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Genomic evidence of introgression and adaptation in a model subtropical tree species, *Eucalyptus grandis*

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Abstract

The genetic consequences of adaptation to changing environments can be deciphered using population genomics, which may help predict species' responses to global climate change. Towards this, we used genome-wide SNP marker analysis to determine population structure and patterns of genetic differentiation in terms of neutral and adaptive genetic variation in the natural range of *Eucalyptus grandis*, a widely cultivated subtropical and temperate species, serving as genomic reference for the genus. We analysed introgression patterns at subchromosomal resolution using a modified ancestry mapping approach and identified provenances with extensive interspecific introgression in response to increased aridity. Furthermore, we describe potentially adaptive genetic variation as explained by environment-associated SNP markers, which also led to the discovery of what is likely a large structural variant. Finally, we show that genes linked to these markers are enriched for biotic and abiotic stress responses.

KEYWORDS

ancestry mapping, environmental association, introgression, population genomics

1 | INTRODUCTION

Hidden within extant genomes are the consequences of natural selection in response to millennia of abiotic and biotic stress. With population genomics, we can decipher genomic signatures of selection and combine them with environmental data to predict how a species will likely respond to climate change and ex situ environments. This approach has gained interest with recent advances in high-resolution environmental and genome-wide genotyping technologies, particularly for non-model organisms (Bragg, Supple,

Andrew, & Borevitz, 2015; Rellstab, Gugerli, Eckert, Hancock, & Holderegger, 2015), to aid in conservation, assisted migration and ecosystem restoration efforts (Ahrens, Byrne, & Rymer, 2019; Gugger, Liang, Sork, Hodgskiss, & Wright, 2018; Jordan, Dillon, Prober, & Hoffmann, 2016; Landguth, Holden, Mahalovich, & Cushman, 2017; Shryock et al., 2015; Supple et al., 2018). Because population genomics can reveal hidden adaptive genetic variation, it allows for management of genetic resources beyond simple species demography (Luikart, England, Tallmon, Jordan, & Taberlet, 2003).

Eucalyptus, frequently used in forest and woodlands restoration (Bennett, 2016; Jordan et al., 2016; Pryor & Johnson, 1981; Supple et al., 2018), is globally the most widely planted hardwood genus (Myburg et al., 2007) covering over 20 million ha worldwide (Iglesias-Trabado & Wilstermann, 2008). Differentiation among the more than 700 eucalypt species spans the speciation continuum, with extensive evidence of reticulate evolution, hybridization and adaptive introgression (Bayly et al., 2013; Jones, Nicolle, Steane, Vaillancourt, & Potts, 2016; McKinnon, Jordan, Vaillancourt, Steane, & Potts, 2004; Pollock, Bayly, Nevill, & Vesk, 2013; Rutherford et al., 2018). Obscured species delineation can complicate management of genetic resources, which are evaluated at the species level. Population genomics could resolve some species boundaries within this genus. Additionally, it could enrich our understanding of hybrid speciation and the role of adaptive introgression in response to changing environments.

Eucalyptus grandis serves as a model species for the genus. Although the wealth of common garden data (Arnold, Burgess, & Allender, 1996; Burgess, 1988; Darrow, 1983; Langat & Kariuki, 2004; Matheson & Mullin, 1987; Skolmen, 1986), and genomic (Myburg et al., 2014; Silva-Junior, Faria, & Grattapaglia, 2015) and transcriptomic (Mangwanda, Myburg, & Naidoo, 2015; Mizrachi, Hefer, Ranik, Joubert, & Myburg, 2010; Oates, Külheim, Myburg, Slippers, & Naidoo, 2015; Vining et al., 2015) resources available for *E. grandis* stem from its commercial significance (Harwood, 2011), it is also an important foundation species along the east coast of Australia. Its natural range encompasses eight Köppen climate classification zones (Köppen, 1884, 2011) including subtropics in Queensland and temperate areas in New South Wales (<http://www.ala.org.au>, accessed 28 November 2019; see Table S1). Despite its ecological and commercial relevance, an in-depth analysis of potentially valuable adaptive genetic variation in relation to abiotic factors is still lacking. Outstanding questions include the following: What are the dominant environmental factors affecting the realized niche? What will most likely be the mode of response to niche changes? Do we see evidence of local adaptation as proposed from historic common garden experiments (Arnold et al., 1996; Burgess, 1988; Darrow, 1983; Langat & Kariuki, 2004; Matheson & Mullin, 1987; Skolmen, 1986)? To address these questions, we first need to understand the population structure and genetic diversity in wild *E. grandis*.

With available genomic and environmental data (Belbin & Williams, 2016) resources, we have begun to decipher population differentiation and genetic diversity in wild populations of *E. grandis* in terms of demography and potentially adaptive genetic variation. Our hypothesis is that genomic loci associated with environmental factors would be enriched for adaptive genetic variation. Furthermore, following the rationale of Steane et al. (2014), population stratification derived from the adaptively enriched genetic space would correlate with environmental clines as opposed to the demography of the species. This is because regions of the genome affected by selection for adaptive traits could have different patterns of inheritance compared to selectively neutral

regions. We report the use of latent factor mixed model (LFMM) analyses to identify SNP markers associated with environmental clines to serve as adaptively enriched marker sets for population differentiation analysis. Population stratification is investigated using principal component analysis (PCA) and sparse non-negative matrix factorization (sNMF). Generalized dissimilarity modelling (GDM) was used to refine which environmental factors are most predictive of the population differentiation observed for adaptively enriched SNP sets. Finally, we present a modified approach for ancestry mapping that does not require prior knowledge of the identity of the introgressing species. This study generates new hypotheses regarding adaptive population differentiation in *E. grandis*, to be tested in common garden trials.

2 | MATERIALS & METHODS

2.1 | Study population, DNA isolation and genotyping

Seed stocks collected from 193 wild-growing trees (Bean, 1989; Gunn, 2001) were imported from the Australian Tree Seed Centre (Commonwealth Scientific and Industrial Research Organisation; Gunn, 2001) and a Queensland Government collection managed by D. Lee (University of the Sunshine Coast, Australia), and germinated in Pretoria, South Africa, under greenhouse conditions. The collection represents most of the latitudinal range, from Windsor Tablelands (16°11'S) in Queensland to Bulahdelah (32°20'S) in New South Wales, with 33 provenances represented (Table 1 and Figure 1). Since most provenances were represented by fewer than 10 individual mother trees (Table S1), multiple half-siblings were selected in each family to capture pollen parent variation (Appendix S1). All seedlings analysed were established in an ex situ gene conservation park in the KwaZulu-Natal Midlands, South Africa.

