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Environmental selection, rather than neutral processes, best explain regional patterns of diversity in a tropical rainforest fish

Katie Gates [1], Jonathan Sandoval-Castillo [1], Chris J. Brauer [1], Peter J. Unmack[2], Martin Laporte [3,4], Louis Bernatchez[3] and Luciano B. Beheregaray [1]✉

To conserve the high functional and genetic variation in hotspots such as tropical rainforests, it is essential to understand the forces driving and maintaining biodiversity. We asked to what extent environmental gradients and terrain structure affect morphological and genomic variation across the wet tropical distribution of an Australian rainbowfish, *Melanotaenia splendida splendida*. We used an integrative riverscape genomics and morphometrics framework to assess the influence of these factors on both putative adaptive and non-adaptive spatial divergence. We found that neutral genetic population structure was largely explainable by restricted gene flow among drainages. However, environmental associations revealed that ecological variables had a similar power to explain overall genetic variation, and greater power to explain body shape variation, than the included neutral covariables. Hydrological and thermal variables were the strongest environmental predictors and were correlated with traits previously linked to heritable habitat-associated dimorphism in rainbowfishes. In addition, climate-associated genetic variation was significantly associated with morphology, supporting heritability of shape variation. These results support the inference of evolved functional differences among localities, and the importance of hydroclimate in early stages of diversification. We expect that substantial evolutionary responses will be required in tropical rainforest endemics to mitigate local fitness losses due to changing climates.

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INTRODUCTION

Empirical studies are fundamental to the advancement of evolutionary theory and are increasingly relevant as we grapple with the novel selective forces of anthropogenic environmental change. Both adaptive and non-adaptive processes contribute to the proliferation of biodiversity, but there remains much to explore about their relative roles (Bernatchez 2016; Wellenreuther and Hansson 2016; Luikart et al. 2018). At a landscape scale, the environment is expected to modulate interactions between evolutionary mechanisms, namely natural selection, genetic drift, and gene flow (Haldane 1948; Slatkin 1987; Manel et al. 2003; Storfer et al. 2007). However, we are only now developing frameworks to untangle coexisting signatures of these processes in natural populations. Such studies are particularly sparse in biodiversity hotspots such as tropical rainforests, where there has not only been substantial debate about diversifying processes (Endler 1982; Mayr and O'Hara 1986; Moritz et al. 2000) but also suggestions of high risk to adaptive diversity from human influences (Moritz 2002; Barlow et al. 2018; França et al. 2020).

As some of the world's most biodiverse and temporally continuous ecosystems, tropical environments merit a central place in eco-evolutionary research. Tropical rainforests alone may contain more than half the world's species (Turner 2001; Primack and Corlett 2005) and are among the greatest terrestrial providers of ecosystem services (Brandon 2014). Attributes such as localised endemism, high niche specificity and history of relative stability may increase threats to diversity under environmental change (Reed 1992; Barlow et al. 2018; Hoffmann et al. 2019). However, there is an inherent logistical difficulty in studying such diverse and often remote ecological communities (Beheregaray 2008; Beheregaray et al. 2015; Clarke et al. 2017), and both terrestrial and freshwater tropics remain remarkably understudied relative to temperate ecosystems (Beheregaray et al. 2015; Wilson et al. 2016). There has also been a long history of contention about the processes generating and sustaining tropical rainforest biodiversity (Endler 1982; Mayr and O'Hara 1986; Haffer 1997; Smith et al. 1997). Biogeographic and palaeoecological research has debated factors permitting both the accumulation of species and the preconditions for divergence; while strong evidence suggests that the stability of rainforest refugia through glacial maxima has helped sustain high species richness (Weir and Schluter 2007; Weber et al. 2014; Cattin et al. 2016), the factors precipitating diversification remain less clear. Arguments for vicariant influences such as refugial isolation and landscape breaks (Wallace 1854; Haffer 1969; Vuilleumier 1971; Mayr and O'Hara 1986; Ayres and Clutton-Brock 1992; Dias et al. 2013) have been increasingly contested with evidence for parapatric and sympatric divergence across

[1]Molecular Ecology Laboratory, College of Science and Engineering, Flinders University, Adelaide, SA 5042, Australia. [2]Centre for Applied Water Science, Institute for Applied Ecology, University of Canberra, Canberra, ACT 2601, Australia. [3]Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, 1030 avenue de la Médecine, Québec, Québec G1V 0A6, Canada. [4]Ministère des Forêts, de la Faune et des Parc (MFFP) du Québec, Québec, QC, Canada. Associate editor: Xiangjiang Zhan. ✉email: Luciano.Beheregaray@flinders.edu.au

ecotones (Endler 1982; Smith et al. 1997; Kirschel et al. 2011; Cooke et al. 2012a, Cooke et al. 2012b, Cooke et al. 2014; Morgan et al. 2020).

While providing important geographical context, polarisation of such views presented in earlier research has sometimes obscured the complexity of evolutionary processes in rainforest taxa (Butlin et al. 2008; Jardim de Queiroz et al. 2017). For example, the difficulty of inferring adaptation in isolated populations against a neutral 'null hypothesis' may have encouraged the view that allopatric divergences were largely drift-driven, despite evidence that local selection can often be more effective in a low gene flow context (Schluter 2001; Nosil 2012; Beheregaray et al. 2015). Moreover, while species-level diversification has received great emphasis, intraspecific approaches are comparatively under-exploited for identifying evolutionary processes such as drift and adaptation (Moritz et al. 2000; Moritz 2002). In tropical studies explicitly assessing neutral and adaptive processes, both have been found important for generating genetic or physiological variation (Freedman et al. 2010; Smith et al. 2011; Cooke et al. 2014; Brousseau et al. 2015; Benham and Witt 2016; Maestri et al. 2016; Termignoni-García et al. 2017; Zhen et al. 2017; Gallego-García et al. 2019; Morgan et al. 2020; Hay et al. 2022). This highlights the need for more nuanced assessments of rainforest diversity, which can be aided by increased integration of molecular and geospatial methods (Moritz et al. 2000; Moritz 2002; Beheregaray et al. 2015).

The field of landscape genomics has exploited rapidly advancing genomic and geospatial toolsets to detect ecological adaptation (Manel and Holderegger 2013; Hoffmann et al. 2015; Li et al. 2017), including in aquatic ecosystems (Grummer et al. 2019). Genotype-environment association (GEA) analyses have proven to be a powerful means to identify candidate loci under selection by specific environmental factors (Rellstab et al. 2015; Waldvogel et al. 2020), even for relatively weak allele frequency shifts (Bourret et al. 2014; Laporte et al. 2016; Forester et al. 2018). Similarly, phenotype-environment associations (PEAs) can allow the identification of ecologically adaptive phenotypes, benefited by multivariate approaches like geometric morphometrics (Zelditch et al. 2012; Maestri et al. 2016). Detection of local adaptation is complicated by the expectation of additional random, and potentially neutral, divergences, so statistical methods correcting for shared population history can benefit these approaches (Gautier 2015; Rellstab et al. 2015). For PEAs, it is also important to consider that plastic responses to environment, rather than evolved differences, can produce divergent physical characteristics (Merilä and Hendry 2014). Therefore, clearer interpretations can be made where it is possible to relate ecologically adaptive genotypes to significant phenotypic polymorphisms (Hu et al. 2020). Such integrative genotype-phenotype-environment (GxPxE) associations increase the opportunity for teasing apart eco-evolutionary mechanisms, and, by closing the gap between genotype, phenotype, and environment, can strengthen inferences about candidate genes underlying ecological adaptations (Smith et al. 2020; Carvalho et al. 2021).

Landscape heterogeneity places unique constraints on the biodiversity structure of taxa with restricted niches, including freshwater obligates. In contrast to temperate ecosystems, high year-round precipitation in tropical rainforests makes freshwater habitats ubiquitous, and their biotic interactions inextricable from those of the broader forest (Lo et al. 2020). However, available habitats and opportunities for gene flow in freshwater are typically restricted to dendritic, hierarchical, island-like, or ephemeral water features (Lévêque 1997; Grummer et al. 2019). The architecture of river networks and the strength and direction of flows can profoundly influence evolutionary dynamics (Paz-Vinas et al. 2015; Thomaz et al. 2016; Brauer et al. 2018), as well as vulnerability to fragmentation (Jiménez-Cisneros et al. 2014; Davis et al. 2018; Brauer and Beheregaray 2020). These factors make understanding

the spatial distribution of aquatic diversity important but complicated, and few riverscape genomic studies have been attempted in tropical freshwater (but see Barreto et al. 2020; Gallego-García et al. 2019).

We therefore capitalise on growing knowledge of eco-evolutionary processes in Australian rainbowfishes (*Melanotaenia* spp, family Melanotaeniidae; e.g. McGuigan et al. 2003; McGuigan et al. 2005; Smith et al. 2013; McCairns et al. 2016; Gates et al. 2017; Brauer et al. 2018; Lisney et al. 2020; Sandoval-Castillo et al. 2020; Smith et al. 2020; Brauer et al. 2023). In this genus, previous work has indicated not only the likely importance of hydroclimate as a driver of diversity, but the utility of integrative methods for assessing aquatic adaptation. Early work found heritable and potentially convergent body shape variation in association with streamflow (*M. duboulayi, M. eachamensis*; McGuigan et al. 2003; McGuigan et al. 2005). More recently, experimental assessments of gene expression have detected selection for plasticity of thermal response mechanisms (*M. duboulayi, M. fluviatilis*, and *M. s. tatei*) (Smith et al. 2013; McCairns et al. 2016; Sandoval-Castillo et al. 2020). Riverscape GEAs have also supported intraspecies ecological divergence related to hydroclimate for *M. splendida tatei* (Attard et al. 2022), for hybridising *Melanotaenia spp.* across an elevational gradient (Brauer et al. 2023), for *M. fluviatilis* (Brauer et al. 2018) and *M. duboulayi* (Smith et al. 2020), with the latter including evidence of GxPxE links.

