ORIGINAL ARTICLE

Latitudinal variation in climate-associated genes imperils range edge populations

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Abstract

The ecological impacts of increasing global temperatures are evident in most ecosystems on Earth, but our understanding of how climatic variation influences natural selection and adaptive resilience across latitudes remains largely unknown. Latitudinal gradients allow testing general ecosystem-level theories relevant to climatic adaptation. We assessed differences in adaptive diversity of populations along a latitudinal region spanning highly variable temperate to subtropical climates. We generated and integrated information from environmental mapping, phenotypic variation and genome-wide data from across the geographical range of the rainbowfish *Melanotaenia duboulayi*, an emerging aquatic system for studies of climate change. We detected, after controlling for spatial population structure, strong interactions between genotypes and environment associated with variation in stream flow and temperature. Some of these hydroclimate-associated genes were found to interact within functional protein networks that contain genes of adaptive significance for projected future climates in rainbowfish. Hydroclimatic selection was also associated with variation in phenotypic traits, including traits known to affect fitness of rainbowfish exposed to different flow environments. Consistent with predictions from the "climatic variability hypothesis," populations exposed to extremes of important environmental variables showed stronger adaptive divergence and less variation in climate-associated genes compared to populations at the centre of the environmental gradient. Our findings suggest that populations that evolved at environmental range margins and at geographical range edges may be more vulnerable to changing climates, a finding with implications for predicting adaptive resilience and managing biodiversity under climate change.

KEYWORDS
adaptive resilience, aquatic biodiversity, climate change, freshwater fish, landscape genomics, rainbowfish

1 | INTRODUCTION

Latitudinal gradients provide ideal natural experiments to test general ecosystem-level theories about a changing climate (Frenne et al., 2013; Hoffmann & Sgrò, 2011; Oldfather et al., 2020; Scheffers et al., 2016). They show annual and seasonal variation in climate, which defines the fundamental niche of organisms (especially for ectotherms), patterns of local and regional adaptation, as well as

genetic diversity (Adams & Hadly, 2013; Currie et al., 2004; Umina et al., 2005). The scope of populations spread over latitudinal gradients to buffer climate change may be shaped by genetic diversity generated through exposure to a variable environment, as inferred by the climatic variability hypothesis, CVH (Deutsch et al., 2008; Janzen, 1967; Tewksbury et al., 2008). The general expectation of the CVH is that populations that evolved under more variable climatic regimes would show less sensitivity to climate change. A balance between the extent of phenotypic plasticity and the capacity for local adaptation, modulated by the environmental gradient and selection (Chevin et al., 2010; Polechová & Barton, 2015), is expected to define the latitudinal limits for how species will persist into the future.

One way to understand whether a species is likely to adapt to climate change is to measure evolved differences in climate-dependent traits along latitudinal gradients (Umina et al., 2005). This is difficult for nonmodel species due to a lack of information about adaptive traits and their distribution across geographical and climatic ranges. The rapidly growing field of landscape genomics circumvents some of the issues of experimentally measuring adaptation in wild populations (Grummer et al., 2019; Manel et al., 2018; Schoville et al., 2012). Landscape genomics integrates spatial and environmental analyses of population genomic data across heterogeneous landscapes to address previously intractable questions, such as forecasting of adaptive capacity (Grummer et al., 2019). Recent developments in environmental and niche mapping and in landscape genomic analyses of associations among genotypic, environmental and phenotypic variation have improved our ability to identify signals underlying adaptive evolution, including signals of polygenic adaptation (Bragg et al., 2015; Forester et al., 2018; Grummer et al., 2019; Wellenreuther & Hansson, 2016). This is important given the increasing evidence that adaptation to environmental change often proceeds by allelic covariances among large numbers of small-effect polygenes, which are individually hard to detect (Bernatchez, 2016; Grummer et al., 2019; Pritchard & Di Rienzo, 2010; Wellenreuther & Hansson, 2016).

Climate-driven impacts are predominantly apparent in aquatic ecosystems, where the pace of change threatens to outstrip the ability of species to adapt (Crozier & Hutchings, 2014; Munday et al., 2013; Scheffers et al., 2016). Of particular concern are freshwater fishes, a group highly susceptible to decline following disturbance and that shows some of the highest extinction rates among vertebrates (Burkhead, 2012). Here we use a range-wide landscape genomics approach to investigate differences in adaptive variation and resilience to climate change in an abundant subtropical freshwater fish, the rainbowfish *Melanotaenia duboulayi*. We achieve this by examining phenotypic and genomic variation in hydroclimate-related traits (both phenotypic and genomic) along a latitudinal gradient. The species is found along a coastal latitudinal region (~22–31°S) in eastern Australia spanning a transitional zone from highly variable temperate hydroclimatic conditions to more consistent subtropical environments. This is also the same region where a climate change shift was reported

for a classic genetic cline in *Drosophila melanogaster* (Umina et al., 2005).

Rainbowfishes are an emerging aquatic system to study adaptation to climate change. Their low dispersal potential and marked genetic structure between river catchments, coupled with broad distribution and local abundance, makes them a powerful field model (Brauer et al., 2018; McGuigan et al., 2003, 2005; Unmack, 2001; Unmack et al., 2013). Previous studies attest to the suitability of rainbowfishes for investigating how climatic variation influences adaptive resilience. From the perspective of assessing fitness linked to contemporary changing environments, rainbowfishes show body shape divergence in fin positioning and length associated with varying hydrology, such as different stream flow rates (McGuigan et al., 2003). This pattern observed in wild-caught rainbowfish is heritable, as it was retained in offspring raised in a common garden experiment (McGuigan et al., 2003). Repeated evolution of this same heritable phenotype in populations of two *Melanotaenia* species (*M. duboulayi* and *M. eachamensis*) suggests that variation in phenotypic traits that influence fitness in rainbowfish might be a product of selection linked to the hydroclimatic environment (McGuigan et al., 2003, 2005). From the perspective of assessing adaptive resilience to future climates, mechanistic experiments of global gene expression have shown substantial plastic transcriptional responses in *M. duboulayi* (Smith et al., 2013), as well as adaptive (i.e. genetically based) plasticity due to ecotype-specific directional selection (Sandoval-Castillo et al., 2020) in a projected 2070 summer temperature. The latter study discovered a very strong association between transcriptome responses in projected future climates and thermal tolerance limits in three rainbowfish species from different climatic regions, with the subtropical *M. duboulayi* showing the strongest adaptive resilience (Sandoval-Castillo et al., 2020). In addition, transgenerational experiments in *M. duboulayi* provided pedigree-based support for a heritable basis to plastic responses in future climates (McCairns et al., 2016). These studies clarified pathways enriched for heat stress genes underpinning the adaptive thermal responses (McCairns et al., 2016; Sandoval-Castillo et al., 2020), suggesting that subtropical rainbowfish can respond and adapt via heritable plasticity to projected summer climates.