Leaves were collected from 6-week-old seedlings for DNA isolation using the NucleoSpin DNA extraction kit (Machery-Nagel GmbH & Co. KG, Düren, Germany), and SNP genotypes were generated for 596 seedlings using the EUChip60K SNP chip (Silva-Junior et al., 2015) by the genomics service provider, GeneSeek (Neogen, Lincoln, NE, USA). Intensity data were first assessed using GenomeStudio V2011.1 (Illumina Inc., San Diego, CA, USA) to re-cluster genotypic classes as described by Silva-Junior et al. (2015), followed by data filtering to retain high-confidence, informative SNPs (minor allele frequency >0.02 and genotyped in at least 90% of samples) and samples with less than 10% missing data using the SNP & Variation Suite™ v8.x (SVS8; Golden Helix, Inc., Bozeman, MT). Identity-by-descent analysis was used to confirm sibship and detect cryptic relatedness among individuals (Appendix S1 and Figure S1). To avoid over-representation of any individual family, a balanced subset of 362 samples with no more than two half-siblings per family was compiled for population differentiation and environmental association analyses.

TABLE 1 Seed stocks and study population

Location/provenance	Region	Subpop. ^a	Latitude	Longitude	Elevation (m)
Windsor Tableland 1	Windsor	North	-16.183	144.983	1,161
Windsor Tableland 2	Windsor	North	-16.200	145.017	1,134
Copperlode	Copperlode	North	-16.967	145.667	368
Tinaroo Creek Road	Atherton	North	-17.083	145.567	925
Tinaroo Dam	Atherton	North	-17.133	145.567	893
Wild River, QLD	Atherton	North	-17.367	145.400	927
Mt. Spec, Paluma	Paluma	North	-18.933	146.117	822
Paluma	Paluma	North	-19.000	146.150	796
Eungella	Eungella	Mackay	-21.075	148.632	294
Finch Hatton Gorge	Finch Hatton Gorge	Mackay	-21.197	148.504	753
Brooweena	Brooweena	South	-25.550	152.267	210
Kin Kin ^b	Sunshine Coast	South	-26.263	152.876	142
Woondum	Sunshine Coast	South	-26.300	152.817	306
Yabba	Sunshine Coast	South	-26.533	152.400	475
Mapleton	Sunshine Coast	South	-26.600	152.867	325
Conondale	Sunshine Coast	South	-26.667	152.600	498
Beerwah	Sunshine Coast	South	-26.850	153.000	20
Mt. Mee	Sunshine Coast	South	-27.133	152.717	368
Mt. Tamborine	Mt. Tamborine & Mt Lindsay	South	-27.917	153.183	396
Mt. Lindsay	Mt. Tamborine & Mt Lindsay	South	-28.350	152.750	352
Cherry Tree	Cherry Tree	South	-28.900	152.817	236
Newfoundland	Newfoundland	South	-29.946	153.162	52
Bagawa	Coffs Harbour	South	-30.117	152.900	299
Wedding Bells	Coffs Harbour	South	-30.167	153.117	95
Lower Bucca	Coffs Harbour	South	-30.178	153.074	108
Orara East	Coffs Harbour	South	-30.225	153.068	195
Orara West (71)	Coffs Harbour	South	-30.332	152.973	189
Orara West (72)	Coffs Harbour	South	-30.341	153.011	353
Tuckers Nob	Coffs Harbour	South	-30.372	152.951	259
Pine Creek	Coffs Harbour	South	-30.414	153.009	18
Gladstone	Coffs Harbour	South	-30.509	152.838	242
Newry	Coffs Harbour	South	-30.536	152.956	21
Bulahdelah	Bulahdelah	South	-32.338	152.149	119

^aSubpopulation assignment based on geographic and genetically distinct groupings.

^bGPS coordinates provided for Kin Kin (-26.200;153.167) appeared improbable, as such the coordinates for Kin Kin the town were used as proxy environment.

2.2 | Population structure and differentiation

Population structure was assessed using sNMF (Frichot, Mathieu, Trouillon, Bouchard, & François, 2014) with the LEA R package (Frichot & François, 2015). The number of ancestral populations, K , tested in each admixture analysis was one to ten with 20 repetitions, and cross-entropy was visually inspected to determine the most plausible value for K either represented as a clear “knee” in the plot or the lowest cross-entropy value. Population differentiation was also assessed using PCA (Price et al., 2006) using the standard

deviation of each marker for normalization in SVS8. Differentiation between subpopulations, regions and provenances (defined in Table 1) was quantified in terms of the F -statistic, F_{ST} , as described by Weir and Cockerham (1984), with 95% confidence intervals in SVS8. Hierarchical population structure analyses, as recommended in Evanno, Regnaut, and Goudet (2005), were done to find within-group structure by repeating sNMF and PCA on subsets of the population defined by the assignment of individuals at the uppermost hierarchical level, that is to identify subtle structure within the Northern and Southern subpopulations.