Despite these advances, genome-wide research has not yet been presented for a tropical representative of the clade. Hence, we focus this study on *Melanotaenia splendida splendida* (eastern rainbowfish), endemic to tropical north-eastern Australia. The species is abundant throughout its distribution, including several river systems in the complex rainforest landscape of the Wet Tropics of Queensland World Heritage Area (Pusey et al. 1995; Russell et al. 2003; Hilbert 2008; see Fig. 1 for sampled rivers). It inhabits a variety of freshwater environments, and is also known for its high morphological diversity, even within connected drainages (Pusey et al. 2004). Although the ecological relevance of this diversity has not yet been tested, the low to moderate dispersal tendency of *Melanotaenia* spp (Brauer et al. 2018; Smith et al. 2020) makes localised adaptation a plausible contributor. Moreover, the rugged terrain of the Great Dividing Range provides diverse conditions and possible selective influences across the sampled habitat (Nott 2005; Pearson et al. 2015). In that region, temperature, precipitation, and streamflow vary with latitude, elevation, terrain structure, and proximity to the coast (Metcalfe and Ford 2009; Stein et al., 2011), and human impacts according to land use (Pert et al. 2010). This environmental and climatic heterogeneity, combined with the recognised biodiversity values, make the Wet Tropics of Queensland an ideal location for testing hypotheses about evolutionary dynamics in tropical freshwaters.

The broad aims of this study were to develop an understanding of adaptive and neutral influences on variation in tropical rainforest freshwater ecosystems. This was approached using landscape genomics to characterise spatial patterns of genetic and morphological diversity, identify links between genotype, phenotype, and environment, and test the impacts of adaptive and non-adaptive forces on divergence across a variable rainforest hydroclimate. Based on previous evidence for climatic factors promoting adaptive diversity among higher latitude rainbowfishes (Brauer et al. 2018; Sandoval-Castillo et al. 2020; Smith et al. 2020), we tested the hypothesis that hydroclimate would also play a strong role in driving intra-species diversity within a tropical ecotype. The following questions were addressed: First, to what extent are genetic and morphological diversity correlated with hydroclimate beyond expectations based on neutral genetic structure? Second, if ecological associations exist, can further associations be drawn to suggest a genetic (heritable) adaptive
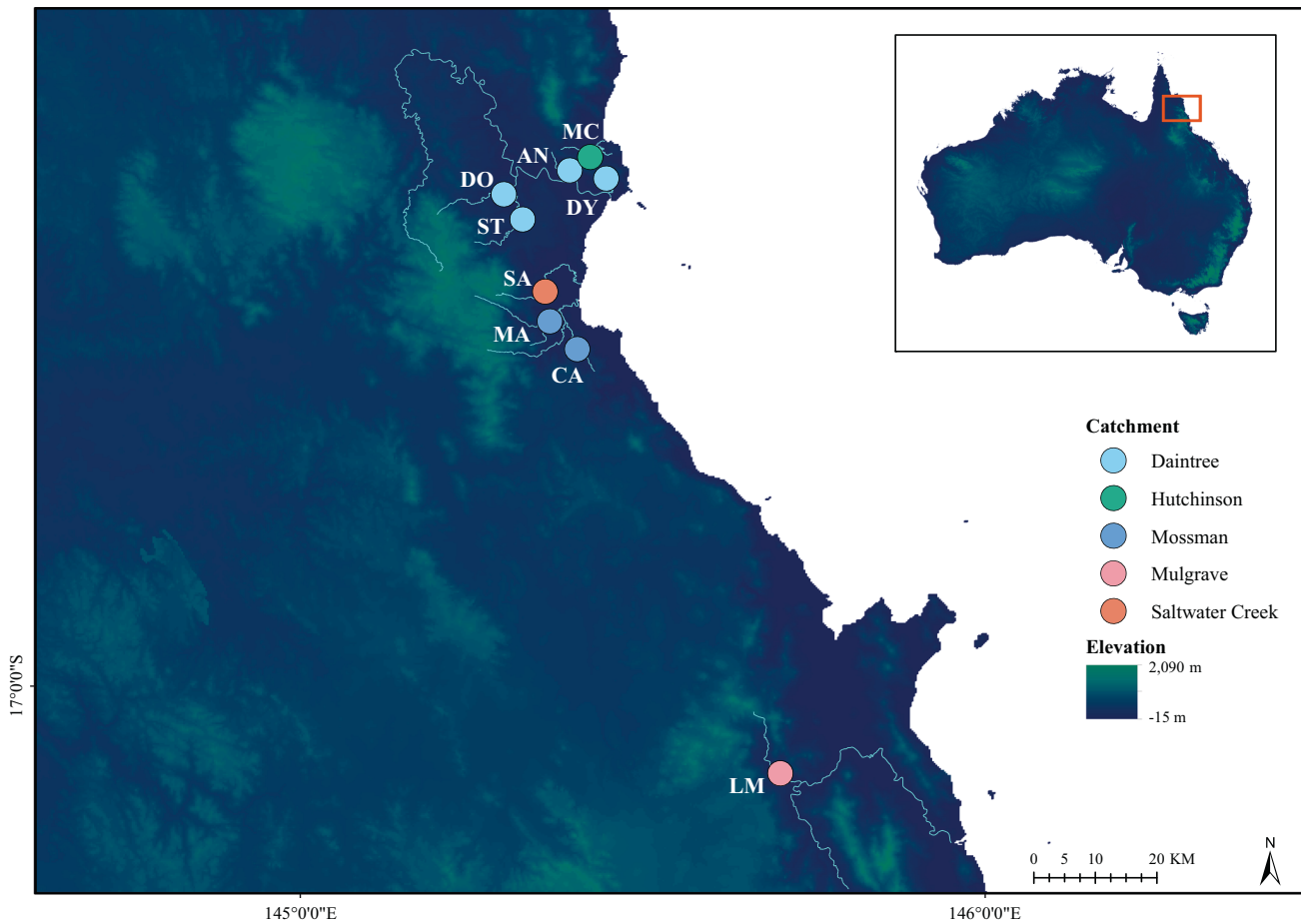
Fig. 1 Sampling location map of *Melanotaenia splendida splendida* collected from the Wet Tropics of Queensland. Point colours correspond to river drainage of origin. Blue lines highlight only the sampled creeks and major rivers of each represented drainage system. Inset: extent indicator of main map relative to the Australian continent. Locality codes: LM Little Mulgrave Creek, CA Cassowary Creek, MA Marrs Creek, SA Saltwater Creek, ST Stewart Creek, DO Douglas Creek, DY Doyle Creek, AN Forest Creek, MC McClean Creek.

component to the relevant morphology? Third, to what extent does catchment structure in this rugged terrain contribute to patterns of divergence? These factors have implications not only for understanding contemporary evolutionary processes in rainforest ecosystems, but also for the interpretation of adaptive resilience to environmental change.

METHODS
Sample collection
During March 2017, wild *Melanotaenia splendida splendida* (eastern rainbowfish) were sampled from nine rainforest creek sites across five drainages in the Wet Tropics of Queensland, north-eastern Australia (Fig. 1; Supplementary Table S1a). Sites were chosen to maximise sampling coverage across the climatic habitat gradient, while targeting accessible creeks with known occurrences of *M. s. splendida*. Live fish were captured by seine netting and transported by road in closed containers fitted with battery-running air pumps to a mobile fieldwork station. Here, 267 fish were euthanised, one at a time, via an overdose of anaesthetic sedative (AQUI-S®: 175 mg/L, 20 min). Of these, 208 individuals (avg. ~23, min. 19 per sampling site; Table S1a) were photographed immediately after death for morphometric data collection (details in Supplementary Methods S1a). Fin clips from all 267 individuals were preserved in 99% ethanol and stored at −80°, of which 210 high quality samples were selected for the final DNA dataset (avg. ~23, min. 20 per site; Table S1a). For 180 individuals (avg. ~20, min. 15 per site), both genomic and morphometric datasets were of high quality, allowing direct comparisons in later GxPxE analyses.

DNA extraction, library preparation and sequencing
We extracted DNA from fin clips using a salting-out protocol modified from Sunnucks and Hales (1996) (S1b). DNA was assessed for quality using a NanoDrop spectrophotometer (Thermo Scientific), for integrity using gel electrophoresis (agarose, 2%), and for quantity using a Qubit fluorometer (Life Technologies). High-quality samples from 212 individuals were used to produce double-digest restriction site-associated DNA (ddRAD) libraries in-house following Peterson et al. (2012) with modifications according to Sandoval-Castillo et al. (2018) (S1c), which have demonstrated efficacy for rainbowfishes (e.g., Brauer et al. 2018). Samples were randomly assigned across sequencing lanes with an average of six replicates per lane for quality control. Four lanes were sequenced at the South Australian Health and Medical Research Institute Genomics Facility on an Illumina HiSeq25000 (single-ended), and one lane at Novogene Hong Kong on an Illumina HiSeq4000 (paired-ended).

Bioinformatics: read trimming, alignment to genome, variant calling, and filtering
We used TRIMMOMATIC 0.39 as part of the DDOCENT 2.2.19 pipeline (Puritz et al. 2014) to demultiplex and trim adaptors from raw sequences, as well as leading and trailing low-quality bases (Phred < 20). Individuals with < 700,000 reads were considered poorly sequenced and were removed from the dataset. Sequences were mapped to a reference genome of the closely related *M. duboulayi* (Beheregaray et al. unpublished data; Brauer et al. 2023) following the GATK 3.7 pipeline (Van der Auwera and D O'Connor 2020). Briefly, we used BOWTIE 2.3.4 (Langmead and Salzberg 2012) to generate a FASTA file reference index and sequence dictionary from the genome and align individual sequences to the

reference. After sorting and converting SAM files to BAM format, potential mapping errors and alignment inconsistencies were corrected using a local realignment around indels. Finally, variants were called from the mapped reads using BCFTOOLS 1.9 (Li 2011). To target high-quality SNPs, we used VCFTOOLS 0.1.15 (Danecek et al. 2011) to filter poorly sequenced reads, non-biologically informative artefacts (*sensu* O'Leary et al. (2018), variants other than SNPs (e.g., indels), and sites with a high likelihood of linkage disequilibrium (full details S1d).

Differentiating putatively neutral versus outlier loci

Conformity of loci to neutral expectations was assessed using BAYESCAN 2.1 (Foll and Gaggiotti 2008), which identifies outlier loci under selection based on allele frequency distributions. Because the model relies on F_{ST}, it requires prior specification of population membership. We therefore ran an analysis using FASTSTRUCTURE 1.0 (Raj et al. 2014) for the full filtered dataset (details S1e). We then ran BAYESCAN using default settings for all filtered loci, with individuals assigned to putative populations based on the best K selected by FASTSTRUCTURE. A putatively neutral dataset was inferred using a false discovery rate <0.05. Such an approach is usually considered appropriate for minimally biased assessments of demographic parameters (Luikart et al. 2003; Luikart et al. 2018). The resulting dataset (14,478 loci, 210 individuals) was used for subsequent analyses of neutral genetic diversity and population structure except where otherwise specified.