Here, we capitalize on the existing resources and knowledge for rainbowfishes to implement an integrative study of adaptive resilience along a latitudinal gradient. We predict that rainbowfish populations that evolved in regions with variable hydroclimate (e.g., at the centre of the environmental gradient) will show higher variation in genes that are most likely to respond to changing climates than those that evolved in more stable regions (e.g., at the environmental edges). To assess this, we compare information from environmental mapping, phenotyping of adaptive traits and genome-wide data for 21 populations of *M. duboulayi*. Our study integrates correlative analyses to identify genotype–phenotype–environment (GxPxE) links along the range of *M. duboulayi*. Testing this three-way association provides power for detecting causal genetic variants underlying ecological adaptation

and for characterizing adaptive resilience to changing environments (Bragg et al., 2015; Grummer et al., 2019; Wellenreuther & Hansson, 2016).

2 | MATERIALS AND METHODS

2.1 | Sampling along latitudes and at the range edges

We collected a total of 420 adult *Melanotaenia duboulayi* from 21 sites that cover the geographical range of this coastal and latitudinally distributed freshwater species (Figure 1; Table S1). We amassed samples from multiple rivers within each broader catchment, and repeated this sampling effort across catchments. This design enables testing for hierarchical population structure predicted due to riverine network arrangement in nonmigratory fish species, such as

the pattern disclosed in the closely related rainbowfish *M. fluviatilis* (Brauer et al., 2018). Individuals were collected using seine nets and traps between 1997 and 2012, and their tissue samples were snap frozen in liquid nitrogen and stored at −70°C in the Australian Biological Tissues Collection at the South Australian Museum, Adelaide. DNA was extracted using a salting out method (Sunnucks & Hales, 1996). The quality and concentration of DNA were estimated via spectrophotometry using a Nanodrop 1000 (Sigma).

Given our latitudinally arranged study region, we are in a position to use "range edge population" in the strict geographical context of the term. Although our findings (discussed herein) might suggest that geographical range edges in our system might be comparable with environmental or climatic range edges, it is important to clarify a distinction between these terms. Geographical range edges (e.g., northernmost, southernmost) are often weakly concordant with environmental or climatic edges (e.g., the extreme position along a climate axis beyond which abundance drops to 0) (Oldfather
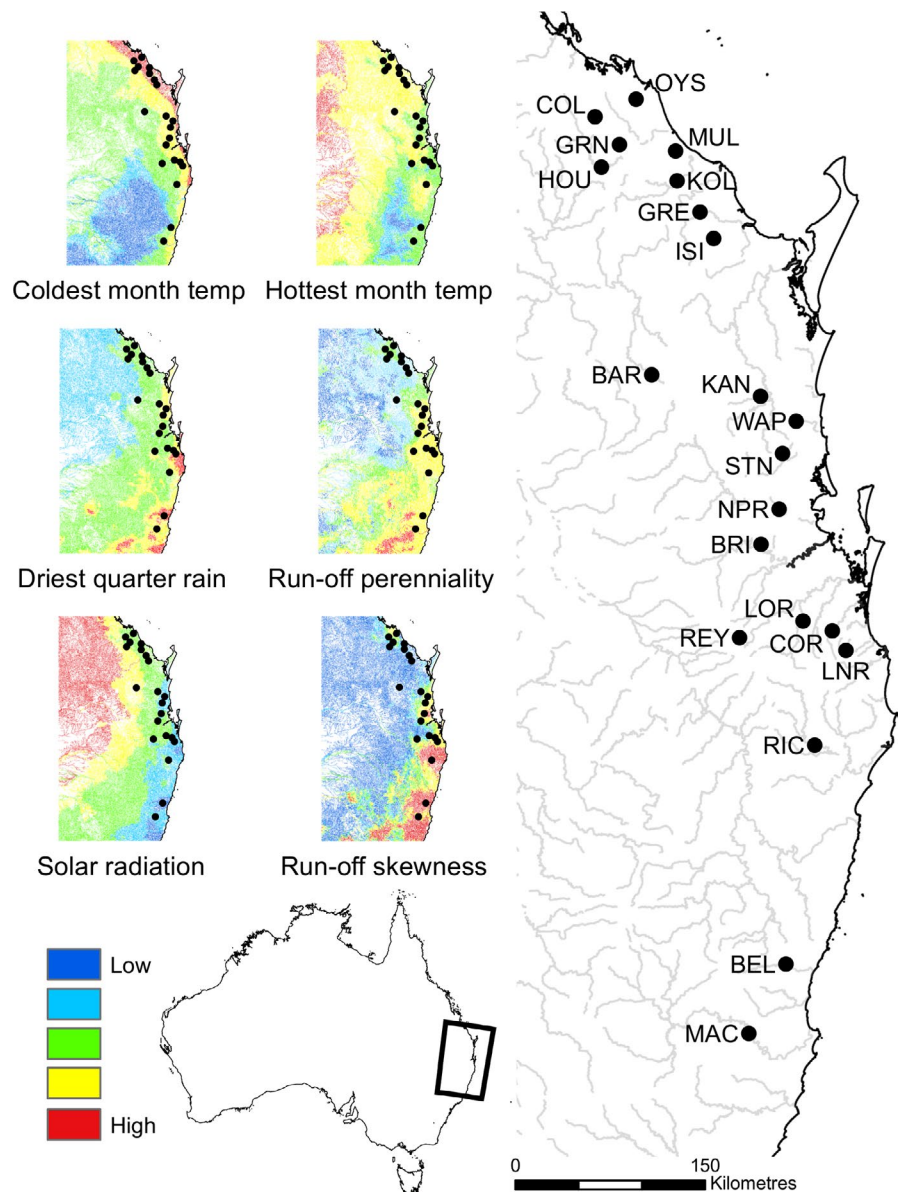

FIGURE 1 Map of eastern Australia showing the 21 sampling sites across the latitudinal range of the rainbowfish *Melanotaenia duboulayi*. Smaller maps on the left show the relative values of six environmental variables used for the GEA (genotype–environment) and GxPxE (genotype–phenotype–environment) association analyses. Location codes are as in Table S1

et al., 2020), because the complexity of spatial structure of range edges is thought to be a product of the interaction among climate heterogeneity, collinearity among climate variables, and spatial scale (Oldfather et al., 2020).