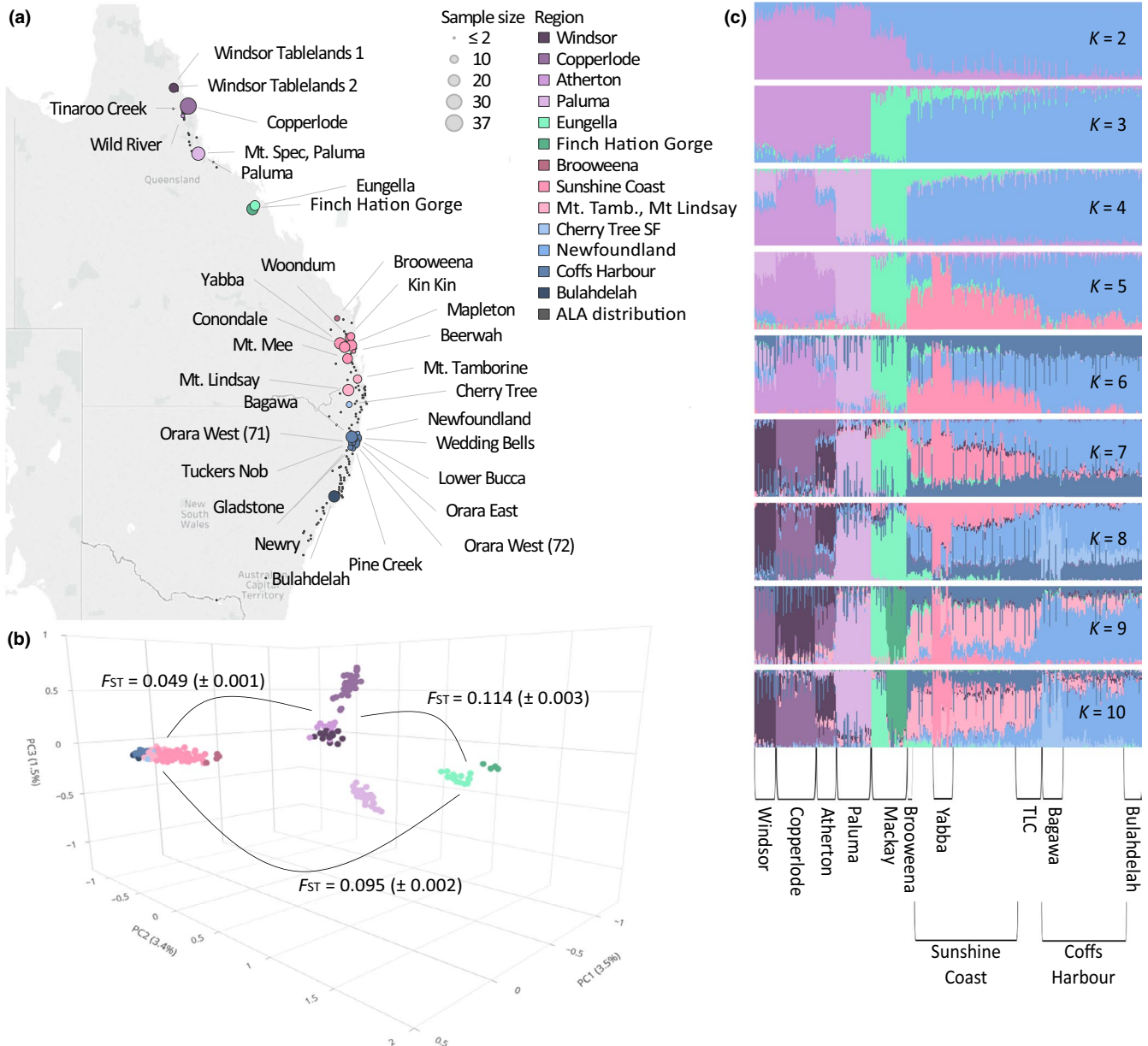


FIGURE 1 Population structure of *E. grandis*. (a) Source locations (coloured circles) with circle sizes indicating numbers of samples. Broad geographic regions of the Northern and Mackay provenances are indicated in shades of purple and green, respectively. Southern regions are coloured in shades of pink and blue. The natural distribution according to Atlas of Living Australia (occurrence data download at <http://www.ala.org.au>, accessed 28 November 2019) is given in dark grey dots. (b) Population structure is given based on the first three components of the PCA, and F_{ST} estimates are indicated with 95% confidence intervals in parentheses. (c) sNMF outcome indicating per individual (x-axis) the genomic proportion assigned to each ancestral population (y-axis) for different values of K . Geographic regions and key provenances are indicated along the x-axis; Mackay–Eungella and Finch Hatton Gorge; TLC–Mt. Tamborine, Mt. Lindsey and Cherry Tree

Elevated F_{ST} estimates for Eungella, Finch Hatton Gorge (the two provenances from the Mackay region) and Brooweena, raised the suspicion that introgression might have occurred in these populations. The *Efficient Inference of Local Ancestry (EILA)* R package (Yang, Li, Buu, & Williams, 2013) was used to test ancestral origins of chromosomal segments. In short, the *EILA* method consisted of three steps: first, each SNP was assessed in sampled individuals and assigned a probability score for ancestry to one of the three possible ancestral populations. Ancestral population 1 (anc1) consisted of

168 *E. grandis* individuals (one individual of each of the 193 families, excluding those from Eungella, Finch Hatton Gorge and Brooweena). Ancestral population 2 (anc2) consisted of genetically diverse individuals from the section *Maidenaria* (10 *E. nitens*, 10 *E. viminalis* and 12 *E. dunnii* individuals), and ancestral population 3 (anc3) represented non-*E. grandis* individuals from the section *Latoangulatae* (10 *E. saligna* and 18 *E. urophylla* individuals). SNP data for anc2 and anc3 were obtained from Silva-Junior et al. (2015), and a subset of 7,736 SNPs with no missing data and MAF >0.02 across all reference

and query samples was used. Species delineation was illustrated by PCA (Figure S2). The second step in the *EILA* process involved identification of breakpoints along the chromosome where ancestry switched from one ancestral population to another. Different values for the breakpoint penalty (λ) were tested (Appendix S1 and Figure S3). Third, each chromosomal segment was assigned to one of the three ancestral populations based on the cumulative ancestry assignment of all the SNPs in that segment. If the segments were too small (breakpoint penalty too low), there would not be enough SNPs for confident ancestry assignment, while larger breakpoint values resulted in reduced resolution along the chromosomes. For this purpose, we report $\lambda = 15$ and $\lambda = 30$ for suspected introgressed provenances and $\lambda = 30$ for all other provenances. With each provenance tested, the individuals from that provenance were removed from the anc1 reference set and all 596 seedlings genotyped were analysed. Ancestry assignment scores were graphically presented using Tableau Professional Edition (Tableau Software Inc., Seattle, WA, USA).

2.3 | Population structure in relation to environmental clines

Environmental association analysis (EAA) was conducted to identify loci associated with abiotic environmental clines, using LFMM analysis, as implemented in the *LEA* R package (Frichot & François, 2015). Environmental predictor variables were obtained from the Atlas of Living Australia (Belbin & Williams, 2016) environmental data layers (<http://www.ala.org.au>, accessed 28 November 2019; Figure S4 and Table S2). Environmental factors were selected to include variables reported for source locations and/or trial sites in historic provenance and growth trials of *E. grandis* (Arnold et al., 1996; Burgess, 1988; Skolmen, 1986; Van den Berg, Verryn, Chirwa, & Van Deventer, 2016). These included five climate variables: cold period minimum temperature (Bio06), warm period maximum temperature (Bio05), dry period average precipitation (Bio14), wet period average precipitation (Bio13) and mean annual aridity index. In addition to the geographic variables elevation and distance to coast, four edaphic variables were included namely soil depth, soil pedality (grade between one and five, predictive of water retention ability; McKenzie, Jacquier, Ashton, & Cresswell, 2000), pre-European soil nitrogen levels and pre-European soil phosphorus levels. First, PCA was performed to reduce dimensionality among correlated environmental variables for the entire species range and two subpopulations, respectively (Appendix S1). Principal components (PCs) were retained for downstream analysis if eigenvalues ≥ 1 , and environmental variables showed significant correlation (loadings ≥ 0.7) with the PC to enable meaningful interpretation. LFMM analyses were then used to find loci associated with the PCs and with individual environmental variables. Due to the potential presence of interspecific introgression in Eungella and Finch Hatton Gorge provenances (Mackay region), differences in correlations among environmental variables in the Northern (latitudes -16.183 to -19.000) and Southern (latitudes

-25.550 to -32.338) subpopulations (Table S3) and distinct gene flow patterns in the two subpopulations, we performed EAA on each subpopulation separately. For LFMM, the K value with the minimum cross-entropy in the sNMF analysis was used to correct for populations structure. This was $K = 4$ in the Northern subpopulation EAA and $K = 5$ in the Southern subpopulation EAA (Figure S7). Lastly, population differentiation, calculated as F_{ST} estimates, derived from the full genetic space was compared with that derived from the environment-associated genetic space. Missing genotypic data can result in increased false positives (Frichot & François, 2015); therefore, EAA was performed using SNPs with less than 10% missing data in each of the subpopulations, and population differentiation analysis, described below, was done using environment-associated SNPs with no missing data.