Genetic diversity and inference of population structure

We estimated neutral genomic diversity for each sampling site using ARLEQUIN 3.5 (Excoffier and Lischer 2010), including mean expected heterozygosity (H_e), mean nucleotide diversity (π), and proportion of polymorphic loci (*PP*). We also calculated Wright's fixation indices (*F*-statistics) in R (RC Team 2019) using HIERFSTAT 0.04–22 (Goudet 2005) for the entire sampling region. The same package was used to calculate pairwise F_{ST} and site-specific F_{ST} among sampling localities. To produce an overview of phylogenetic relationships among individuals, a Neighbour-Joining tree was constructed in PAUP* 4.0 (Swofford and Sullivan 2003) using TN93 distances (Tamura and Nei 1993). We also produced a scaled covariance matrix of sampling site-based 'population' allele frequencies (Ω) using BAYPASS 2.2 (Gautier 2015) core model, based on the full SNP dataset rather than the neutral subset. We further interrogated population structure using clustering approaches, including FASTSTRUCTURE, and Discriminant Analysis of Principal Components (DAPC) in R package ADEGENET 2.0.0 (Jombart 2008; Jombart and Ahmed 2011). Finally, we estimated asymmetrical rates of recent migration (m) between inferred populations using BA3-SNPS 1.1 (Wilson and Rannala 2003; Mussmann et al. 2019), a Bayesian Markov chain Monte Carlo approach. Full details of the above analyses, including preparation of input files, are in Supplemental Methods (S1f).

Characterising environmental variation

Environmental variables characterising the regional aquatic landscape (resolution of 9 s/250 m) were obtained from the National Environmental Stream Attributes v1.1.3, a supplementary product of the Australian Hydrological Geospatial Fabric (Stein et al. 2011). From >400 available attributes, we selected only those which varied among sampling sites, were uncorrelated, were measured at a relevant scale, and were considered to have broad ecological relevance for freshwater organisms (further details S1g). The six selected variables were: stream segment aspect (ASPECT), river disturbance index (RDI), average summer mean runoff (RUNSUMMERMEAN), average annual mean rainfall (STRANNRAIN), average annual mean temperature (STRANNTEMP), and total length of upstream segments calculated for the segment pour-point (STRDENSITY) (Table S1g, Fig. S1g). These were used as a basis for the subsequent analyses of genotype-environment associations (GEA), phenotype environment associations (PEA) and GxPxE associations.

Genotype-environment associations

We used GEAs to assess the effect of environment on genotype of *M. s. splendida* within the climatically heterogeneous Daintree rainforest. Since GEA approaches can vary markedly in their detection of candidate genes depending on demography, sampling design, and strength of selection (de Villemereuil et al. 2014; Rellstab et al. 2015; Forester et al. 2018), we chose to use two analytical approaches with different advantages. These included a Bayesian hierarchical model (BAYPASS 2.2 auxiliary covariate model; Gautier 2015), and constrained ordination (redundancy analysis; RDA) performed in

R package VEGAN 2.5–6 (Oksanen et al. 2019). For both methods, we tested associations between the full SNP dataset (14,540) and the six scaled, uncorrelated environmental variables (see above) while controlling for putatively neutral genetic variation. The algorithm used by BAYPASS is well suited to study systems involving nested or hierarchical population structure (Gautier 2015), which is particularly common in dendritic habitats such as freshwater (Thomaz et al. 2016). We tested for GEA associations accounting for assumed population demographic structure (scaled sampling site-based allelic covariance; Ω), previously identified using the software's core model (details in Supplemental methods S1h). Meanwhile, RDAs have been shown to have both a low rate of false positives and a high rate of true positives under a range of demographic histories, sampling designs, and selection intensities when compared with other popular GEA methods (Forester et al. 2018). Despite these strengths, we do emphasise that all detected environmental associations remain putative due to the unknown and potentially confounding factors present in natural study systems.

We first ran a global RDA using the full SNP dataset as the multivariate response matrix, and the six environmental variables (Fig. S1g), centred and scaled, as the explanatory matrix. Then, to control for demographic structure, partial RDAs (pRDAs) were used to model relationships between alternative (neutral) explanatory variables and genotypic responses, ordinating only the residual genotypic responses against environmental explanatory variables. To this end, three pRDAs were performed to include different neutral (or neutral proxy) covariable matrices, (1) significant principal components (PCos) of scaled Ω, (2) significant PCos of pairwise F_{ST}, and (3) significant PCos of waterway distances. For each pRDA, we used the full set of SNP genotypes as a response matrix, and an explanatory matrix containing only environmental variables previously associated with genotype ($p < 0.1$) in the global RDA (full details S1h).

Geometric morphometric characterisation and analyses

Eighteen landmarks were positioned on digital images of *M. s. splendida* collected during field sampling using TPSDIG2 2.31 (Rohlf, 2017). Landmarks (Fig. 2) were selected to maximise anatomical homology, repeatability, and representation of potentially ecologically relevant characteristics, based on recommendations by Zelditch et al. (2012) and Farré et al. (2016) (details S1i). Digitised TPS files were imported into MORPHOJ 1.07a (Klingenberg 2011) for exploratory analyses. Individual landmark configurations were subjected to Procrustes superimposition, that is, a scaling of homologous coordinates by size, rotation, and placement in space. The dataset was checked for outliers to ensure correct order and location of landmarks, and a covariance matrix was generated for the full dataset of individual Procrustes fits. To characterise major features of shape variation, a PCA was performed on the resulting covariance matrix. Due to size variation among individuals, an allometric regression was used to test the association between size (log centroid) and shape (Procrustes coordinates), pooled within population-based subgroups earlier identified by neutral genetic analyses. While allometric shape differences can result from adaptive evolution, ontogenic allometry may also be observed throughout the lifespan (Pélabon et al. 2013), potentially confounding signals of selection. Therefore, due to a strong relationship between size and shape (Supplementary Fig. S1i), residuals from size regression were used for the subsequent canonical variate analyses (CVAs), also performed in MORPHOJ. To test for relationships between body shape and locality of origin, we ran CVAs of Procrustes distances against sampling site and against catchment. This method calculates the total variation among groups, scaling for relative within-group variation. Statistical significance was assessed using 1000 permutation rounds.

Phenotype-environment associations

To assess the effect of environmental gradients on body shape of *M. s. splendida* within the Daintree rainforest, we adapted the RDA approach used for the GEAs (described above) to implement phenotype-environment analyses (PEAs). We used the same set of environmental explanatory variables (above), this time testing body shapes (PCs of individual Procrustes distances determined as significant by Broken-Stick method) as response variables. We again controlled for putatively neutral genetic structure (allelic covariance Ω; pairwise F_{ST}; waterway distance), plus the additional covariable of body size (log centroid size). Inputs for the body shape response variable and size covariable were
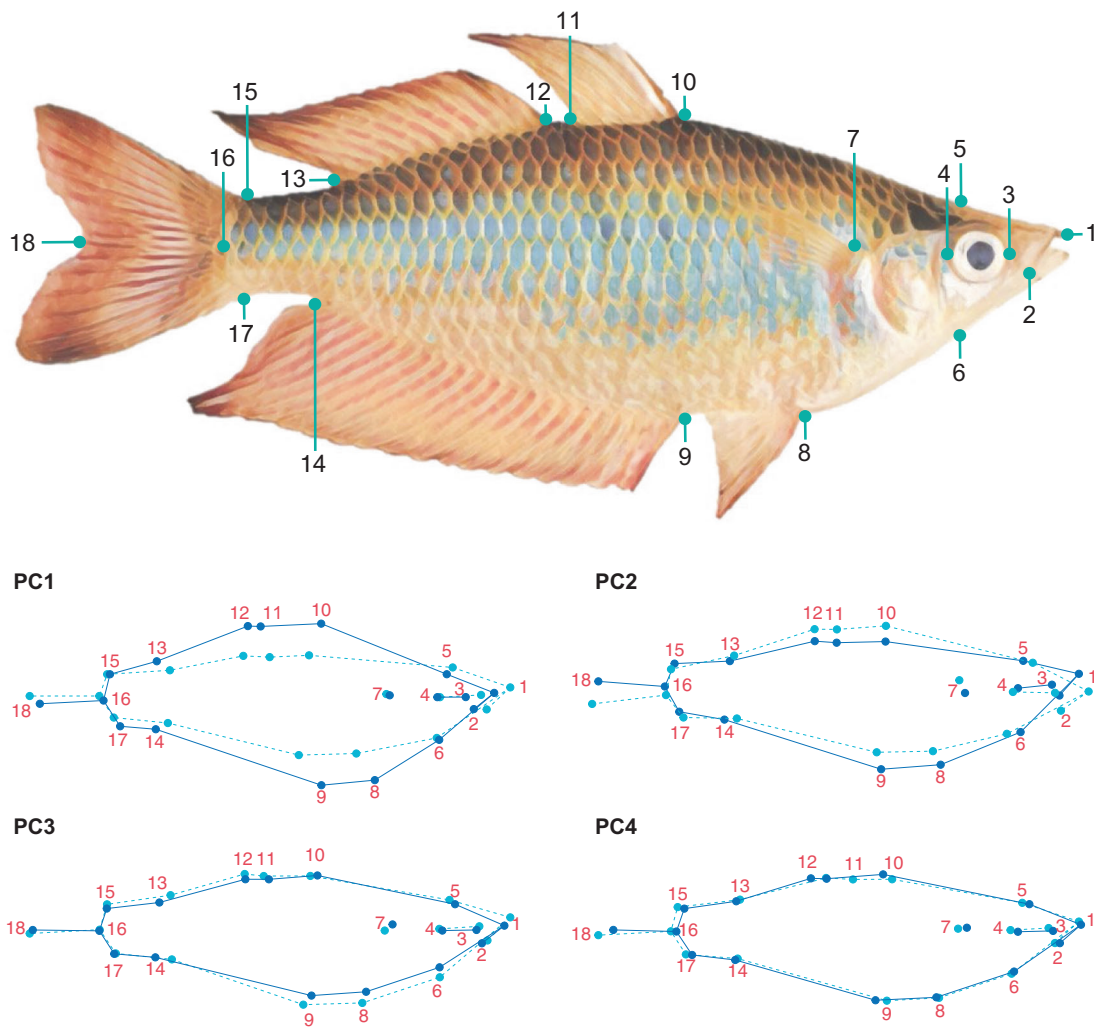
Fig. 2 UPPER: The 18 landmarks used for geometric morphometric analysis of the eastern rainbowfish *Melanotaenia splendida splendida*. 1: Anterior tip of head, where premaxillary bones articulate at midline; 2: Posterior tip of maxilla; 3: Anterior margin in maximum eye width; 4: Posterior margin in maximum eye width; 5: Dorsal margin of head at beginning of scales; 6: Ventral margin at the end of the head; 7: Dorsal insertion of pectoral fin; 8: Anterior insertion of the pelvic fin; 9: Anterior insertion of the anal fin; 10: Anterior insertion of the first dorsal fin; 11: Posterior insertion of the first dorsal fin; 12: Anterior insertion of the second dorsal fin; 13: Posterior insertion of the second dorsal fin; 14: Posterior insertion of the anal fin; 15: Dorsal insertion of the caudal fin; 16: Posterior margin of the caudal peduncle (at tip of lateral line); 17: Ventral insertion of the caudal fin; 18: Posterior margin of the caudal fin between dorsal and ventral lobes. LOWER: Fig. 4. Wireframe graphical representation of significant principal components of body shape variation based on 18 landmarks for 207 *Melanotaenia splendida splendida* individuals sampled across nine rainforest sampling localities in the Wet Tropics of Queensland. Dark and light blue frames respectively represent body shapes at high and low extremes of each significant axis (scale factor = 0.75).

created in R, using functions developed by Claude (2008) (full details in S1j).