2.2 | Environmental mapping

High-resolution environmental data were obtained for six variables that characterize the heterogeneous aquatic landscape of eastern Australia (Figure 1). Environmental data were obtained from the Australian Hydrological Geospatial Fabric (GeoscienceAustralia, 2011; Stein et al., 2014). The six catchment-scale hydroclimatic variables used were: average minimum temperature during the coldest month (CATCOLDMTHMIN), mean maximum temperature during the hottest month (CATHOTMTHMAX), mean rainfall during the driest quarter (CATDRYQRAIN), percentage contribution to mean annual discharge by the six driest months of the year (RUNPERENNIALITY), mean annual solar radiation (CATANNRAD) and skewness (median/mean) of annual run-off (RUNSKEWNESS). These variables were selected to capture hydroclimatic variation across the latitudinal study area (Figure 1). They are of relevance for adaptation to flow environments in rainbowfish (McGuigan et al., 2003) and are known to influence both neutral and adaptive genetic diversity in Australian freshwater fishes (Attard et al., 2018; Brauer et al., 2016), including in a related species of rainbowfish from an adjacent temperate river basin (Brauer et al., 2018).

2.3 | Genome-wide data collection

Population genomic data were generated in the form of SNPs (single nucleotide polymorphisms) via a double digest genotyping by sequencing (GBS) approach (Poland et al., 2012; modified from Elshire et al., 2011) using enzymes *Pst*I and *Mse*I. Individual samples were pooled in equimolar amounts into groups of 48 samples. Library preparations for SNP genotyping were performed at the Institut de Biologie Intégrative et des Systèmes (IBIS) and sequenced at the Genome Quebec/McGill University Innovation Center in 10 separate lanes of a HiSeq2000 (Illumina) as 100-bp single-end sequencing. Individual sample data were demultiplexed and trimmed such that they were all 94 bp long using NGS QC TOOLKIT (version 2.2.3) (Patel & Jain, 2012). Raw sequences were also subjected to quality filtering with the same toolkit to remove any sequence reads that did not meet the minimum requirement of ≥90% of bases exceeding a PHRED quality score of 20. Individual SNP loci were identified de novo via STACKS version 1.3 (Catchen et al., 2011, 2013). Stacks were only accepted based on a minimum stack depth of three reads with no more than 2-bp mismatches. The deleveraging algorithm was used to resolve multiple merged reads and the removal algorithm was implemented to drop highly repetitive stacks. Loci identified for each individual were merged into an overall catalogue of loci for all samples, based on a maximum mismatch of five positions among

reads, using the pipeline component CSTACKS. Only individual loci that appeared in the catalogue were accepted as true. Usable loci for population-level analyses were selected based on the requirements that they appear in more than 80% of the individuals of at least 18 of the 21 populations, with a local minor allele frequency above 0.01, and only SNPs that were biallelic. Also, only the first SNP per locus was included in the analysis to minimize the likelihood of linkage between SNPs. Numbers of reads retained at each filtering step are shown in Table S2.

2.4 | Population structure analysis

Population differentiation (F_{ST}), expected heterozygosity (H_E), and observed heterozygosity (H_O) were estimated in ARLEQUIN version 3.5 (Excoffier & Schneider, 2005) and F_{IS} was estimated with the R package HIERFSTAT (Goudet, 2005) (Tables S1 and S7). Presence of genetic structure within our data set was investigated with the Bayesian model-based clustering method in the program FASTSTRUCTURE using no a priori information about cluster numbers and limits (Raj et al., 2014). We used GENODIVE 2.0b27 (Meirmans & Van Tienderen, 2004) to perform a hierarchical analysis of molecular variance (AMOVA) grouping sampled sites according to catchment to test for the presence of hierarchical structure.

2.5 | Geometric morphometrics

Voucher specimens of *M. duboulayi* were sourced from the South Australian Museum (Adelaide), the Australian Museum (Sydney) and the Queensland Museum (Brisbane). All specimens were pinned flat horizontally and digitized with a Canon EOS 50D SLR with EF-S 60-mm lens. Eleven homologous landmarks (McGuigan et al., 2003, 2005) were captured using TPSDIG2 version 2.17 (Rohlf, 2015): anterior point of upper snout, origin of first dorsal fin (DF), insertion of first DF, origin of second DF, insertion of second DF, posterior point of the caudal peduncle dorsally, posterior point of the caudal peduncle ventrally, insertion of anal fin, origin of anal fin, origin of pelvic fins and gill juncture. A total of 210 fish from 15 sites were digitized. These cover a large portion of the species range and include sites Stoney Creek (STO, −26.933,289 and 152.766,800) and Littabella Creek (LIT, −24.714,698 and 152.083,000) which were only used for the phenotype × environment analysis because no SNP data were available for these localities. Four additional curve points along the lateral line were placed to mathematically unbend the few specimens that became distorted during storage using TIPSUTIL64 version 1.68 (Rohlf, 2015). Generalized procrustes analysis (Rohlf & Slice, 1990) was used to remove nonshape-related information such as size, position and orientation. The presence of outliers and allometry was tested using MORPHOJ 1.06c (Klingenberg, 2011). We found that 16.9% of shape differences ($p < 0.05$) were explained by the centroid size and thus the resulting allometric effect was removed by regression and the residuals were kept for further analyses. A

principal component analysis of the weight matrix was made in MOR-PHOJ 1.06c to examine overall patterns of shape change (results not shown). The first two principal components (PCs) explained 59.5% of the total variance. PC1 showed latitudinal transition while PC2 separated the sex of fish. As a measure of population differentiation, we used a canonical variate analysis (CVA) in PAST 3.12 (Hammer et al., 2001) that correctly assigned 70.5% of individuals to their site of origin after jackknifing (Table S3). After the initial analyses described above, we used partial least-square analysis (PLS) in TPSPLS version 1.2 (Rohlf, 2003) to assess covariance between shape and the 65 SNP loci that had allele frequencies strongly associated with the six climate-related environmental variables (CATCOLDMTHMIN, CATHOTMTHMAX, CATDRYQRAIN, RUNPERENNIALITY, CATANNRAD and RUNSKEWNESS) and the additional variable LATITUDE. All environmental variables were standardized prior to analysis and a result from PLS analysis was visualized in GGPLOT2 version 2.1.0 in R (R Development Core Team, 2015).