Correlations between genetic differentiation, geographic distance and environmental clines were tested using GDM. This makes use of F_{ST} population differentiation estimates, calculated for the full genetic space or environment-associated genetic spaces, as response variable. Although small sample sizes can affect F_{ST} estimates, the large number of markers used in our analyses helped to circumvent this (Willing, Dreyer, & Van Oosterhout, 2012). Still, provenances represented by single families were excluded from the models. First, models were generated using the *gdm* R package (Ferrier, Manion, Elith, & Richardson, 2007; Manion et al., 2018) for the entire balanced population (model A) and the population excluding introgressed provenances, Eungella, Finch Hatton Gorge and Brooweena (model B). Models were also generated and compared for the Northern and Southern subpopulations using population differentiation as given by the total genetic space and environment-associated SNP sets. For each model generated, three *l*-spline basis functions (Ferrier et al., 2007; Ramsay, 1988) were used to fit the relationship of each predictor variable, environmental factor or geographic distance, with the response variable, genetic distance as given by F_{ST} estimate. Plots of the *l*-spline function with the maximum value for each predictor variable were used to determine the relative importance of each predictor variable, given by the maximum value of the plot, and the rate of change in the response variable along the environmental gradient of the predictor variable as given by the slope of the plot (Fitzpatrick et al., 2013). Permutation tests were done to quantify the importance of each predictor variable calculated as the change in deviance explained by the full model compared to that explained by the model where the variable was permuted. In addition, significance of each predictor variable was calculated as bootstrapped p -values.

2.4 | Identification of chromosomal rearrangements

The population structure analysis by PCA using SNPs associated with North PC1 separated all provenances into three classes along the y -axis (Figure 3c). A similar pattern was observed by Faria et al. (2019) while investigating chromosomal rearrangements. This raised the questions: Is there evidence of structural variants in the

North PC1-associated SNP data set? Lack of recombination between loci within chromosomal rearrangements, due to lack of sequence homology, can manifest as genomic regions with high linkage disequilibrium (LD). As such, the North PC1-associated SNPs were interrogated to identify genomic regions of high LD, measured as the squared correlation (R^2) between allelic values at two loci using SVS8. Subsequently, SNPs on chromosome 11, including a large region where $R^2 > 0.8$, were used for population structure analysis to corroborate the putative presence of a structural variant.

2.5 | Functional dissection of environment-associated genomic regions

To identify enriched biological processes in the environment-associated gene space compared to the total SNP-captured gene space, genes overlapping a 4 kb window around SNPs (2 kb up- and downstream of each locus) were extracted using *bedtools* (Quinlan & Hall, 2010). This was based on previously determined genome-wide LD decay estimates for *E. grandis* (Silva-Junior & Grattapaglia, 2015). Gene annotation and functional enrichment were performed as described by Pinard, Fierro, Marchal, Myburg, and Mizrahi (2019), with the exception that functional enrichment was tested for each environment-associated gene set against the total SNP-captured gene set instead of all annotated genes in the reference genome. Enrichment was also tested for a combined set of genes in linkage with SNPs that had association with at least one of the environmental clines tested in each of the subpopulations ("North all environment-associated" and "South all environment-associated"). The enrichment test was also repeated for genes in LD with a random selection of 513 informative SNP loci ("Random") and SNPs that did not show association with any of the environmental variables in the Northern subpopulation ("North non-associated").

3 | RESULTS

3.1 | Population structure, differentiation and demography

Of the 64 639 SNP markers assayed across 596 seedlings representing 193 families, 25 099 were informative in at least 90% of the samples with minor allele frequency (MAF) >0.02 . Three samples were excluded with more than 10% missing data and 15 samples did not cluster with samples from the same provenance or region in hierarchical PCA. These PCA outliers were excluded from population structure and environmental association analyses, but retained for ancestry mapping. The 578 samples were reduced to 362 so that each family was represented by no more than two half-siblings. Of the 193 families, 24 were represented by only one seedling (Figure S1).

Based on PCA and sNMF, $K = 3$ was the best supported number of subpopulations in the first-tier population structure analysis

(Figure 1). Population differentiation estimates by F_{ST} (Figure S5) supported clustering observed in the PCA with the highest level of differentiation observed for the Mackay subpopulation (Figure 1b). The Northern subpopulation, consisting of 109 samples from eight source locations, appeared to be fragmented into four broad regions ($K = 4$; Figure 1c, Figures S6 and S7). In contrast, the Southern subpopulation structure was less fragmented as illustrated by a lack of distinct clustering in the PCA and sNMF, and lower F_{ST} estimates for the seven geographic regions represented by 220 seedlings (Figure 1c, Figures S6 and S7). Only one provenance, Brooweena, appeared clearly distinct from the rest of the subpopulation (Figure S6b).

3.2 | Evidence of introgression in three provenances

To test the hypothesis that the high F_{ST} estimates observed for Eungella, Finch Hatton Gorge and Brooweena were due to interspecific introgression, *EILA* was used to test for *E. grandis* and non-*E. grandis* ancestry of chromosomal segments in each seedling. Different introgression patterns were observed in all individuals from the three provenances presumed to be introgressed (Figure 2 and Figures S8–S10). Chromosomes 7 and 8 had the least non-*E. grandis* assignment. The 15 samples excluded from population structure and association analyses as they did not group with the expected provenance, had ancestry profiles indicative of more recent interspecific hybridization, for example Eungella_20 (Figure 2 and Figure S8).

3.3 | Population structure in relation to environmental clines

3.3.1 | Generalized dissimilarity modelling across the species range

The effect of geographic distance and environmental variables on population differentiation across the species range was modelled using GDM. Model A, which included introgressed provenances, explained 71.4% of deviance. Exclusion of the introgressed provenances (model B) increased the explained deviance to 87.6% (Table S4). Geographic distance between provenances was the only variable that explained significant variation in genetic distance in either model (Figure S11).