Genotype-phenotype-environment analysis

If environmental selection for a particular phenotype has promoted evolutionary adaption, then the relevant phenotypic divergence should be accompanied by a genotypic response. We therefore tested whether any of the putative adaptive (environmentally associated) genetic variation could be attributed to environmentally associated morphological variation throughout the study region. This could indicate both a heritable component to the associated body shape traits (as opposed to the alternative hypothesis of phenotypic plasticity), as well as provide further support for their adaptive advantages. In R, we ran a global RDA using the four significant PCs of individual Procrustes distances as explanatory variables, and 864 putative adaptive alleles (identified in the genotype-environment RDA controlling for Ω) as the multivariate response. The analysis was then repeated as a partial RDA using individual body size (log centroid) as a covariable (details S1k), the results of which isolated only the genotype-phenotype interactions best explained by environmental selection.

Functional annotation

From the *M. duboulayi* reference genome (Beheregaray et al. unpublished data; Brauer et al. 2023), we extracted 300 bp flanking sequences on either side of the same 864 candidate SNPs. These were aligned to the UniProtKB/Swiss-Prot database using BLASTx (BLAST + 2.12.0; Camacho

Table 1. Genetic diversity measures and F-statistics for the eastern rainbowfish *Melanotaenia splendida splendida* at nine rainforest localities, based on 14,478 putatively neutral loci.

Location	Site Code	Drainage system	n	H_E	H_O	PP	FI_S	F_{ST}
Little Mulgrave Creek	LM	Mulgrave	23	0.283	0.271	0.323	0.018	0.204
Cassowary Creek	CA	Mossman	23	0.297	0.295	0.314	−0.011	0.177
Marrs Creek	MA	Mossman	20	0.307	0.293	0.305	0.019	0.178
Saltwater Creek	SA	Saltwater Creek	24	0.321	0.307	0.264	0.019	0.261
Stewart Creek	ST	Daintree	25	0.278	0.259	0.391	0.031	0.065
Douglas Creek	DO	Daintree	24	0.289	0.272	0.376	0.038	0.060
Doyle Creek	DY	Daintree	24	0.294	0.280	0.358	0.030	0.095
Forest Creek	AN	Daintree	22	0.289	0.268	0.377	0.054	0.059
McClean Creek	MC	Hutchinson	25	0.279	0.271	0.252	0.009	0.382

n sample size for final DNA dataset, H_E expected heterozygosity, H_O observed heterozygosity, PP proportion of polymorphic loci, F_{IS} site-specific inbreeding coefficient, F_{ST} site-specific F_{ST}.

et al. 2009). Matches with e-value < = 1e-3 were annotated with gene ontology (GO) terms and associated functional descriptions.

RESULTS
Genome-wide SNP data, diversity, and population structure
Sequencing produced ~550 million ddRAD reads for 242 *M. s. splendida* individuals (including replicates). After variant filtering and removal of lower-quality samples, we retained 14,540 putatively unlinked SNPs (Table S2a), of which 14,478 could be considered neutral for the purposes of population genomic analyses (Fig. S2a). The final dataset comprised 210 high-quality individuals across nine sampling sites. Neutral genomic diversity (Table 1) was moderately high for most sites, with expected heterozygosity (H_E) ranging from 0.278 to 0.321 (mean = 0.293), and proportion of polymorphic loci (PP) ranging from 0.252 to 0.391 (mean = 0.329). Population subdivision accounted for a substantial proportion of the neutral variation, with global $F_{ST} = 0.165$, and $F_{IT} = 0.205$. None of the site-specific F_{IS} values (Table 1) were significant. Pairwise F_{ST} comparisons (Fig. 3a; Table S2b) indicated relatively little differentiation between localities within the same drainage (0.017–0.029; mean = 0.024) compared with localities in different drainages (0.071–0.208; mean = 0.120), consistent with a segregating effect of drainage boundaries. Similarly, greater correlations in allelic covariance (Fig. 3b) were observed among, rather than within drainages. Both pairwise and site-specific F_{ST} values indicated that the most neutrally divergent sampling localities were the northernmost McClean Creek (Hutchinson Drainage), followed by the more centrally located Saltwater Creek (Saltwater Creek Drainage). In addition to being the smallest drainage systems sampled, both are located along the coastal boundary of the species distribution (Fig. 1).

Low differentiation within drainages and high differentiation among drainages was also reflected by clustering analyses. Both FASTSTRUCTURE (Fig. 3c) and DAPC (Fig. S2c) grouped individuals by their drainage system of origin, resulting in an optimal K of five for both analyses. Pairs of drainages in relatively close geographic proximity (i.e., Daintree and Hutchinson; Saltwater and Mossman) grouped more closely in the DAPC, indicating similarities in genetic variation which may result from a more recent shared history. The neighbour-joining tree (Fig. S2d), representing putative individual-level evolutionary relationships, presented each drainage system as reciprocally monophyletic and supported a hierarchical pattern of spatial connectivity. Contemporary gene flow among drainages was very low, with inferred migration rates (m) ranging from 0.0033–0.0224 between the inferred populations, versus 0.9521–0.9865 within the populations (Fig. S2e, Table S2e).

Genotype-environment associations
Without considering neutral influences, global redundancy analyses (RDAs) found six environmental variables associated with 23% of the observed genetic variation among individuals ($p = $ <0.001; Fig. S2f). After controlling for locality-specific neutral variation, GEAs remained highly significant ($p = $ <0.001). Controlling for scaled allelic covariance Ω (Figs. 4a; S2e), associations with five environmental variables accounted for 16.6% of total SNP variation, from which 864 loci were identified as candidates for environmental selection ($p \le 0.0027$; Fig. S2g). The environmental explanatory variables STRANNRAIN and STRANNTEMP were the most influential in the model. When controlling for the alternative neutral covariable of pairwise F_{ST} (Fig. S2e, h), associations with six environmental variables accounted for 12.1% of total SNP variation, with STRANNRAIN and STRANNTEMP likewise emerging as the most influential. When controlling for the neutral proxy of waterway distance (Fig. S2e, i), associations with six environmental variables accounted for 15.7% of SNP variation, in this instance with STRANNTEMP and ASPECT as the most influential. However, STRANNRAIN and STRANNTEMP were once again the most important in the BAYPASS GEA approach (auxiliary covariate model; Fig. S2j), which identified a more conservative 176 loci as candidates. Of these, 88 were uniquely associated with STRANN-RAIN, 56 with STRANNTEMP, 12 with ASPECT, ten with RDI, nine with STRDESITY, and one with RUNSUMMERMEAN. Twenty percent of these candidates (36 loci) were shared with the pRDA approach.

Morphological variation among localities and environmental gradients
Across the sampled range of rainforest *M. s. splendida*, four PCs of body shape (Figs. 2b, S2k) were identified as significant by Broken-Stick modelling. Major shape changes along these axes included differences in body depth (PCs 1 and 4), dorsal and ventral curvature (PCs 2 and 3), fin length and position (PCs 3 and 4), and upturn of head and mouth (PCs 2 and 3). Despite some overlap of individual variation among localities, CVAs revealed significant differences ($p < 0.05$) in Procrustes distances among most sampling sites, and among all drainages/populations (Fig. S2l, Table S2l). Interestingly, the sites for which shape difference could not be significantly distinguished (Forest Creek, Daintree drainage; and McClean Creek, Hutchinson drainage) were not within the same drainage system (or neutrally inferred population grouping) but were the closest sites in geographical proximity. The most shape-divergent localities were Little Mulgrave (Mulgrave drainage) and Doyle Creek (Daintree drainage).