2.6 | Detection of selection

We used multivariate and univariate genotype–environment association (GEA) analyses to test for selection associated with hydroclimatic variation, to assess adaptive divergence among populations and to identify GxPxE links. Partial redundancy analysis (RDA), a multivariate constrained ordination method, was implemented as detailed by Brauer et al. (2018). This powerful GEA approach to identify polygenic adaptation accounts for spatial genetic structure, is robust across a range of demographic scenarios, and offers the best balance between low false positive and high true positive rates in landscape genomic studies (Forester et al., 2018). Across their range, *M. duboulayi* occur in a series of adjacent coastal drainages with a history of connectivity that has given rise to nonlinear spatial genetic patterns among populations. To control for these patterns in the RDA, we combined multivariate regression with Moran's eigenvector maps (MEMs) to identify a set of spatial genetic neighbourhoods describing the spatial components of genetic variation across a range of spatial scales (Galpern *et al.* 2014). We first calculated a Bray–Curtis dissimilarity matrix based on individual SNP genotypes using the VEGAN R package (Oksanen et al., 2015). Spatial *XY* coordinates and the dissimilarity matrix were then used to estimate MEM eigenvectors using the *mgQuick* function from the MEMGENE R package (Peres-Neto & Galpern, 2019), before using a forward selection procedure to identify significant MEMGENE predictors for use as conditioning variables in the RDA. Initially, RDA was performed with all six environmental variables before using a forward-stepwise selection procedure implemented in the PACKFOR R package (Dray et al., 2016) to remove uninformative predictors from the model. Variance inflation factor (VIF) analysis was then used to exclude highly correlated variables using a VIF threshold of 10 (Dyer et al., 2010). The final RDA then evaluated the reduced environmental model, controlling for spatial structure using the retained MEM variables. The significance of the model, as well as marginal

significance of each environmental variable, was assessed by 1,000 ANOVA permutations using the ANOVA.cca function from the VEGAN R package (Oksanen et al., 2015). This function applies a marginal permutation procedure to the residuals of the reduced model after the variance explained by the conditional spatial MEM variables has been removed (Oksanen et al., 2015). The mean locus score across all loci was calculated for each of the first three RDA axes, and individual loci with a score greater than three standard deviations from the mean were considered as GEA candidates (Forester et al., 2015). In addition to RDA, we also used the spatially explicit generalized linear mixed-model approach of Guillot et al. (2014), gINLAnd. This univariate method aims at detecting SNPs displaying outstanding correlation with some environmental variables while controlling for the potential confounding effect of genetic autocorrelation resulting from shared population history. Using population allele frequencies and *XY* coordinates, gINLAnd first uses a random subset of 500 SNPs to estimate parameters describing the spatial covariance structure of the allele frequency data. Then for each locus in the full SNP data set, the spatial model is compared to a model where allele frequencies are additionally dependent on the fixed effect of an environmental variable. The analysis was run for each of the six environmental variables, and log-Bayes factors were used to identify GEA candidates after accounting for spatial structure (log-BF > 10). Finally, to reduce even further the rate of false positives when assessing the signal of selection, we combined the results of the two GEA methods with the outcomes of three traditional F_{ST} outlier approaches. These were the FDIST2 method implemented in the package LOSITAN (Antao et al., 2008), the Bayesian approach of Foll and Gaggiotti (2008) within BAYESCAN, and a hierarchical outlier test implemented in ARLEQUIN (Excoffier et al., 1992). All three methods have been shown to have varying strengths and weaknesses for accurate detection of loci under selection (reviewed by Narum & Hess, 2011) so we took the cautionary approach of combining output data from all three. Only the SNPs that were identified by at least one GEA method and multiple outlier tests were considered as candidate adaptive SNPs. This conservative strategy might increase the risk of removing true candidate adaptive SNPs from the data set, but it better accounts for the issue of false-positive detection of signals of selection in genome scan studies (Grummer et al., 2019).

2.7 | GxPxE analysis

The genomic data were also compared with phenotypic and environmental data (GxPxE) using a two-step approach. Morphological and genetic data in common were available for 126 individuals representing nine populations (BEL, RIC, KAN, REY, GRE, ISI, GRN, MUL, OYS). First, we used the software LFMM version 1.4 (Latent Factor Mixed Models) (Frichot et al., 2013) to test for an association between each of the 17,047 SNPs and the first axis of a Procrustes principal component analysis (Perreault-Payette et al., 2017). The latter was the only shape axis meaningful according to the broken-stick distribution (Legendre & Legendre, 1998). We controlled for

population structure and used a false discovery rate with alpha of 0.01. In a second step, the major allele frequency (MAF) of the GEA candidates was extracted for all genotyped individuals that showed a shape gradient. As a surrogate for multilocus MAF candidate genes, we selected the first four PC axes based on the brokenstick distribution. Then we tested if environmental variables could explain the genetic variation of these candidate SNPs (GxPxE) after controlling for spatial autocorrelation (geographical coordinates of population sites) by performing a partial RDA (Benestan et al., 2016; Frichot et al., 2013; Legendre & Legendre, 1998).

2.8 | Functional annotation

The functional significance of outlier SNPs detected as potentially under selection was assessed using the database for annotation, visualization and integrated discovery (DAVID) (Huang et al., 2008). Functional clusters were inferred for the annotated outlier genes and compared to background genes to test for over-representation of particular categories. DAVID requires candidates to be annotated to provide gene IDs for functional assignment. To achieve the best outcome in terms of inferred gene annotation we first queried the NCBI protein database using the BLAST2GO program via the standard BLASTX setting. The minimum E value score was set to 1.0E-04 with the annotation cut-off threshold set to 55 and the GO level weighting set to 5. Annotation was further supplemented by performing simple BLAST searches against the Swissprot database and against reference transcripts assembled and annotated from a previous RNA sequencing study of the same species (Smith et al., 2013).