3.3.2 | Environment association analysis (EAA)

Correlation patterns among environmental variables differed between the Northern and Southern subpopulations (Table S4). Furthermore, fewer SNPs were associated with environmental clines in the south than in the north (Table S4). Although GDM was successful for Southern subpopulation environment-associated SNP

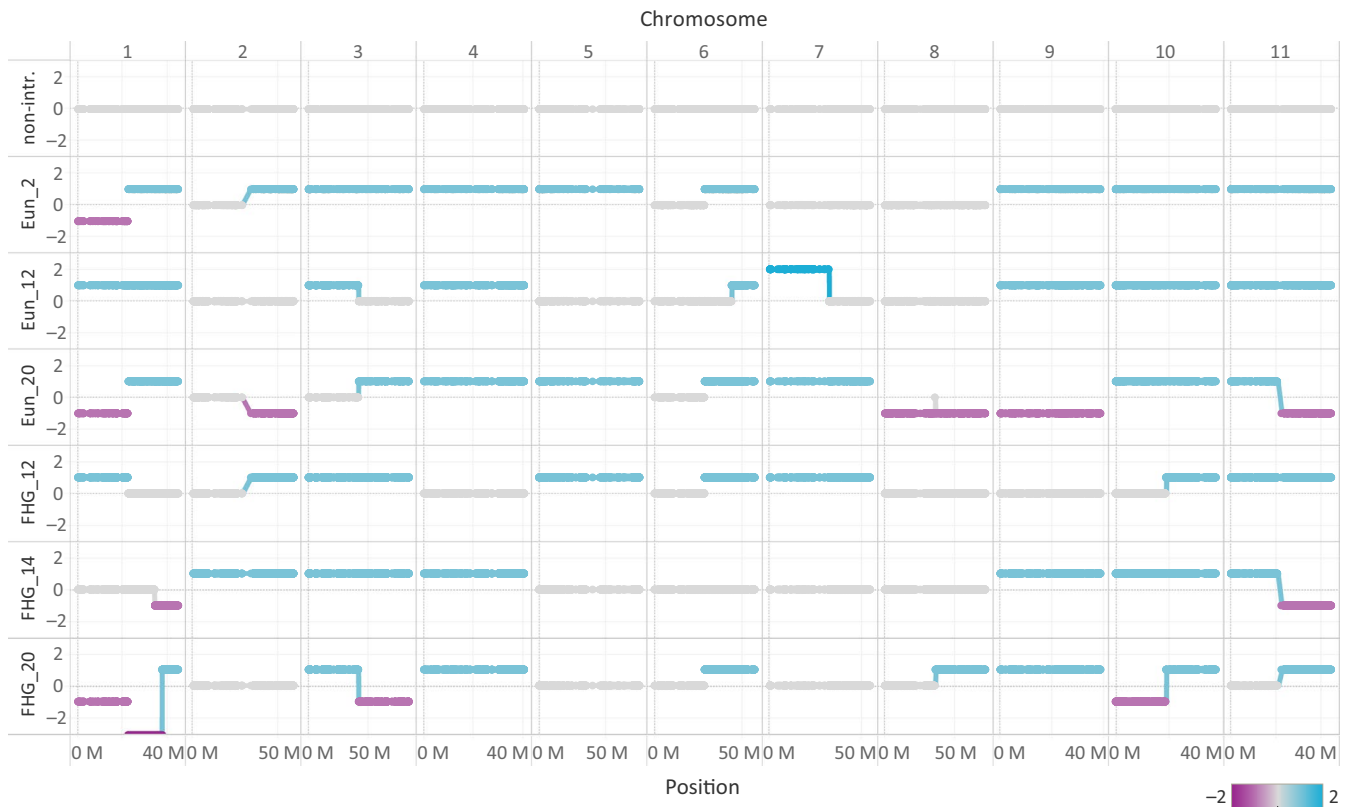


FIGURE 2 Ancestry assignment of chromosomal segments. Assignments are shown to *E. grandis* (anc1, grey), non-*E. grandis* *Latoangulatae* (anc3, cyan) or *Maidenaria*-like (anc2, purple) using *EILA* for one seedling from the Copperlode provenance with no introgression (non-intr.), and selected individuals from Eungella (Eun) and Finch Hatton Gorge (FHG). Each colour dot represents a SNP (7,736 SNPs shown along the 11 chromosome with Mbp positions on the x-axis). Both alleles assigned to *E. grandis* (anc1) = 0; one allele assigned to *E. grandis* and the other to anc2 = -1, both alleles assigned to anc2 = -2, one allele assigned to *E. grandis* and the other to anc3 = 1, both alleles assigned to anc3 = 2, one allele assigned to anc2 and the other to anc3 = -2.5. Breakpoint penalty $\lambda = 30$

sets (Figure S12), the models did not perform as well as those for the north.

Population differentiation patterns derived from PC1-associated markers in the North showed highest correlation with distance to coast, and this was supported by the GDM where changes in distance to coast were most predictive of population differentiation patterns (Figure S13) and the most important predictor variable in the permutation test. Distance to coast was also important in PC2-associated population differentiation (Table S4). As expected, dry period average precipitation (Bio14), soil depth, soil pedality and warmest period maximum temperature (Bio05) were significant predictors in GDMs for Bio14-, soil depth-, soil pedality- and Bio05-associated population differentiation patterns, respectively (Figure S13). Markers associated with PC1 in the Northern subpopulation also revealed unexpected population differentiation patterns (Figure 3c). Population structure PCA split each provenance into three groups along the y-axis. Since this pattern could be indicative of a segregating chromosomal rearrangement (Faria et al., 2019), a genome-wide scan was done for large regions of high LD, which revealed a ~700 kb block containing 17 SNP markers in high LD ($R^2 > 0.8$) on chromosome 11. Population structure across the species range based on North PC1-associated

markers on chromosome 11 separated provenances into three clusters (Figure 3d).

Gene Ontology term enrichment for biological processes was tested for 13 environment-associated SNP sets against the full SNP-captured gene space of 13,014 genes (Table S5). Defence response terms were enriched in all query sets, and signalling processes were enriched for environment-associated loci in the Southern subpopulation (Table S6). No enrichment was detected for the control data sets.