Global RDAs found that ~24% of body shape variation (based on four significant shape PCs) was associated with environment
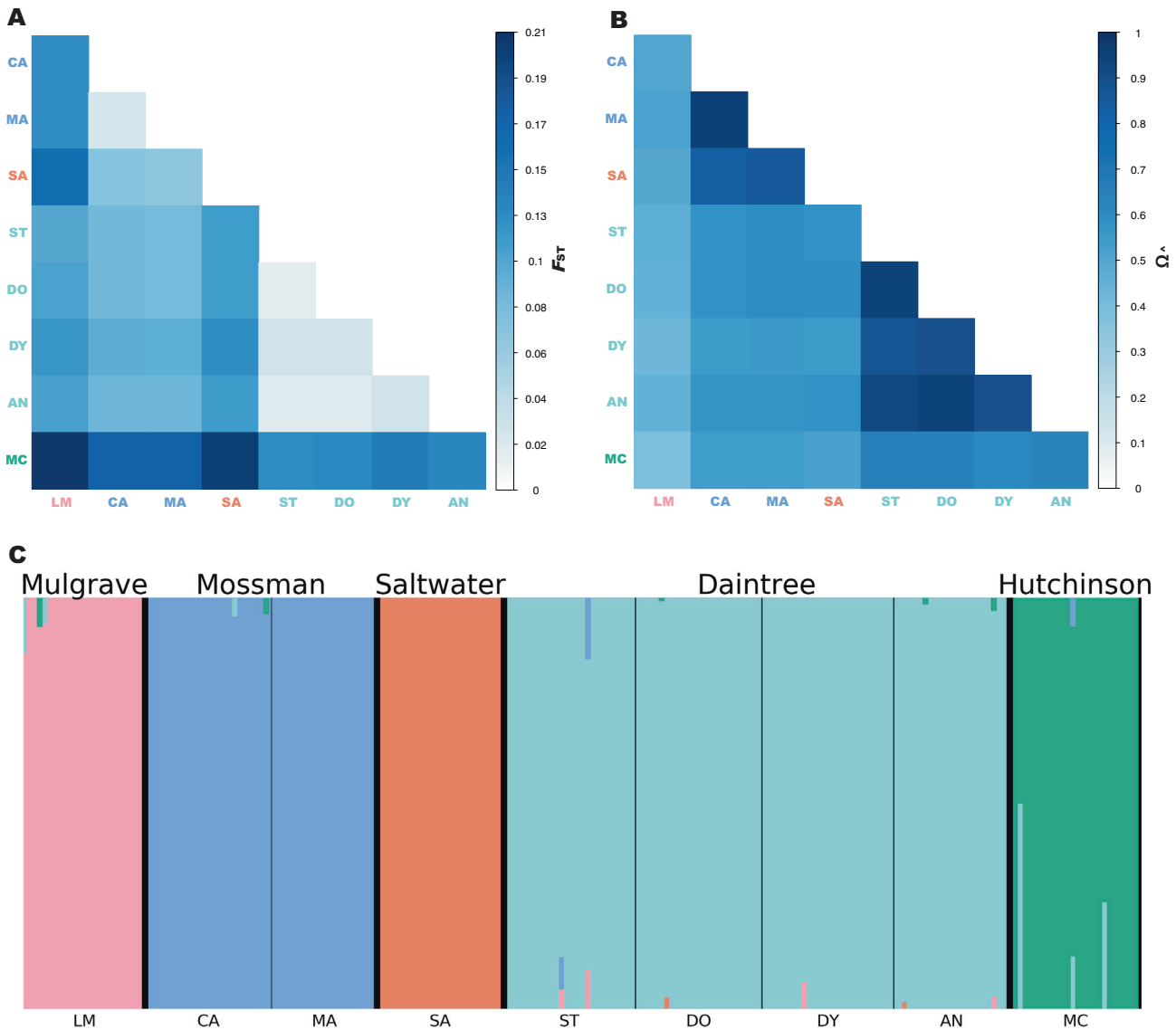
Fig. 3 Genomic differentiation and population structure of the eastern rainbowfish *Melanotaenia splendida splendida*. A Heatmap of pairwise F_{ST} based on 14,478 putatively neutral SNPs; **B** Correlation map for BAYPASS core model scaled covariance matrix Ω based on allele frequencies of the full dataset of 14,540 SNPs; and **C** Cluster plot based on FASTSTRUCTURE analysis of 14,478 putatively neutral SNPs, where colours represent inferred ancestral populations of individuals based on an optimal K of five. Large type refers to drainage systems, which are separated by thicker black lines. Small type refers to sampling localities, separated by thinner black lines. Locality abbreviations follow Table 1.

($p = $ <0.001; Fig. S2m). After controlling for possible allometric (log centroid size) and neutral genetic (locality-specific allelic covariance Ω) influences using pRDA, 14% of body shape variation remained significantly associated with four environmental variables, with STRANNTEMP and STRDENSITY the most influential ($p = $ <0.001; Figs. 4b; S2e). The body shape components most strongly associated with environment were PC2, relating to dorsal flattening, ventral curvature, and upturn of head; and PC4, relating to width and position of first and second dorsal fins and anal fin, body depth, and length of caudal peduncle (see Fig. 2 for graphical representation). pRDAs controlling for alternative covariables of pairwise F_{ST} and waterway distance (each together with body size), respectively found 11.9% and 11.6% of shape variation associated with environment (Fig. S2e, n, o).

Associations among genotype, phenotype, and environment
The GxPxE analysis using global RDA revealed a significant association ($p = $ <0.001) between putatively adaptive genetic

variation and morphology, to which 6.8% of divergence in those loci could be attributed. After controlling for possible allometric effects (centroid size) using pRDA, this figure was only slightly reduced to 6.5% (Fig. 4c). The PCs of body shape that had the strongest influence on the model were PC2, followed by PC4. Based on these associations, we identified 61 candidate loci for climate-adaptive morphological variation with $p = $ <0.0455 (Fig. S2p). In other words, these loci are predicted to confer a heritable selective advantage under localized environmental conditions based on their association with body shape.

BLAST annotation results
Of 864 candidate adaptive loci, 128 were successfully aligned to protein sequences in the UniProtKB/Swiss-Prot database and annotated with GO terms (Table S2q). Five of these were also identified as candidates by the GxPxE analysis: cdh2, farsa, Fbxl3, MON1B, SPTAN1.
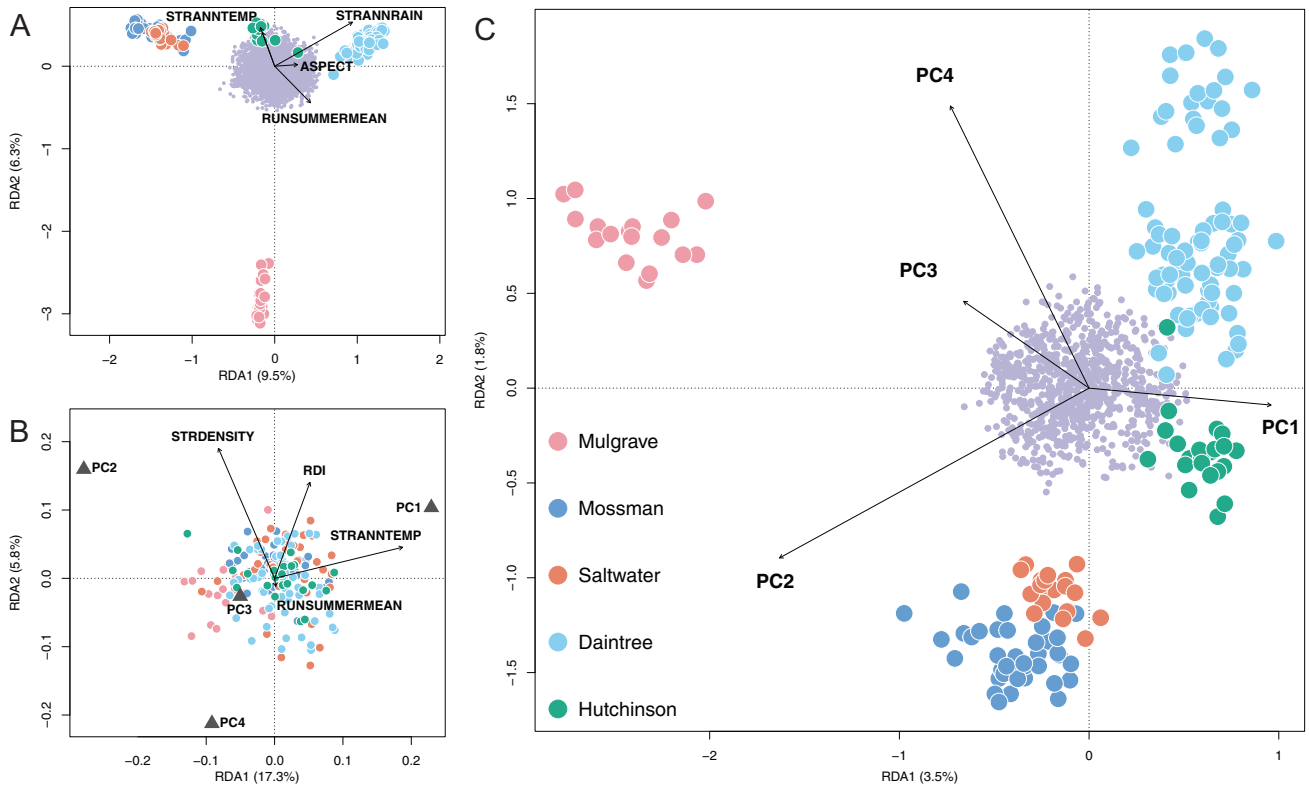
Fig. 4 Ordination plots summarising the first two axes of partial redundancy analyses (pRDAs) for _Melanotaenia splendida splendida_ individuals sampled across nine rainforest sampling localities in the Wet Tropics of Queensland. A Genomic variation (based on 14,540 SNPs) explained by five associated environmental variables, after partialing out the locality-specific effect of Ω (allelic covariance). Environmental predictors accounted for 16.6% of total variation ($p = $ <0.001). **B** Body shape variation (based on 18 morphometric landmarks) explained by four associated environmental variables, after partialing out the locality-specific effect of Ω (allelic covariance) and the individual effect of body size (log centroid). Environmental predictors accounted for 14% of total variation ($p = $ <0.001). **C** Genomic variation (based on 864 putative climate-adaptive alleles) explained by four associated principal components of body shape, after partialing out the individual effect of body size (log centroid). Body shape accounted for 6.5% of climate-associated genetic variation ($p = $ <0.001). For all plots, large points represent individual-level responses, and are coloured by drainage system of origin. Small purple points represent SNP-level responses. Vectors represent the magnitude and direction of relationships with explanatory variables.

DISCUSSION

As some of the most diverse, iconic, and potentially vulnerable ecosystems in the world, tropical rainforests remain remarkably understudied. Their complex and often inaccessible nature has created ongoing challenges to identifying the processes which drive and maintain biodiversity. Here, we contribute insight into these processes on an intraspecies level by addressing influences on genetic and morphological variation across the rainforest range of an Australian tropical fish (_Melanotaenia splendida splendida_). A clear association was found between both genetic and morphological variation and the drainage divisions of this highly structured catchment system, indicating an important role of gene flow limitations on population divergence. Despite this, a larger component of divergence was better explained by local environmental conditions, and especially by variables relating to hydroclimate. This pattern was particularly pronounced for the morphological component of diversity, providing further evidence for its functional relevance. Meanwhile, GxPxE associations identified highly significant relationships between major components of body shape divergence and ecologically associated genetic variants. Based on these consistencies, we propose that local evolutionary adaptation is a favourable contributor to the high phenotypic diversity renowned of _M. s. splendida_ (Pusey et al. 2004). We also infer that hydroclimatic adaptation has been a central mechanism for local divergence in this species, posing future challenges under rapid climatic change.