3 | RESULTS

3.1 | Genome-wide data and population structure

The population genomic data set was based on a total of 1,139,567,973 raw sequencing reads recovered from 420 individuals, with 1,071,262,531 of these reads retained following quality trimming. Individuals with fewer than 500,000 reads were removed from further analysis, leaving 396 individuals with sufficient read depth across 21 demes (average per sample = 2,679,976; min = 530,234, max = 8,705,835). The read depth average per locus per individual was 22.6 reads (min = 5.5, max = 122.7). Following the filtering pipeline, a total of 17,047 SNPs were retained from the 781,545 variant sites detected in the entire data set (Figure 1; Tables S1 and S2).

The species' overall genetic architecture is characterized by marked population structure (Table S7) and is best represented by six population clusters (Figure S1). Connectivity was inferred mostly between sampled sites within the same catchment, a pattern expected since most catchments separately drain into the Pacific Ocean. The AMOVA results supported a pattern of hierarchical structure in the study system, with a much greater amount of genetic variation (42.3%) explained by differences among catchments
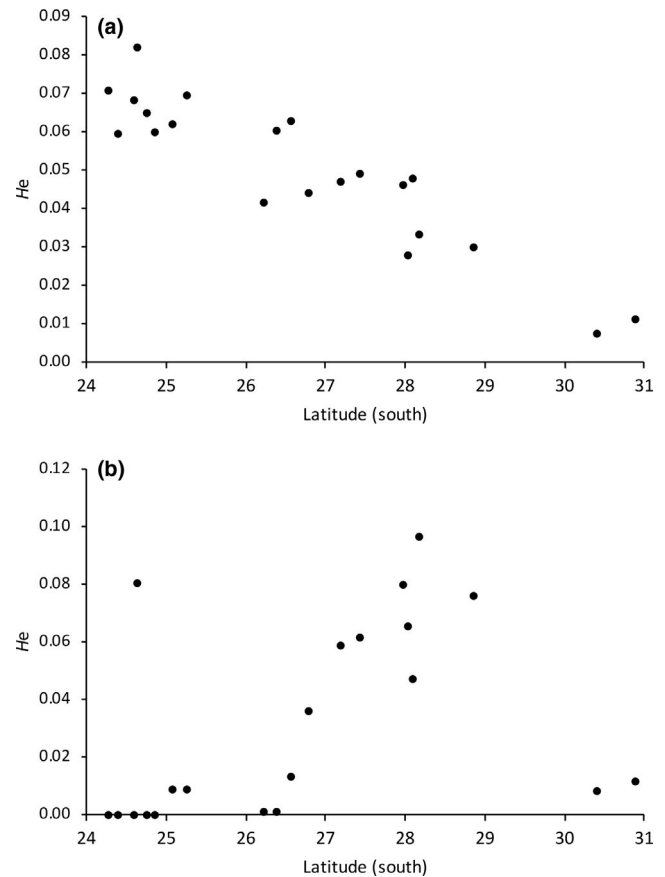

FIGURE 2 Association between latitude and population-level heterozygosity (H_E) for 21 sampled locations of *Melanotaenia duboulayi* based (a) on a total 17,047 SNPs ($r = 0.845$) and (b) on GEA-identified SNPs ($r = 0.150$)

than by differences among sites within catchments (8.4%) (Table S4). Genetic variation showed a strong latitudinal cline in genetic diversity ($r = 0.84$; Figure 2a), with a reduction in heterozygosity (a proxy for population size) towards the southern range. There was also marked phenotypic divergence between populations, with 70.5% of individuals uniquely assigned to their sampling site (Table S3). Phenotypic divergence was largely accounted for by differences in heritable traits linked to hydrodynamics (McGuigan et al., 2003, 2005), such as fin positioning and fin length (Figure 3).

3.2 | Adaptive divergence and candidates for selection

The partial RDA indicated 879 SNPs with allele frequencies associated with a model that included four hydroclimate-related environmental predictors, whereas gINLAnd detected 65 SNPs associated with at least one of the six environmental predictors (Figure 4; Figures S2 and S3). A total of 20 SNPs were common to both approaches. Patterns of genetic diversity for the GEA candidates do not follow the demographically driven latitudinal cline identified for the total SNP data set (Figure 2b), a finding suggestive of local adaptive divergence. For instance, high diversity in GEA candidates

FIGURE 3 Partial least-square (PLS) plot displaying the covariance between body shape of *Melanotaenia duboulayi* (Block 1), the six environmental variables (coldest minimum monthly temperature, hottest maximum monthly temperature, mean rainfall in the driest quarter of the year, run-off perenniality, mean annual solar radiation, run-off skewness) and latitude (Block 2). Data points from each site are colour coded; site abbreviations follow Table S1. Shape changes in warp grids illustrating the two most divergent population groups are shown below the PLS plot. The phenotypic divergence across the range is better explained by adaptive traits previously shown to be linked to hydrodynamics; for example, morphotype on the left has shorter predorsal length and longer second dorsal fin compared to the one on the right
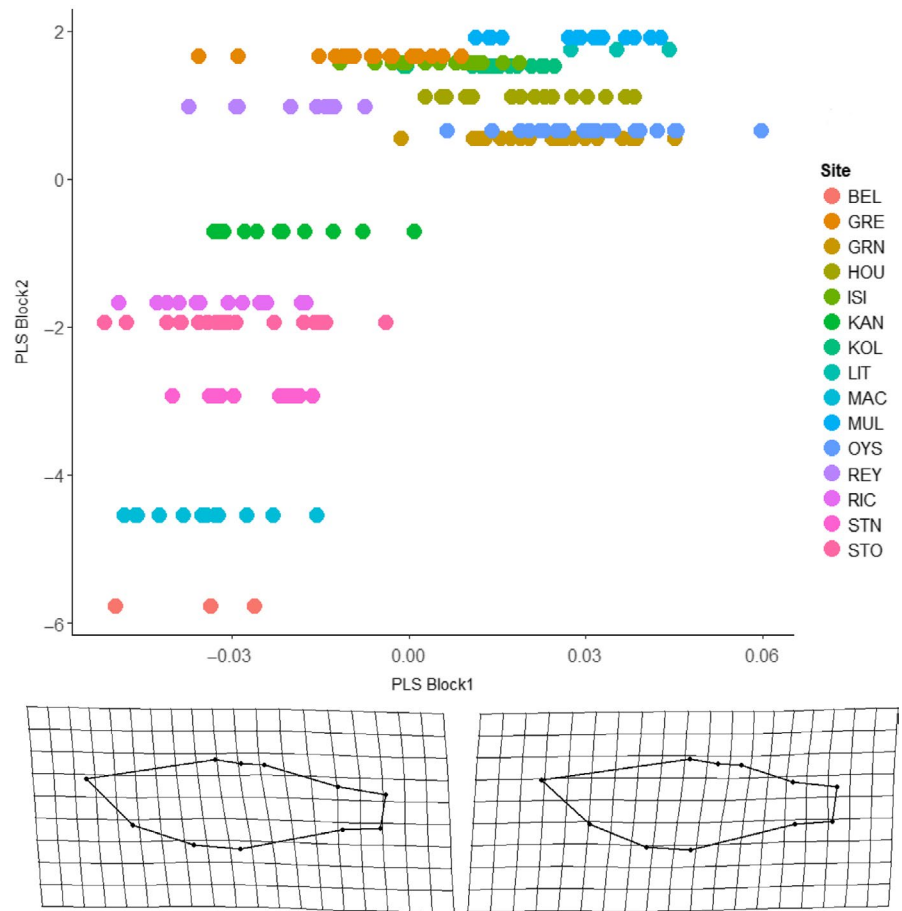
was maintained in midrange populations, whereas range edge populations showed no variation in hydroclimate-associated genes (Figure 2b). Seventeen SNPs were identified by at least one GEA and by multiple outlier tests based on population differentiation; these are robust candidates for SNP loci under selection (Grummer et al., 2019; Lotterhos & Whitlock, 2015; Figure 4; Table S2). Seven of those candidate SNPs could be annotated to functional proteins. These are considered herein as the "seven strong genes" for hydro-climatic adaptation (Figure 5).