4 | DISCUSSION

We conducted a population genomics study on the *E. grandis* native species range. With 33 provenances genotyped, this study represents the most comprehensive analysis of the natural genetic diversity for the species (Jones, Shepherd, Henry, & Delves, 2006; Silva-Junior & Grattapaglia, 2015; Song et al., 2016). With the exception of Silva-Junior and Grattapaglia (2015) who used variants detected from pooled resequencing data of two provenances, this is also the best genome coverage achieved with one informative SNP for every 25.5 kb of the genome. Because we aim to test hypotheses

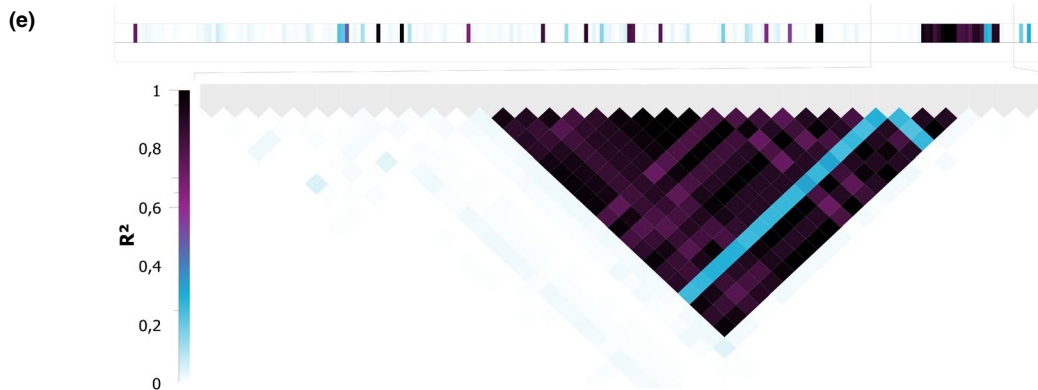
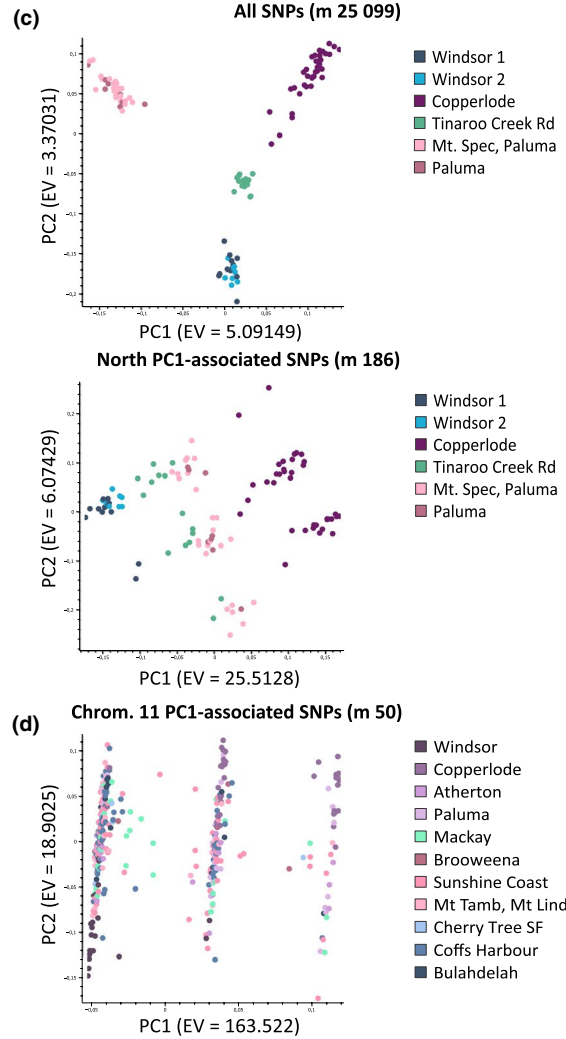
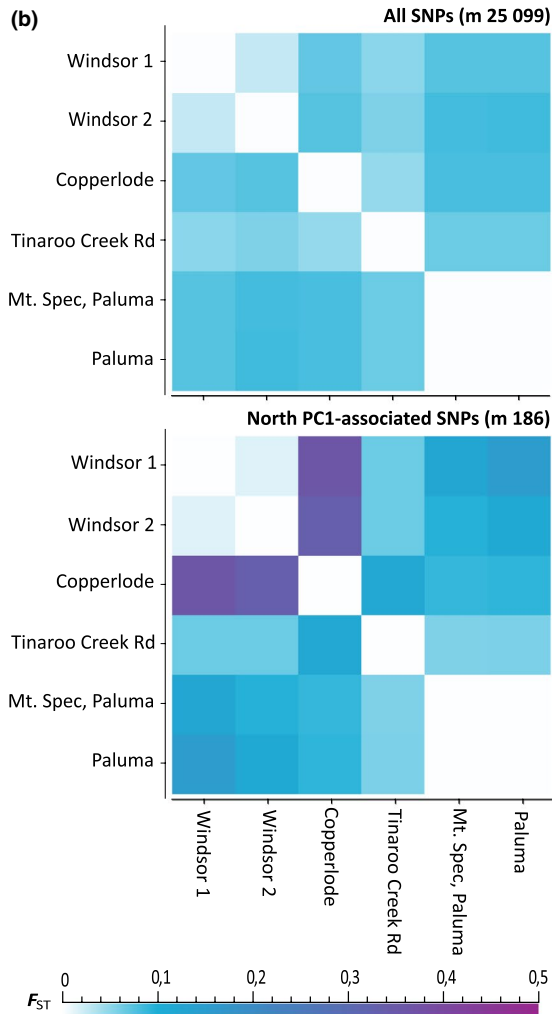
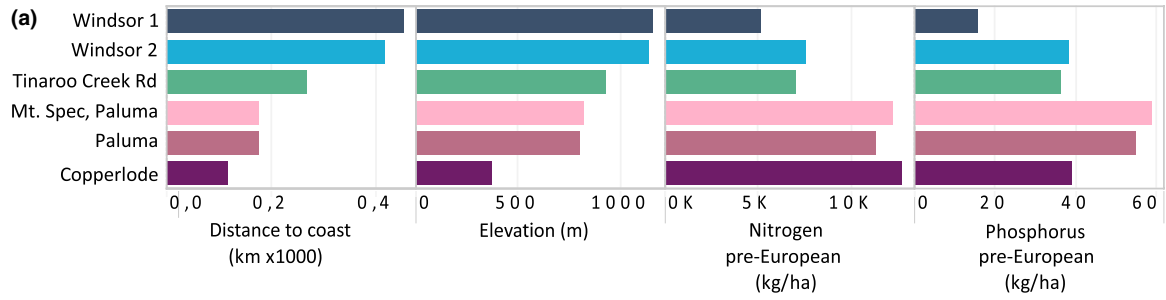