Environmental selection as a driver of rainforest freshwater diversity

Although there has been substantial historical emphasis on vicariant drivers of tropical rainforest diversity, an increasing number of genomic studies have revealed a dominant influence of contemporary environment (Ntie et al. 2017; Termignoni-García et al. 2017; Zhen et al. 2017; Lam et al. 2018; Jaffé et al. 2019; Miller et al. 2020; Morgan et al. 2020). Most of these works have focussed on terrestrial species, finding strong associations with either temperature or precipitation. In the Wet Tropics of Queensland, hydroclimatic variations dependent on latitude, elevation, terrain, and human impacts (Metcalfe and Ford 2009; Terrain NRM 2016) mean that hydroclimatic selection could be expected to contribute to geographic patterns of diversity in freshwaters. Consistent with this hypothesis, we found strong evidence for environmental influences on both genetic and body shape divergence of _M. s. splendida_, even after accounting for approximations of neutral demographic structure. Highly significant genotype-environment associations (GEAs) were supported by both RDA and BAYPASS analytical approaches. Depending on the covariables included, partial RDAs attributed ~12–17% of allelic variation to associations with key environmental variables, in contrast to the ~10–15% of variation which could be equally well or better explained by neutral conditional variables. Although it is difficult to draw direct comparisons, such strong GEAs support, and even exceed, those previously described for related

temperate and subtropical Australian rainbowfishes (*M. fluviatilis*, Brauer et al. 2018; *M. duboulayi*, Smith et al. 2020).

Large associations with environment were also found between body shape and environment in phenotype-environment associations (PEAs), with greater overlap among sites indicating that morphology may be more conserved than genotype. Environment accounted for ~7–14% of body shape variation in partial RDAs after accounting for conditional variables of neutral genetic structure and centroid size. These conditional variables accounted for a much larger 44–50% of shape variation, but intriguingly, most of this related to a large effect of size rather than of neutral genetic structure, which could only explain ~4% of shape variation alone. In contrast to the relatively large contribution of neutral structure in the GEAs, this pattern was surprising, yet plausible, under the premise of greater functional constraints on morphology than on genome-wide variation. While many genomic changes may have little functional relevance (e.g., synonymous substitutions, pseudogenes, noncoding sequences), it has been suggested that the effects of random drift on phenotypes, and particularly on morphology, are less likely to be truly neutral (Ho et al. 2017; Zhang 2018; but see Wideman et al. 2019). That is, if a physiological trait is subject to strong selection (directional or otherwise), it is unlikely to conform to neutral patterns unless genetic drift is also extremely strong (McKay et al. 2001; Clegg et al. 2002). Considering that body shape variation in teleosts has well-established roles related to swimming biomechanics, sensory ability, sexual behaviour, and various life history traits (Hanson and Cooke 2009; Langerhans and Reznick 2010; Killen et al. 2016), it is congruous that only a small proportion of variation would be explained by demography.

Few studies in the tropics have so far attempted to link signals of local genetic adaptation with patterns of phenotypic divergence. However, notable overlaps in genetic and morphological associations with environment have been detected by Morgan et al. (2020) for the rodent *Praomys misonnei* in relation to precipitation and vegetation structure, and by Miller et al. (2020) for the frog *Phrynobatrachus auritus* in relation to seasonality of precipitation. Here, we found a strong association between 6.5% of environmentally associated genetic loci and body shape PCs. While the relationship between these variables remains putative, a plausible explanation is that genes linked to the 61 implicated loci are contributing to body shape differences among sampled sites. Although only five of these loci were annotated to protein sequences, two have known phenotypic relevance in zebrafish (*Danio rerio*), including cdh2 (Cadherin-2) and SPTAN1 (Spectrin alpha chain, non-erythrocytic 1). Cdh2 is involved in early development (morphogenesis; Tay et al. 2010), while SPTAN1 is expressed in the fin, eye, cranial ganglion, and post-vent region (Farnsworth et al. 2021), with knockout affecting motor nerve formation (Voas et al. 2007; Susuki et al. 2011). These findings could suggest a heritable component of phenotypic diversity in *M. s. splendida*, congruent with previous evidence for the heritability of rainbowfishes' hydrodynamic morphology (*M. eachamensis*; McGuigan et al. 2003) and transgenerational heritability of transcriptional plasticity (*M. duboulayi*, McCairns et al. 2016). In the former example, similar phenotypic differences linked to hydrology were maintained by offspring produced in a common garden environment, providing evidence for evolved functional differences. The association of these signals thus adds an additional layer of support for the influence of local environment on evolutionary trajectories in the Wet Tropics.

In considering which environmental variables may have been the most influential in shaping diversity, repeated associations with thermal and hydrological variables indicated a strong role for hydroclimate. Average annual rainfall and average annual temperature were the strongest environmental predictors of genotype regardless of the GEA software, statistical approach, or neutral covariable used. The PEAs also emphasised the role of

hydroclimate, with average annual temperature and stream density explaining the greatest shape variation. As with the GEAs, average annual rainfall was strongly associated with body shape in global RDA modelling. However, its covariation with body size meant effects could not be reliably separated from the alternative hypothesis of allometric shape change. Regardless, both GEA and PEA results accord with globally applicable expectations for climate as a driver of functional diversity (Hawkins et al. 2003; Siepielski et al. 2017), and emerging evidence for its importance in terrestrial tropical adaptation. As is common with environmental association, it is difficult to determine whether tested environmental variables are directly affecting studied organisms' physiologies, or are interacting via affiliated but unmeasured environmental factors (Rellstab et al. 2015). However, even indirect associations with abiotic variables can reflect connected effects at other ecological levels (Blois et al. 2013). Thermal and hydrological associations in this freshwater context support the evolutionary relevance of climatic variance to wet tropical diversity, a key finding in light of the 'ecology vs isolation' debate.

Putative trait adaptation to local environment
Body shape may be one of the best indicators of a fish's inhabited niche (Gatz Jr 1979; Wainwright 1996; Shuai et al. 2018), and shape changes with important associations in this system match several well-described physiological adaptations in other teleosts, including rainbowfishes (McGuigan et al. 2003; McGuigan et al. 2005; Smith et al. 2020). Here, shape PCs 4 and 2 had the strongest relationship with environmentally associated alleles, making them among the most likely to have a heritable adaptive relevance. Interestingly, PC4 was mostly characterised by a change in fin positions, with some striking similarities to those described by McGuigan et al. (2003) and McGuigan et al. (2005) for congeneric *M. duboulayi* and *M. eachamensis*. These studies found that across lineages, streamflow conditions were consistently associated with insertion points of the first dorsal and pelvic fins, as well as the width of the second dorsal fin base. Here, changes on PC4 similarly included insertion of the first dorsal fin and width of the second dorsal fin base, and the associated precipitation and stream density variables can be related directly to stream flow (Carlston 1963).

Shape change on PC2 was not only relevant in the GxPxE analyses but was also the most important shape variable directly associated with environment (PEAs). Positive values coincided with a more upturned head, smaller eye, reduced dorsal hump, and distended pelvic region. Much of this divergence appeared latitudinally, with upturned shape extremes more common in the higher rainfall northerly catchments of Hutchinson, Daintree, and Saltwater. In a variety of teleost species, an upturned head and flattened dorsal region have been associated with a tendency for surface dwelling and feeding (Wootton 2012), surface breathing in oxygen-deficient waters (Lewis Jr 1970; Kramer and McClure 1982), and predation intensity (Langerhans et al. 2004; Eklöv and Svanbäck 2006). While an arching body shape has also been associated with rigor mortis in fishes (Hooker et al. 2016), the immediate imaging of individuals at the time of death, consistent among sampling sites, is likely to have prevented locality-specific differences. *M. s. splendida* is known for an omnivorous feeding strategy, including floating material such as invertebrates (Pusey et al. 2004). Notably, the surface-feeding tendency of the related *M. duboulayi* has been associated with differences in vegetative cover, possibly due to thermoregulatory influences or predator density (Hattori and Warburton 2003). Therefore, while this component of shape variation could be explained by a variety of factors, promising hypotheses include local selective differences due to relative abundance of food sources, predator presence, or vegetation structure.

In addition to the described adaptive signals occurring throughout the region, our results suggested an important effect of drainage structure in demographic divergence. Both demographic and environmental association analyses indicated gene flow barriers

across contemporary drainage boundaries, delineating populations and affecting broader patterns of diversity. This was reflected by low inter-drainage migration rates and genetic clustering within drainages. Some additional, shallow substructure was detected among sampling sites within drainages, possibly resulting from isolation by distance or other resistance within the stream network. Similar hierarchical configurations have been previously described for subtropical and temperate rainbowfishes (*M. fluviatilis*, Brauer et al. 2018; *M. duboulayi*, Smith et al. 2020), reflecting a recognised pattern of connectivity in lotic environments (Grummer et al. 2019). We therefore propose that, in addition to hydroclimatic factors, the geographic arrangement and relative size of individual watersheds has promoted evolutionary divergence.

Considerations for the ongoing maintenance of adaptive diversity in tropical rainforests

Both the strong effects of hydroclimate on intraspecies diversity, and the geographical confinement created by catchment structure, indicate that climate warming could place strong selective pressure on rainforest populations of *M. s. splendida*. Climate projections for the Wet Tropics cluster region indicate increases in average temperature, as well as more extreme weather events including rainfall intensity (Hilbert et al. 2014). If a large component of local diversity has developed in either a direct or indirect response to climate, we can expect that alteration of current environmental conditions will necessitate an adaptive response (Fitzpatrick and Keller 2015; Bay et al. 2017). It is notable that signals of adaptive divergence were directionally similar for genotype and morphology, and significant overlaps were revealed by GxPxE results. But as previously discussed, there were also some differences among associated environmental variables, their respective contributions, and the relative influences of neutral processes. These factors suggest similar but non-identical ecological dynamics are contributing to genetic and morphological diversity across the studied riverscapes. It therefore seems likely that while management strategies informed by either component of diversity should produce common benefits, a knowledge of both components would benefit more comprehensive management.

Melanotaenia splendida splendida is one of the most abundant fishes in the Queensland Wet Tropics (Pusey et al. 2004), and our results indicated relatively high genetic variation in most populations. Moreover, the total species range extends beyond rainforest limits (ALA 2020). Hydroclimate-associated variation in this species may highlight the need for further adaptation in response to climate change; but, perhaps more concerning are the implications for already vulnerable tropical freshwater species. Species with small effective population sizes and low genetic diversity are likely to have less standing variation available for selection (Frankham 2015; Ralls et al. 2018), and opportunities for future adaptation have a greater chance of being outweighed by random genetic drift (Perrier et al. 2017). While not all tropical rainforests exhibit as structured terrain as the Queensland Wet Tropics, mountainous features are common to most continental tropics. Moreover, rainforests are becoming globally affected by less predictable flow dynamics (Jiménez-Cisneros et al. 2014) and accumulating human modifications (Davis et al. 2018). In the context of dendritic systems, even relatively small structural changes can divide the habitat area over which gene flow can occur (Davis et al. 2018; Blanchet et al. 2020). We therefore suggest that the maintenance of existing connectivity should be prioritised in tropical rainforest river networks, and support a proactive strategy of evolutionary rescue for particularly vulnerable taxa (*sensu* Ralls et al. 2018).