3.3 | GxPxE analysis

High correlation was detected between body shape variation across the species range and the six climate-related environmental predictors (r = 0.785; Figure 3). We then compared the genomic data with phenotypic and environmental data (GxPxE) using a two-step approach. This identified 36 candidate adaptive loci related to body shape, which were subjected to an analysis of association with environment. After correcting for spatial autocorrelation, the model was globally significant, explained 14% of the variation of the candidate loci and included three significant variables: annual solar radiation, driest quarter rainfall and run-off skewness (Figure S4). There were no matches between the 36 GxPxE and the GEA candidate SNPs (Figure 4). One annotated GxPxE gene was in common with the

candidate data set based on outlier tests (*lbx1a*). This gene is involved in limb muscle development (Neyt et al., 2000) and thus represents a strong functional candidate for swimming.

3.4 | Functional annotation

Linking functions of candidate genes with ecological factors was facilitated by the availability of a high-quality transcriptome (Sandoval-Castillo et al., 2020) for *Melanotaenia duboulayi*. Twenty-seven of the 65 candidate loci could be assigned gene ontology (GO) terms. Roughly the same proportion (6,012 out of 17,047) could be annotated from the background set of all SNP loci and were used in a functional enrichment analysis. This identified four functional clusters significantly over-represented within the candidates. The clusters included important metabolic and regulatory pathways related to cellular functioning and tissue development shown in other fishes to be associated with adaptation to temperature extremes and flow variation (Table S5; McCairns et al., 2016; Smith et al., 2013).

The seven strong candidate genes have functions expected to be highly important for dealing with variation in temperature and stream flow (see Table S6). Two of these genes were associated with coldest monthly temperature (NHERF1, HIF1A), two with hottest monthly temperature (NLRC3, SIK3) and three with run-off perenniality (ARHGAP1, TRPM7, MCM3AP). Remarkably, these candidates
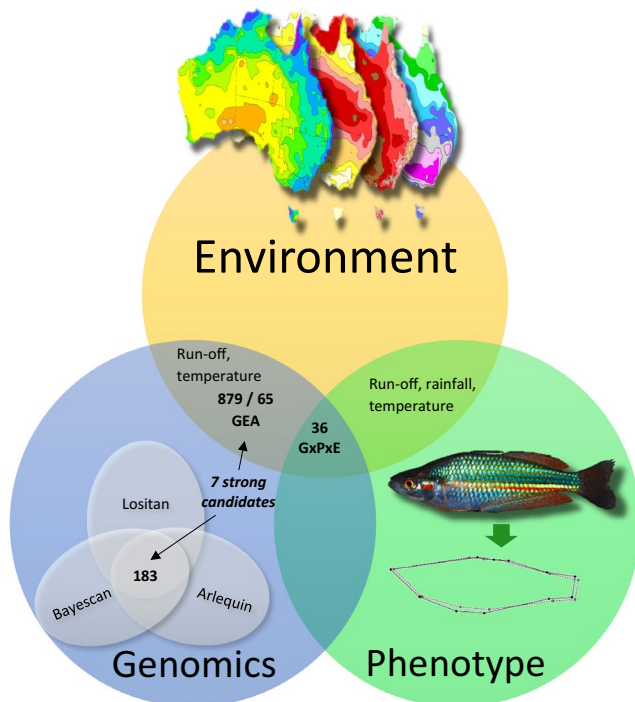
FIGURE 4 Venn diagram showing results of associations between environment, genotype and phenotype for populations along the range of *Melanotaenia duboulayi*. Numbers relate to the candidate adaptive SNPs. GEA relates to multivariate (RDA) and univariate (gINLAnd) analyses that identified candidates due to genotype–environment associations. Results for BAYESCAN, LOSITAN and ARLEQUIN are based on outlier tests. GxPxE relates to genotype–phenotype–environment associations. Also shown are the seven strong candidates (i.e., those identified by GEA and multiple outlier tests and annotated to functional proteins) and the main categories of environmental variables underpinning association results

have frequencies that covary closely with their respective hydroclimatic predictor variables when populations are ordered from minimum to maximum for those predictors (Figure 5). The association can clearly be seen in each case to vary from fixed for the major allele at one extreme of the predictor variable, through heterozygous in the midrange, and then fixed for the alternative allele at the opposite extent of the variable. Thus, diversity is higher within populations exposed to median levels of each environmental predictor than in other populations, indicating balancing selection in midrange populations (Whitehead & Crawford, 2006).