FIGURE 3 Environmental association analysis for PC1 of environmental variables in the Northern subpopulation of *E. grandis*. (a) Northern subpopulation environmental factors contributing to PC1 (Table S3) with loadings >0.7. Colour shades for different provenances in the Northern subpopulations match that in c, and provenances are ordered as furthest from coast to closest to coast. Environmental data layers were obtained from Atlas of Living Australia (occurrence data download at <http://www.ala.org.au>, accessed 28 November 2019; Figure S4). (b) Heatmap of F_{ST} estimates as determined using all 25 099 SNPs (top) and 186 PC1-associated SNPs (bottom). (c) PCA for population structure based on all 25 099 SNPs (top) and 186 PC1-associated SNPs (bottom) with eigenvalues indicated in parentheses. (d) Species-wide population structure based on Northern subpopulation PC1-associated SNPs on chromosome 11 separates the population into three genotypic classes. (e) Heatmap of pairwise LD, measured as squared correlation (R^2), for 186 PC1-associated SNPs on chromosome 11 with an enlargement of the 736 kb LD block of 17 SNP markers

generated in this study in comprehensive common garden trials, our sampling strategy was limited to available single-tree seedlot collections with sufficient seed for the establishment of such trials, and seedlings were used as proxy of the genotypes of standing trees from which seeds were derived. Consequently, some provenances were not adequately represented to allow their inclusion for population differentiation estimates. Still, we were able to interrogate the genetic space for putatively adaptive genetic variation, and uncover, to our knowledge, the first genomic evidence of ongoing interspecific introgression in *E. grandis*. We also describe the first observation of a potential chromosomal structural variant in the genus.

Suspected introgression was assessed at subchromosomal resolution by modifying the *EILA* approach (Yang et al., 2013) for mapping the ancestry of genomic segments. In most studies investigating genomic signatures of hybridization, the parental species are known and genotyped (e.g. Christe et al., 2017; Suarez-Gonzalez, Hefer, Lexer, Cronk, & Douglas, 2018). Since the introgressing species in our study was unknown, we devised a method to leverage the comprehensive species-wide genetic variation captured in our data set against interspecific genetic variation represented by non-*E. grandis* species as published by Silva-Junior et al. (2015). Power to detect non-*E. grandis* ancestry was limited in the closely related species, *E. saligna*, where genomic assignments were equivalent to one allele originating from *E. grandis* and the other from non-*E. grandis* species of section *Latoangulatae* (Figure S3). This was in part due to the recent divergence of the two species, and partly due to the restricted number of *E. saligna* individuals represented in anc3. Despite this limitation, the modified approach performed well in the samples of known ancestry at breakpoint penalty 30 and higher.

Genome-wide ancestry mapping revealed extensive introgression in Finch Hatton Gorge and Eungella provenances in the Mackay subpopulation (Figure 2 and Figures S8 and S9). Introgressed genomic segments in seedlings from the Brooweena provenance, which is at the northern edge of the Southern subpopulation, were fewer and larger than introgressed segments seen in Finch Hatton Gorge and Eungella, even at $\lambda = 15$ (Figure S10), suggesting that this represents a different introgression event. Changes in precipitation were most predictive of the population differentiation pattern of the species when the introgressed provenances were included in the GDM (model A), particularly at lower dry period average precipitation (Bio14) values (Figure S11). The eastern coastline of Australia was wetter and greener than present, up to 5,000 years ago, when the onset of El Niño Southern Oscillation conditions resulted in increased aridity and variable climates (Donders, Haberle, Hope,

Wagner, & Visscher, 2007; Marx, McGowan, & Kamber, 2009). Our hypothesis is that *E. grandis* occurred as a continuous population until the region between the present-day Northern and Southern subpopulations became drier, and hybrid individuals began to outperform the pure species. In response to increased aridity, the Mackay provenances potentially fall within the continuum between introgression and hybrid speciation (Nieto Feliner et al., 2017).

Natural hybridization has been reported in several eucalypts (Griffin, Burgess, & Wolf, 1988; McKinnon, Vaillancourt, Jackson, & Potts, 2001; Whitham, Morrow, & Potts, 1994; Willis et al., 2004). For *E. grandis*, hybrids with other species in section *Latoangulatae* have been reported based on morphological features (Jacobs, 1981). However, as shown by Holman, Hughes, and Fensham (2003), morphological traits attributed to hybridization may in fact be the result of within-species adaptation to an environmental cline. This study provides the first molecular evidence of extensive interspecific introgression in *E. grandis* populations. Our study may also shed some light on the frequency of natural hybridization events in eucalypts. We observed up to 87 seedlings with non-*E. grandis* genomic segments (16% of genotyped individuals, excluding Eungella, Finch Hatton Gorge and Brooweena provenances) representing 58 families (Table S7). However, this does not seem to translate into pervasive introgression outside Mackay and Brooweena, suggesting reduced fitness in hybrids or rare hybridization events followed by neutral evolution of introgressed segments in the rest of the species' range. Differential introgression patterns in the introgressed provenances may underlie genomic regions containing genes involved in reproductive isolation and differential adaptation between *E. grandis* and the introgressing species (see review by Harrison & Larson, 2014), prompting further interrogation of non-introgressed versus introgressed genomic segments in the future.

We identified adaptively enriched SNP sets associated with environmental clines to expose subtle adaptively enriched population structure not discernible using the total SNP set of 25,099 loci (enriched for "neutral" variants). In light of the differences in gene flow patterns and differing correlations among environmental factors in the two subpopulations, we chose an EAA approach that would detect environment-associated loci given low levels of dispersal, variable demographic scenarios and varying selection intensity (De Villedenreuil, Frichot, Bazin, François, & Gaggiotti, 2014; Forester, Jones, Joost, Landguth, & Lasky, 2016; Lotterhos & Whitlock, 2015). We excluded introgressed provenances from the EAA, since the presence of hybridization could result in spurious associations (Hoban et al., 2016). Our approach

was modelled on that of Ingvarsson and Bernhardtsson (2019) who used LFMM (Frichot & François, 2015; Frichot, Schoville, Bouchard, & François, 2013) to identify loci associated with environmental clines and performed GDM to compare the genetic change caused by isolation by distance to that caused by changes in climate. In the absence of common garden trials to test putatively adaptive genetic variation, our strategy was to find indirect support for our delineation of the adaptively enriched genetic space. Following the rationale of Steane et al. (2014), our hypothesis was that adaptively enriched SNP sets would reveal population differentiation patterns that correlate with environmental clines and deviate from the demography of the species, as observed for North PC1-associated SNPs (Figure 3c). Furthermore, we hypothesized that genes in LD with SNPs in the adaptively enriched sets would have functional enrichment for biological processes related to biotic and abiotic stress responses when compared against the total SNP-captured gene space.