CONCLUSION

Our work indicates that the interplay between contemporary hydroclimatic variation and drainage connectivity has helped shape regional diversity in the tropical rainforest fish *M. s. splendida*. Consistent with a growing body of work in Australian rainbowfishes, both genomic and morphological divergence appeared relevant to local adaptation, including heritable and hydrologically associated traits identified in related species. Moreover, three-way associations detected among genotype, phenotype, and environment supported the possibility of heritable, climate-adaptive shape variation in *M. s. splendida*. Empirical evidence for the role of temperature and precipitation driving phenotypic divergence has been mounting in tropical rainforest research, however this is likely the first freshwater example to benefit from a high-resolution genomic dataset. Where possible, we suggest that future work should continue to integrate environmental, genomic, and phenotypic datasets to disentangle evolutionary processes applicable to both conservation and theoretical development.

Data archiving

The relevant data have been appropriately archived and are available at figshare: https://figshare.com/s/c4b75d40fea91ab38beb. Data will be made publicly available upon acceptance.

REFERENCES

ALA. 2020. Atlas of Living Australia occurrence records. https://bie.ala.org.au/species/ Accessed January 2020

Attard CR, Sandoval-Castillo J, Brauer CJ, Unmack PJ, Schmarr D, Bernatchez L et al. (2022) Fish out of water: genomic insights into persistence of rainbowfish populations in the desert. Evolution 76:171–183

Ayres JM, Clutton-Brock T (1992) River boundaries and species range size in Amazonian primates. Am Naturalist 140:531–537

Barlow J, França F, Gardner TA, Hicks CC, Lennox GD, Berenguer E et al. (2018) The future of hyperdiverse tropical ecosystems. Nature 559:517–526

Barreto SB, Knowles LL, Affonso PRAdM, Batalha-Filho H (2020) Riverscape properties contribute to the origin and structure of a hybrid zone in a Neotropical freshwater fish. J Evolut Biol 33:1530–1542

Bay RA, Rose N, Barrett R, Bernatchez L, Ghalambor CK, Lasky JR et al. (2017) Predicting responses to contemporary environmental change using evolutionary response architectures. Am Nat 189:463–473

Beheregaray LB (2008) Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. Mol Ecol 17:3754–3774

Beheregaray LB, Cooke GM, Chao NL, Landguth EL (2015) Ecological speciation in the tropics: insights from comparative genetic studies in Amazonia. Front Genet 5:477

Benham PM, Witt CC (2016) The dual role of Andean topography in primary divergence: functional and neutral variation among populations of the hummingbird, Metallura tyrianthina. BMC Evolut Biol 16:22

Bernatchez L (2016) On the maintenance of genetic variation and adaptation to environmental change: considerations from population genomics in fishes. J Fish Biol 89:2519–2556

Blanchet S, Prunier JG, Paz-Vinas I, Saint-Pé K, Rey O, Raffard A et al. (2020) A river runs through it: the causes, consequences, and management of intraspecific diversity in river networks. Evolut Appl 13:1195–1213

Blois JL, Zarnetske PL, Fitzpatrick MC, Finnegan S (2013) Climate Change and the Past Present and Future of Biotic Interactions. Science 341:499–504

Bourret V, Dionne M, Bernatchez L (2014) Detecting genotypic changes associated with selective mortality at sea in Atlantic salmon: polygenic multilocus analysis surpasses genome scan. Mol Ecol 23:4444–4457

Brandon K (2014) Ecosystem services from tropical forests: review of current science. Center for Global Development, Washington, DC, USA

Brauer CJ, Beheregaray LB (2020) Recent and rapid anthropogenic habitat fragmentation increases extinction risk for freshwater biodiversity. Evolut Appl 13:2857–2869

Brauer CJ, Unmack PJ, Smith S, Bernatchez L, Beheregaray LB (2018) On the roles of landscape heterogeneity and environmental variation in determining population genomic structure in a dendritic system. Mol Ecol 27:3484–3497

Brauer CJ, Sandoval-Castillo J, Gates K, Hammer MP, Unmack PJ, Bernatchez L et al. (2023) Natural hybridization reduces vulnerability to climate change. Nat Clim Change 13:282–289

Brousseau L, Foll M, Scotti-Saintagne C, Scotti I (2015) Neutral and adaptive drivers of microgeographic genetic divergence within continuous populations: the case of the neotropical tree Eperua falcata (Aubl.). PLoS ONE 10:e0121394

Butlin RK, Galindo J, Grahame JW (2008) Sympatric, parapatric or allopatric: the most important way to classify speciation? Philos Trans R Soc B: Biol Sci 363:2997–3007

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K et al. (2009) BLAST+: architecture and applications. BMC Bioinforma 10:1–9

Carlston CW (1963) Drainage density and streamflow. US Government Printing Office, Washington, DC, USA

Carvalho CS, Forester BR, Mitre SK, Alves R, Imperatriz-Fonseca VL, Ramos SJ et al. (2021) Combining genotype, phenotype, and environmental data to delineate site-adjusted provenance strategies for ecological restoration. Mol Ecol Resour 21:44–58

Cattin L, Schuerch J, Salamin N, Dubey S (2016) Why are some species older than others? A large-scale study of vertebrates. BMC Evolut Biol 16:90

Clarke DA, York PH, Rasheed MA, Northfield TD (2017) Does biodiversity–ecosystem function literature neglect tropical ecosystems? Trends Ecol Evol 32:320–323

Claude J (2008) Morphometrics with R. Springer Science & Business Media, New York, NY, USA

Clegg SM, Degnan SM, Moritz C, Estoup A, Kikkawa J, Owens IP (2002) Microevolution in island forms: the roles of drift and directional selection in morphological divergence of a passerine bird. Evolution 56:2090–2099

Cooke G, Chao N, Beheregaray L (2012a) Natural selection in the water: freshwater invasion and adaptation by water colour in the Amazonian pufferfish. J Evolut Biol 25:1305–1320

Cooke GM, Chao NL, Beheregaray LB (2012b) Divergent natural selection with gene flow along major environmental gradients in Amazonia: insights from genome scans, population genetics and phylogeography of the characin fish Triportheus albus. Mol Ecol 21:2410–2427

Cooke GM, Landguth EL, Beheregaray LB (2014) Riverscape genetics identifies replicated ecological divergence across an Amazonian ecotone. Evolution 68:1947–1960

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA et al. (2011) The variant call format and VCFtools. Bioinforma (Oxf, Engl) 27:2156–2158

Davis CD, Epps CW, Flitcroft RL, Banks MA (2018) Refining and defining riverscape genetics: How rivers influence population genetic structure. Wiley Interdiscip Rev: Water 5:e1269

Dias MS, Cornu J-F, Oberdorff T, Lasso CA, Tedesco PA (2013) Natural fragmentation in river networks as a driver of speciation for freshwater fishes. Ecography 36:683–689

Eklöv P, Svanbäck R (2006) Predation risk influences adaptive morphological variation in fish populations. Am Naturalist 167:440–452

Endler JA (1982) Problems in distinguishing historical from ecological factors in biogeography. Am Zool 22:441–452

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567

Farnsworth DR, Posner M, Miller AC (2021) Single cell transcriptomics of the developing zebrafish lens and identification of putative controllers of lens development. Exp Eye Res 206:108535

Farré M, Tuset VM, Maynou F, Recasens L, Lombarte A (2016) Selection of landmarks and semilandmarks in fishes for geometric morphometric analyses: a comparative study based on analytical methods. Sci Mar 80:175–186

Fitzpatrick MC, Keller SR (2015) Ecological genomics meets community-level modelling of biodiversity: mapping the genomic landscape of current and future environmental adaptation. Ecol Lett 18:1–16

Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics 180:977–993

Forester BR, Lasky JR, Wagner HH, Urban DL (2018) Comparing methods for detecting multilocus adaptation with multivariate genotype–environment associations. Mol Ecol 27:2215–2233

França FM, Benkwitt CE, Peralta G, Robinson JP, Graham NA, Tylianakis JM et al. (2020) Climatic and local stressor interactions threaten tropical forests and coral reefs. Philos Trans R Soc B 375:20190116

Frankham R (2015) Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Mol Ecol 24:2610–2618

Freedman AH, Thomassen HA, Buermann W, Smith TB (2010) Genomic signals of diversification along ecological gradients in a tropical lizard. Mol Ecol 19:3773–3788

Gallego-García N, Forero-Medina G, Vargas-Ramírez M, Caballero S, Shaffer HB (2019) Landscape genomic signatures indicate reduced gene flow and forest-associated adaptive divergence in an endangered neotropical turtle. Mol Ecol 28:2757–2771

Gates K, Sandoval-Castillo J, Bernatchez L, Beheregaray LB (2017) De novo transcriptome assembly and annotation for the desert rainbowfish (Melanotaenia splendida tatei) with comparison with candidate genes for future climates. Mar Genomics 35:63–68

Gatz Jr AJ (1979) Community organization in fishes as indicated by morphological features. Ecology 60:711–718

Gautier M (2015) Genome-wide scan for adaptive divergence and association with population-specific covariates. Genetics 201:1555–1579

Goudet J (2005) Hierfstat, a package for R to compute and test hierarchical F-statistics. Mol Ecol Notes 5:184–186

Grummer JA, Beheregaray LB, Bernatchez L, Hand BK, Luikart G, Narum SR et al. (2019) Aquatic landscape genomics and environmental effects on genetic variation. Trends Ecol Evol 34:641–654

Haffer J (1969) Speciation in Amazonian forest birds. Science 165:131–137

Haffer J (1997) Alternative models of vertebrate speciation in Amazonia: an overview. Biodivers Conserv 6:451–476

Haldane J (1948) The theory of a cline. J Genet 48:277–284

Hanson KC, Cooke SJ (2009) Why does size matter? A test of the benefits of female mate choice in a teleost fish based on morphological and physiological indicators of male quality. Physiol Biochem Zool 82:617–624

Hattori A, Warburton K (2003) Microhabitat use by the rainbowfish Melanotaenia duboulayi in a subtropical Australian stream. J Ethol 21:15–22

Hawkins BA, Field R, Cornell HV, Currie DJ, Guégan J-F, Kaufman DM et al. (2003) Energy, water, and broad-scale geographic patterns of species richness. Ecology 84:3105–3117