4 | DISCUSSION

Understanding how climatic and environmental variation influences natural selection and adaptive resilience across species ranges remains a major challenge. We implemented an integrative landscape genomics approach across a broad latitudinal region to assess adaptive variation and resilience in rainbowfish, an emerging aquatic model system for studies of climate change adaptation. As expected for a small fish with poor dispersal ability (e.g., Brauer

et al.,2016, 2018), we detected strong and hierarchical population structure consistent with the spatial arrangement of riverine catchments. After partially controlling for spatial population structure and shared population history (but see below for potential limitations), we focused our analyses on the potential effects of climatic and hydrological heterogeneity. These factors are the main drivers of ecosystem structure and function (Hawkins *et al.* 2003; Willig *et al.* 2003) and should influence the combination of adaptive traits favoured in future aquatic communities. From the perspective of contemporary climates, we found evidence for morphological and genomic variation associated with latitudinal gradients in stream flow and temperature. This included variation in heritable traits that affect hydrodynamic fitness in rainbowfish. From the perspective of future climates, some of the genes associated with hydroclimatic selection were found to interact within gene networks in rainbowfish shown to be of physiological relevance for projected future climates. We also found that populations exposed to extremes of important environmental variables showed stronger adaptive divergence and less variation in climate-associated genes.

Rainbowfishes evolved heritable adaptations in fin positioning and caudal red muscle in response to selection linked to different flow environments (McGuigan et al., 2003, 2005). The enriched functional clusters from our GEA candidates could hypothetically underpin these phenotypic differences in rainbowfish. This suggestion is consistent with a short-term climate change experiment that assessed transcriptomic variation (i.e., global variation in gene expression) in wild-caught *Melanotaenia duboulayi* (Smith et al., 2013). That study identified the same GO terms (e.g., proteins PDLIM2, ZNF385a and COX16) found in the most enriched category of our GEA data set. Functional genes in the current study are involved in cytoskeletal organization and signalling (NHERF1, TRMP7), skeletogenesis (SIK3), control of DNA replication in neuronal tissue (MCM3AP), innate immune response (NLRC3) and metabolic responses (ARHGAP1) particularly in hypoxic environments (H1F1A). Although further studies are needed to demonstrate a causal relationship between candidate genes and adaptation, the three-way (GxPxE) association detected here involving traits that affect fitness in rainbowfish (McGuigan et al., 2003) supports a scenario of adaptive divergence linked to hydroclimatic selection along the latitudinal range of *M. duboulayi*.

A growing number of studies have demonstrated that transcriptional responses can have a heritable basis, and that might influence long-term adaptation to divergent thermal environments (Leder et al., 2015; Sandoval-Castillo et al., 2020; Whitehead & Crawford, 2006). In *M. duboulayi*, evidence that changes in gene expression associated with future climates are precursors to local adaptation come from comparative analyses across multiple climatic ecotypes (Sandoval-Castillo et al., 2020) and from a long-term experiment of adaptive evolution in *M. duboulayi* (McCairns et al., 2016). In the latter, rainbowfish of known pedigree that were laboratory-reared under a 2070 projected summer temperature showed heritable differences in temperature reaction norms for the expression of 12 candidate genes. Interestingly, six of these expression

FIGURE 5 Covariance patterns of hydroclimatic adaptation in the seven strong candidate genes. Allele frequencies of each gene are plotted across 21 sampled sites of *Melanotaenia duboulayi* with their respective environmental predictor variables. Allele frequencies in each deme are ordered with respect to: (a) run-off perenniality from smallest to largest, (b) coldest minimum monthly temperature from smallest to largest, abd (c) hottest maximum monthly temperature, from smallest to largest. Squares denote sites sampled at the edges of the species range: the two southernmost sites are shown in green, and the four northernmost sites are shown in yellow (site codes follow Figure 1)
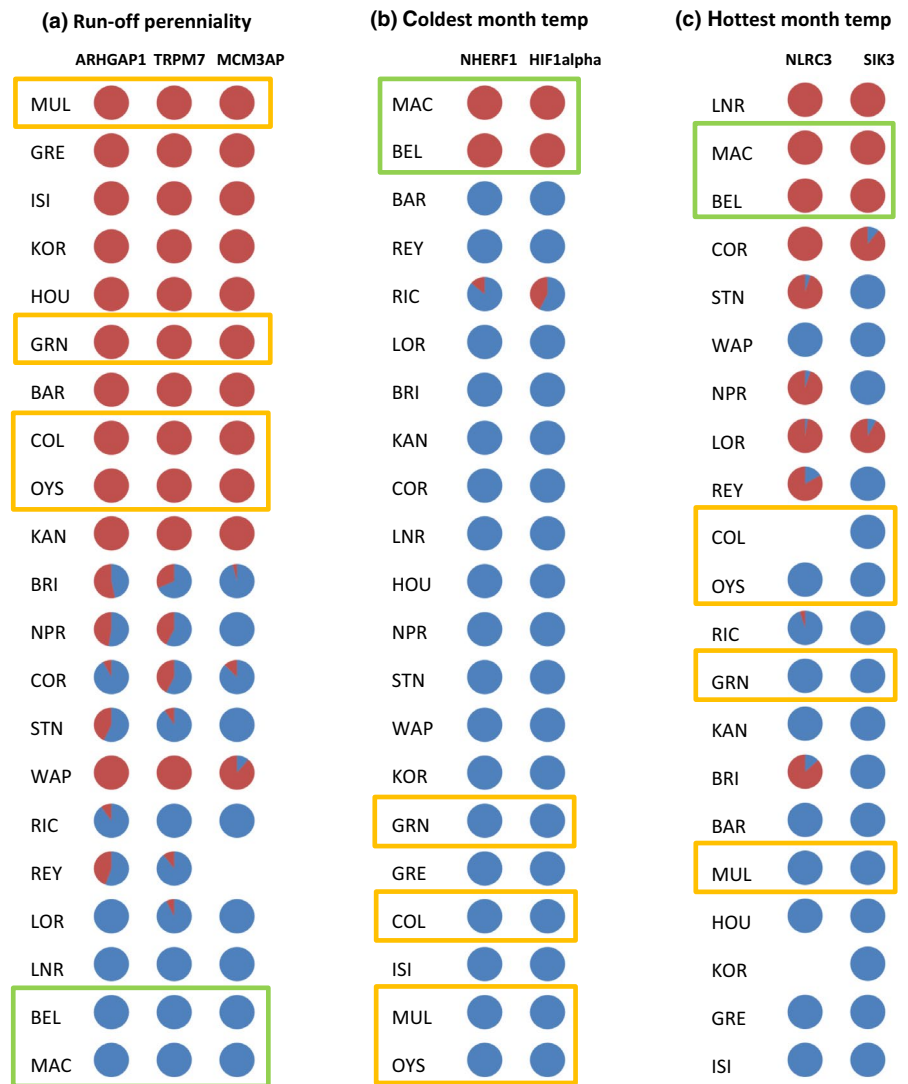
candidate genes that are physiologically relevant for climatic adaptation (McCairns et al., 2016) co-occur in 10 molecular pathways with at least one of our GEA candidates (Figure S5). Inference based on these protein–protein interaction networks reveal important aspects about adaptation to hydroclimatic environments, underlining the multifaceted nature in which transcriptional variation may influence adaptive resilience to climate change.

Theoretical and empirical studies have demonstrated that variation in natural selection across species ranges can maintain fitness-related diversity in populations (Bergland et al., 2014; Bernatchez, 2016; Hill et al., 2011; Whitehead & Crawford, 2006). Our study limitations include using a genome scan that interrogated a relatively small fraction of the genome, and working with a scenario of high population divergence and complex demographic history that can generate patterns of associations with environmental variation. Despite applying a highly conservative strategy to test for selection, we detected a reasonable number of candidate adaptive SNPs associated with variation in the environment or with variation in the phenotype and environment. This finding is consistent with the polygenic architecture of complex adaptive

traits (Jha et al., 2015), where each underlying gene explains a small proportion of trait variance. Our results indicate that, despite the strong latitudinal and demographic cline observed across the range of *M. duboulayi* (Figure 2a), balancing selection has probably maintained putatively adaptive polygenic diversity in populations around the centre of the environmental gradient (which in our system also correspond to geographical midrange populations) (e.g., GEA loci in Figure 2b; seven strong loci in Figure 5). The latitudinal cline detected in neutral genetic diversity is probably influenced by biogeographical history as the rainbowfish family has a tropical origin in New Guinea, with species and populations that expanded into more marginal temperate habitats in Australia being much less abundant (Unmack, 2001; Unmack et al., 2013). This pattern is shared by several eastern Australian freshwater fishes due to the history of sea level changes in this region (Unmack, 2001). Varying hydroclimatic selection at local scales, however, has accounted for GEA and GxPxE interactions, as well as for divergence in heritable phenotypic traits that underpin hydrodynamic fitness in rainbowfish. These findings are consistent with predictions derived from the CVH (Deutsch et al., 2008; Janzen, 1967; Tewksbury et al., 2008) for species found

along latitudinal gradients: range margin populations exposed to extremes of important environmental variables had stronger divergence and less variation in genes that are most likely to show evolutionary responses to changing climates compared to those exposed to varying levels of the same variables.

4.1 | Implications for populations at climatic and geographical range edges

Our study builds on the hypothesis that latitudinal differences in hydroclimatic regimes have selected for traits that influence regional patterns of adaptive resilience. It provides empirical support to theoretical work that shows that limits to adaptation for populations at range margins are associated with the efficacy of the environmental gradient and the intensity of selection relative to stochastic effects (Bridle et al., 2010; Polechová & Barton, 2015). These simulation studies emphasize that the evolutionary potential of low dispersing species is more highly influenced by standing genetic variation than for species where gene flow can bring adaptive alleles from other parts of a species range. Thus, the double impact of fragmented riverine landscapes and reduced diversity at ecologically important gene regions might critically imperil freshwater species at their ecological range margins (Bridle et al., 2010).

The ecological consequences of climate change are increasingly well documented across the Earth's terrestrial and aquatic ecosystems (Scheffers et al., 2016), but the effects of climate on natural selection across species ranges remain largely unknown (Siepielski et al., 2017, but see Bay et al., 2018 and Exposito-Alonso et al., 2019). A meta-analysis of estimated selection gradients revealed variation in climate-predicted selection throughout many of Earth's biomes (Siepielski et al., 2017). In agreement with our findings, that study showed how local and regional variation in climate regime, and in particular precipitation, best explained patterns of selection. Evolutionary responses are generally considered as requisite for long-term persistence of biodiversity, especially during ongoing and projected scenarios of increasing frequency and severity of extreme climate events (Frenne et al., 2013; Hoffmann & Sgrò, 2011; Scheffers et al., 2016; Waldvogel et al., 2020). Assessing vulnerability of populations to climate change requires an understanding of which environmental aspects influence traits underpinning adaptive resilience to changing climates (Siepielski et al., 2017; Waldvogel et al., 2020). By using a study system that has evolved phenotypic adaptations to climate-related variables, we showed that the diversity at climate-associated genes is lower in populations at environmental and geographical range edges. Thus, forecast changes in climate trajectories are likely to impact these populations more than those at the centre of the environmental gradient. Our results add to recent studies that reported variable and context-dependent evolutionary responses to climatic change across populations of the same species (Bay et al., 2018; Razgour et al., 2019), including strongest climate-driven selection at the edges of the species' environmental limits (Exposito-Alonso et al., 2019).

Rapid habitat alteration is a global ecological phenomenon reflecting an unprecedented rate of change in climate and landscape use (Bond et al., 2011; Carpenter et al., 1992; Peñuelas et al., 2013; Roy et al., 2005). Our work describes a strategy for cataloguing adaptive genetic diversity to climate change across the range of ecologically important nonmodel species. Such catalogues can be improved with the addition of population data sets based on whole genomes, which can provide a high-resolution record of variants across the genome and structural information about causative genes. Linking genomics with eco-evoutionary models (e.g., Waldvogel et al., 2020) would provide powerful opportunities for tracking and predicting adaptive responses and vulnerability to climate change along latitudinal gradients.

AUTHOR CONTRIBUTIONS
The study was conceived by L.B.B., with input from L.B. The data were generated and analysed by S.S., M.S., C.J.B., G.G., M.L. and L.B.B. Samples were obtained by P.J.U., L.B.B and S.S. The manuscript was written by L.B.B. and S.S. All authors contributed to data interpretation and commented on the manuscript.

DATA AVAILABILITY STATEMENT
Raw demultiplexed sequences for the *Melanotaenia duboulayi* individuals, SNP genotypes, environmental data, phenotypic data and custom R scripts to replicate the GEA and GxPxE analyses can be accessed on Dryad: https://doi.org/10.5061/dryad.73n5tb2v2.

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SUPPORTING INFORMATION

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