Given the SNP density and predicted LD decay (Silva-Junior & Grattapaglia, 2015), we did not remove the small proportion of SNPs that were in high LD. This fortuitous decision led to the discovery of a putative large structural variant on chromosome 11. Fluctuations in LD can be ascribed to the genomic architecture of, for example centromeric regions; however, the abrupt decay from near-perfect LD to no LD directly adjacent to the region on chromosome 11 supports the hypothesis that this is more likely a chromosomal rearrangement (Todesco et al., 2020). To our knowledge, this is the first report of such a structural polymorphism in *Eucalyptus*. Since all three karyotypes were observed across the species' range (Figure 3d), we conclude that the polymorphism predates the split between the three subpopulations. Although it encompasses a genomic segment of approximately 700 kb (Figure 3e), it still only represents a single variant, and thus, we cannot exclude the possibility that the elevated allele frequency of the minor homokaryotype in Copperlode is due to neutral evolutionary processes such as genetic drift, as opposed to it conveying some adaptive advantage.

This study highlights the variable selection landscape in which a single species can evolve. We observed contrasting population stratification patterns between the Northern and Southern subpopulations in terms of neutral and potentially adaptive genetic variation. The fragmented distribution in the Northern subpopulation represents an aggregated landscape that may result in more discernible local adaptation (Forester et al., 2016). Loci associated with PC1 (Figure 3) differentiated the subpopulation according to the correlated environmental cline. In contrast, we observed a lack of adaptive differentiation in the South. This could be due to the strength of local adaptation; in the presence of undisrupted gene flow and highly heterogeneous landscapes, as observed in the Southern subpopulation, local adaptation strength is predicted to be low (Forester et al., 2016). However, our inability to detect discernible adaptive population differentiation in the Southern subpopulation may also have been due to insufficient sampling depth for some southern provenances. In addition, the high value of K derived from PCA and sNMF, compared to $K = 4$ obtained for the Northern subpopulation,

may have resulted in overcorrection for demography and increased false negatives (Forester et al., 2016).

Elevation and distance to coast are steady environmental variables in terms of accuracy in measurement and consistency over very long periods of time. All other factors, especially climatic variables, are subject to fluctuations within and between seasons. Distance to coast and elevation are also complex variables and perhaps represent a better proxy of the ecosystem in which the species is adapting. Since these two factors were important and significant in most of the GDM analyses that resulted in well-fitted models, they could serve as reliable proxies for the environmental cline shaping local adaptation in *E. grandis*. To pinpoint more accurately specific loci underlying adaptation to climates across seasons and years would require higher marker density, increased sample size, daily climate measures over several years and greater computation power than we could achieve in the present study (Harfouche et al., 2019).

Protein domains underlying adaptive variation have been shown to be highly expanded in eucalypts (Kersting, Mizrahi, Bornberg-Bauer, & Myburg, 2015). *E. grandis* also has among the highest prevalence of tandem gene duplications, known to harbour genes involved in adaptive processes (Butler et al., 2018; Hanada, Zou, Lehti-Shiu, Shinozaki, & Shiu, 2008), of any sequenced plant genome (Myburg et al., 2014). This could complicate GO enrichment analysis for genes presumed to underlie adaptation. The problem is confounded when the sampled genetic space has been biased to single-copy genomic regions as is the case for the informative SNPs used in this study (Silva-Junior et al., 2015). In spite of these potential difficulties, terms representing very large gene families in the *E. grandis* genome (e.g. "protein phosphorylation" and "signal transduction" terms amount to 3,845 and 2,544 genes, respectively) were enriched in one of the larger query sets, "South all environment-associated." Signal transduction is particularly relevant in fluctuating environments since proteins involved in these pathways are responsible for relaying intra- and extracellular stimuli detected by cellular receptors to responses in the organism (Huber, 2007). Protein phosphorylation and signal transduction are also important in biotic stress response, which aligns with the "defence response" GO term that was enriched in all environment-associated sets, suggesting that the adaptive gene space includes genomic regions underlying responses to abiotic and biotic stress.

5 | CONCLUSIONS

We have shown that particular provenances of *E. grandis* may have captured adaptive genetic variation from other species through hybridization. Adaptive introgression, if not properly assayed, could reduce the accuracy of species distribution models under future climate conditions (Aitken, Yeaman, Holliday, Wang, & Curtis-McLane, 2008). Hybrid success is generally low in environments to which the parental species are adapted (Rieseberg et al., 2003). Still, with low dispersal rates (Potts, 1990) and long generation times,

introgression may prove to be the leading mode for eucalypts to adapt to rapid climate change. Our modified ancestry mapping approach, using genus-wide genetic variation to identify genomic regions originating from alternative species, could prove valuable in studies of eucalypts and other species for which genus-wide genetic variation data are available. This is also a useful tool for breeding populations where hybrids between geographically separated species with weak reproductive isolation barriers occur. The method permits an estimate of the extent of introgression and identification of broad genomic regions that tend to be retained preferentially, which could be used to decipher the genomic architecture underlying reproductive isolation and local adaptation.

The predominant environmental factors predictive of population differentiation patterns not explained by geographic separation included distance to coast, elevation, wettest period precipitation (Bio14) and pre-European soil phosphorus content. Although we only considered abiotic environmental variables, GO enrichment analysis of genes in LD with associated SNPs provides support that these loci underlie adaptation to biotic as well as abiotic stresses. Other studies of adaptive genetic variation in the genus have identified abiotic factors affecting species distributions (Ahrens et al., 2019; Dillon et al., 2014; Jordan, Hoffmann, Dillon, & Prober, 2017; Steane et al., 2014), and we hope to add some understanding regarding the complexity of the selection forces driving evolution in eucalypts. Due to the economic importance of the species, large common garden *E. grandis* trials are being established by Camcore (NC State University, Raleigh, NC) across various exotic environments, which will include 108 of the families represented in our study. Two of the sites established in South Africa will be expanded to include all of the families sampled here, with $n \approx 5,000$ seedlings, each. These trials will serve as experimental populations with which to test hypotheses generated from this discovery project.

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AUTHOR CONTRIBUTIONS


The idea was developed by MO and AM. The design of the study was contributed by all authors. Biological material was contributed by DL. The data were generated and analysed by MO, and the first draft of the manuscript was written by MO. The final manuscript was read, edited and approved by all authors.

DATA AVAILABILITY STATEMENT

The genomic data analysed in this study are available online via the Dryad archives under accession <https://doi.org/10.5061/dryad.p8cz8w9kp> (Mostert-O'Neill et al., 2019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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