Hay AC, Sandoval-Castillo J, Cooke GM, Chao NL, Beheregaray LB (2022) Riverscape Genomics Clarifies Neutral and Adaptive Evolution in an Amazonian Characin Fish (Triportheus albus). Front Ecol Evol 10:825406

Hilbert DW (2008) The Dynamic forest landscape of the Australian Wet Tropics: present, past and future. In: Stork N, Turton S (eds) Living in a dynamic tropical forest landscape. Blackwell Publishing, Carlton, VIC, Australia, pp 107–123

Hilbert DW, Hill R, Moran C, Turton SM, Bohnet I, Marshall NA et al. (2014) Climate change issues and impacts in the Wet Tropics NRM cluster region. James Cook University, Cairns

Ho WC, Ohya Y, Zhang J (2017) Testing the neutral hypothesis of phenotypic evolution. Proc Natl Acad Sci USA 114:12219–12224

Hoffmann A, Griffin P, Dillon S, Catullo R, Rane R, Byrne M et al. (2015) A framework for incorporating evolutionary genomics into biodiversity conservation and management. Clim Change Responses 2:1

Hoffmann AA, Rymer PD, Byrne M, Ruthrof KX, Whinam J, McGeoch M et al. (2019) Impacts of recent climate change on terrestrial flora and fauna: some emerging Australian examples. Austral Ecol 44:3–27

Hooker O, Barry J, Van Leeuwen T, Lyle A, Newton J, Cunningham P et al. (2016) Morphological, ecological and behavioural differentiation of sympatric profundal and pelagic Arctic charr (Salvelinus alpinus) in Loch Dughaill Scotland. Hydrobiologia 783:209–221

Hu Z-M, Zhong K-L, Weinberger F, Duan D-L, Draisma SGA, Serrão EA (2020) Linking ecology to genetics to better understand adaptation and evolution: a review in marine macrophytes. Front Mar Sci 7:545102

Jaffé R, Veiga JC, Pope NS, Lanes ÉC, Carvalho CS, Alves R et al. (2019) Landscape genomics to the rescue of a tropical bee threatened by habitat loss and climate change. Evolut Appl 12:1164–1177

Jardim de Queiroz L, Torrente-Vilara G, Quilodran C, Rodrigues da Costa Doria C, Montoya-Burgos JI (2017) Multifactorial genetic divergence processes drive the onset of speciation in an Amazonian fish. PLoS ONE 12:e0189349

Jiménez-Cisneros B, Oki T, Arnell N, Benito G, Cogley J, Döll P et al. (2014) Freshwater resources in climate change 2014: Impacts, adaptation, and vulnerability. Part a: global and sectoral aspects. In: Field C, Barros V, Dokken D, Mach K, Mastrandrea M, Bilir T et al. (eds) Contribution of working group II to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, UK, pp 229–269

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405

Jombart T, Ahmed I (2011) adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics 27:3070–3071

Killen SS, Glazier DS, Rezende EL, Clark TD, Atkinson D, Willener AS et al. (2016) Ecological influences and morphological correlates of resting and maximal metabolic rates across teleost fish species. Am Naturalist 187:592–606

Kirschel AN, Slabbekoorn H, Blumstein DT, Cohen RE, de Kort SR, Buermann W et al. (2011) Testing alternative hypotheses for evolutionary diversification in an African songbird: rainforest refugia versus ecological gradients. Evolution 65:3162–3174

Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. Mol Ecol Resour 11:353–357

Kramer DL, McClure M (1982) Aquatic surface respiration, a widespread adaptation to hypoxia in tropical freshwater fishes. Environ Biol Fishes 7:47–55

Lam AW, Gueuning M, Kindler C, Van Dam M, Alvarez N, Panjaitan R et al. (2018) Phylogeography and population genomics of a lotic water beetle across a complex tropical landscape. Mol Ecol 27:3346–3356

Langerhans RB, Layman CA, Shokrollahi AM, DeWitt TJ (2004) Predator-driven phenotypic diversification in Gambusia affinis. Evolution 58:2305–2318

Langerhans RB, Reznick DN (2010) Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. In: Domenici P, Kapoor BG (eds) Fish locomotion: an eco-ethological perspective. Science Publishers, Enfield, NH, USA, pp 200–248

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357

Laporte M, Pavey SA, Rougeux C, Pierron F, Lauzent M, Budzinski H et al. (2016) RAD sequencing reveals within-generation polygenic selection in response to anthropogenic organic and metal contamination in North Atlantic Eels. Mol Ecol 25:219–237

Lévêque C (1997) Biodiversity dynamics and conservation: the freshwater fish of tropical Africa. Cambridge University Press, Cambridge, UK

Lewis Jr WM (1970) Morphological adaptations of cyprinodontoids for inhabiting oxygen deficient waters. Copeia 2:319–326

Li H (2011) A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinforma (Oxf, Engl) 27:2987–2993

Li Y, Zhang X-X, Mao R-L, Yang J, Miao C-Y, Li Z et al. (2017) Ten years of landscape genomics: challenges and opportunities. Front Plant Sci 8:2136

Lisney TJ, Collin SP, Kelley JL (2020) The effect of ecological factors on eye morphology in the western rainbowfish, Melanotaenia australis. J Exp Biol 223:jeb223644

Lo M, Reed J, Castello L, Steel EA, Frimpong EA, Ickowitz A (2020) The influence of forests on freshwater fish in the tropics: a systematic review. BioScience 70:404–414

Luikart G, England PR, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of population genomics: from genotyping to genome typing. Nat Rev Genet 4:981

Luikart G, Kardos M, Hand BK, Rajora OP, Aitken SN, Hohenlohe PA (2018) Population genomics: advancing understanding of nature. In: Rajora OP (ed) Population genomics. Springer Nature Switzerland, Cham, Switzerland, pp 3–79

Maestri R, Fornel R, Gonçalves GL, Geise L, de Freitas TRO, Carnaval AC (2016) Predictors of intraspecific morphological variability in a tropical hotspot: comparing the influence of random and non-random factors. J Biogeogr 43:2160–2172

Manel S, Holderegger R (2013) Ten years of landscape genetics. Trends Ecol Evol 28:614–621

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol 18:189–197

Mayr E, O'Hara RJ (1986) The biogeographic evidence supporting the Pleistocene forest refuge hypothesis. Evolution 40:55–67

McCairns RJS, Smith S, Sasaki M, Bernatchez L, Beheregaray LB (2016) The adaptive potential of subtropical rainbowfish in the face of climate change: heritability and heritable plasticity for the expression of candidate genes. Evolut Appl 9:531–545

McGuigan K, Chenoweth SF, Blows MW (2005) Phenotypic divergence along lines of genetic variance. Am Naturalist 165:32–43

McGuigan K, Franklin CE, Moritz C, Blows MW (2003) Adaptation of rainbow fish to lake and stream habitats. Evolution 57:104–118

McKay JK, Bishop JG, Lin J-Z, Richards JH, Sala A, Mitchell-Olds T (2001) Local adaptation across a climatic gradient despite small effective population size in the rare sapphire rockcress. Proc R Soc Lond Ser B: Biol Sci 268:1715–1721

Merilä J, Hendry AP (2014) Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. Evolut Appl 7:1–14

Metcalfe DJ, Ford AJ (2009) Floristics and plant biodiversity of the rainforests of the Wet Tropics. In: Stork N, Turton S (eds) Living in a dynamic tropical forest landscape. Blackwell Publishing, Carlton, VIC, Australia, pp 123–132

Miller C, Taboue GT, Fokam E, Morgan K, Zhen Y, Harrigan R et al. (2020) Environmental variation predicts patterns of phenotypic and genomic variation in an African tropical forest frog. Authorea. Preprint at https://doi.org/10.22541/au.159645990.05594317

Morgan K, Mboumba J-F, Ntie S, Mickala P, Miller CA, Zhen Y et al. (2020) Precipitation and vegetation shape patterns of genomic and craniometric variation in the central African rodent Praomys misonnei. Proc R Soc B 287:20200449

Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it. Syst Biol 51:238–254

Moritz C, Patton J, Schneider C, Smith T (2000) Diversification of rainforest faunas: an integrated molecular approach. Annu Rev Ecol Syst 31:533–563

Mussmann SM, Douglas MR, Chafin TK, Douglas ME (2019) BA3-SNPs: Contemporary migration reconfigured in BayesAss for next-generation sequence data. Methods Ecol Evol. 10:1808–1813

Nosil P (2012) Ecological speciation. Oxford University Press, Oxford, UK.

Nott J (2005) The origin and evolution of Australia's eastern highlands. In: Bermingham E, Dick CW, Moritz C (eds) Tropical rainforests: past, present, and future. University of Chicago Press, Chicago, IL, USA, pp 322–335

Ntie S, Davis A, Hils K, Mickala P, Thomassen H, Morgan K et al. (2017) Evaluating the role of Pleistocene refugia, rivers and environmental variation in the diversification of central African duikers (genera Cephalophus and Philantomba). BMC Evolut Biol 17:1–17

O'Leary SJ, Puritz JB, Willis SC, Hollenbeck CM, Portnoy DS (2018) These aren't the loci you'e looking for: principles of effective SNP filtering for molecular ecologists. Mol Ecol 27:3193–3206

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D et al. (2019) vegan: Community Ecology Package. R package version 2.5-6

Paz-Vinas I, Loot G, Stevens VM, Blanchet S (2015) Evolutionary processes driving spatial patterns of intraspecific genetic diversity in river ecosystems. Mol Ecol 24:4586–4604

Pearson RG, Connolly NM, Boyero L (2015) Ecology of streams in a biogeographic isolate—the Queensland Wet Tropics, Australia. Freshw Sci 34:797–819

Pélabon C, Bolstad GH, Egset CK, Cheverud JM, Pavlicev M, Rosenqvist G (2013) On the relationship between ontogenetic and static allometry. Am Naturalist 181:195–212

Perrier C, Ferchaud AL, Sirois P, Thibault I, Bernatchez L (2017) Do genetic drift and accumulation of deleterious mutations preclude adaptation? Empirical investigation using RAD seq in a northern lacustrine fish. Mol Ecol 26:6317–6335

Pert P, Butler J, Brodie J, Bruce C, Honzak M, Kroon F et al. (2010) A catchment-based approach to mapping riparian ecosystem services using riparian habitat: a case study from the Wet Tropics, Australia. Ecol Complex 7:378–388

Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS ONE 7:e37135

Primack RB, Corlett R (2005) Tropical rain forests: an ecological and biogeographical comparison. