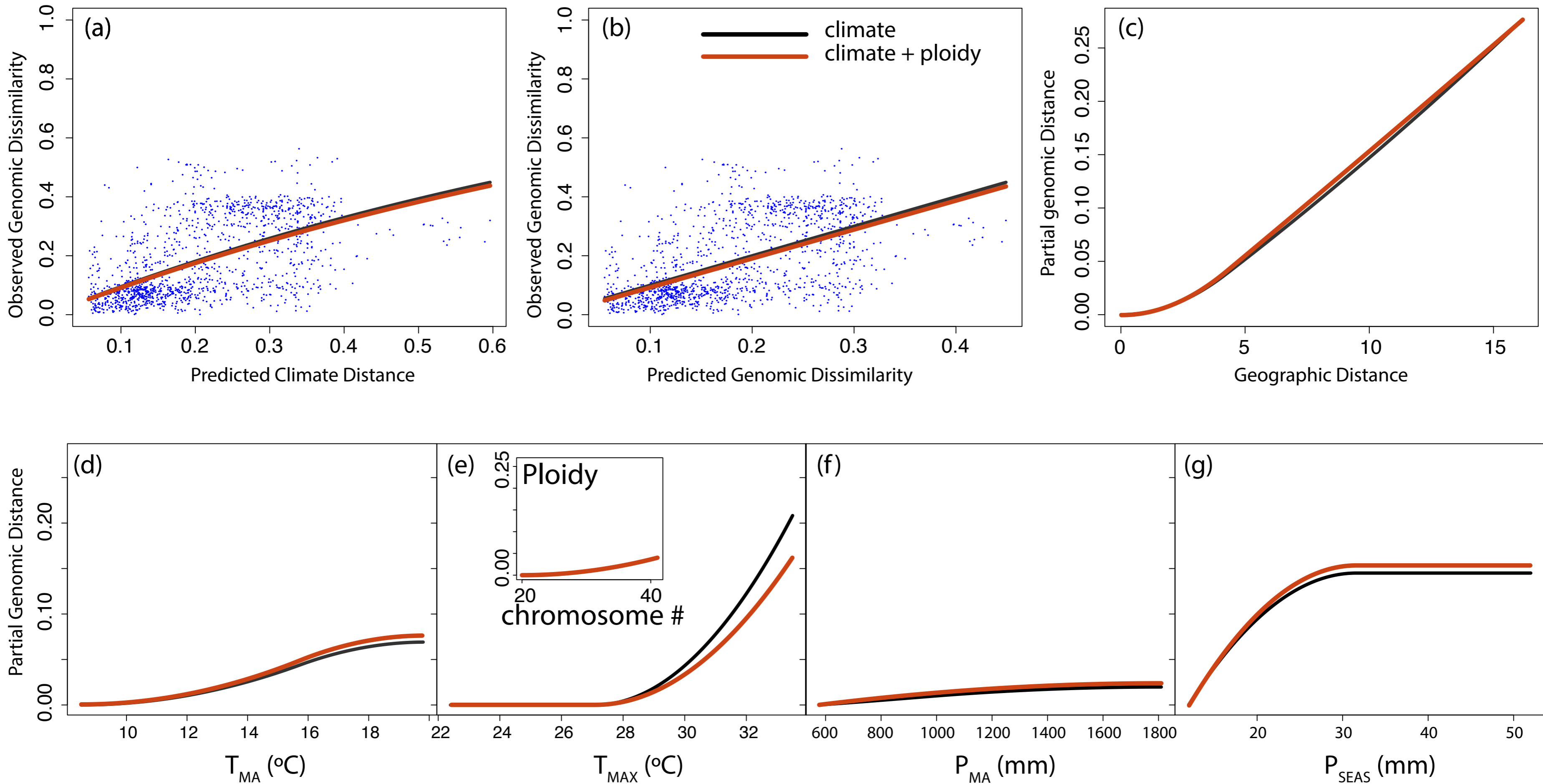
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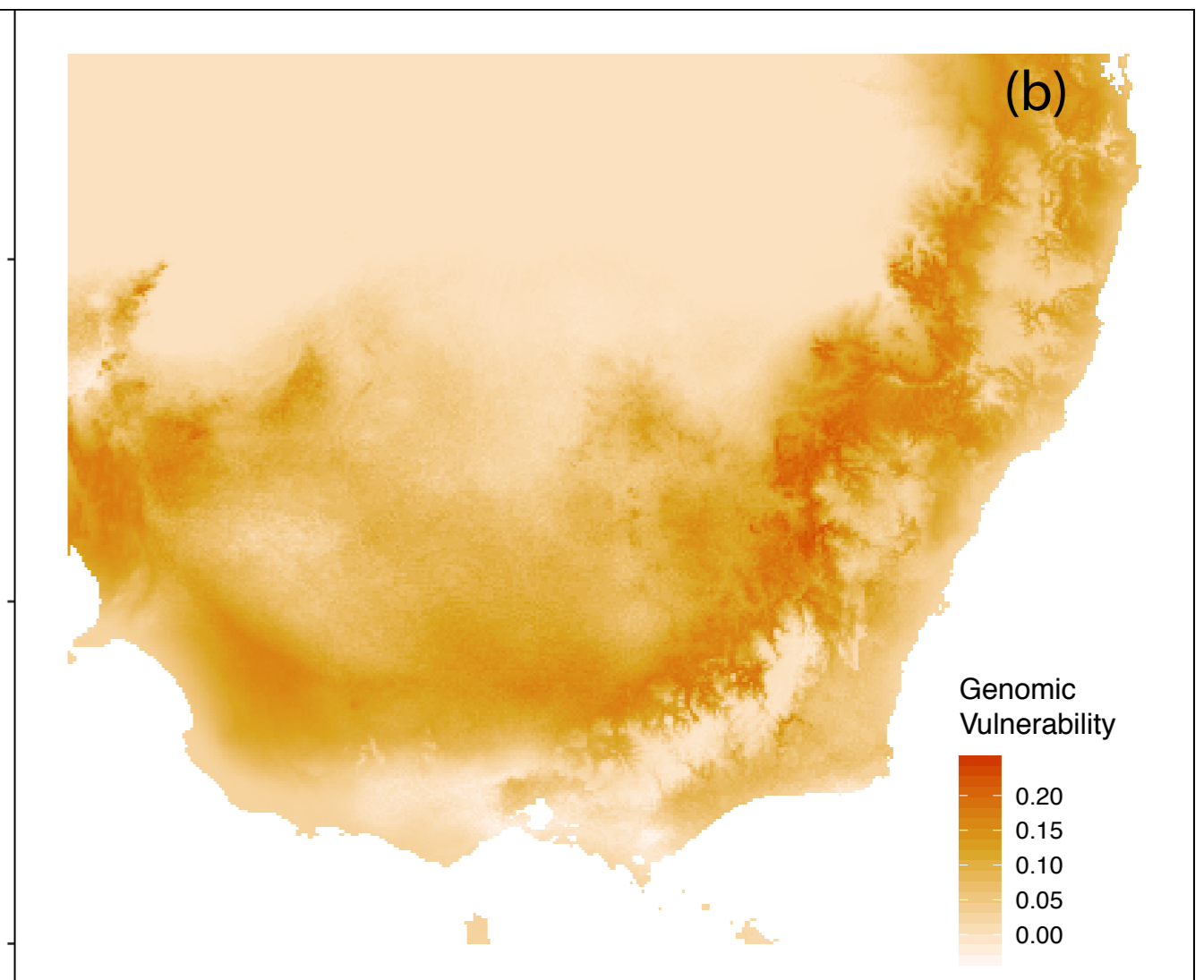
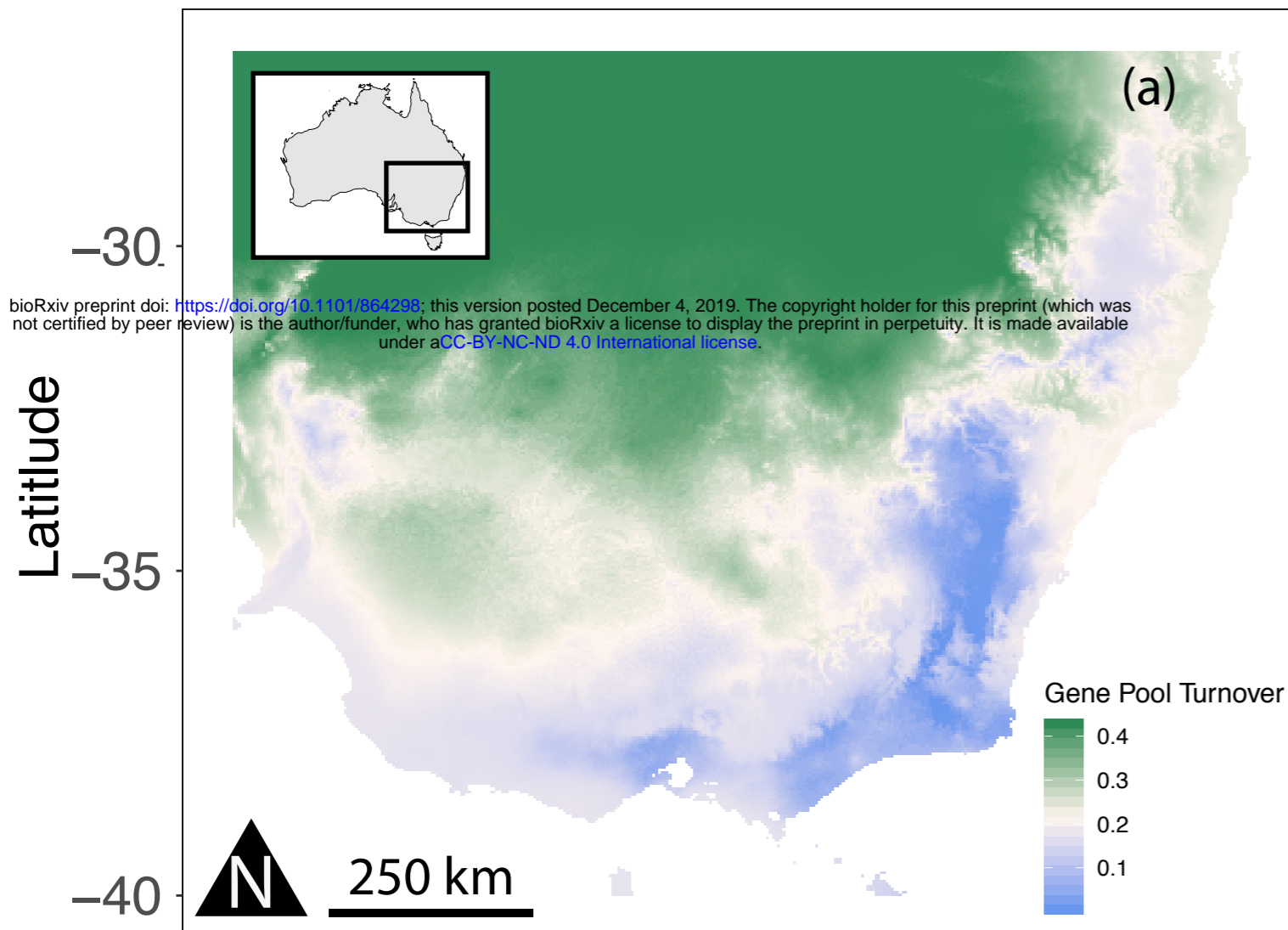








climate variables



climate + ploidy prediction

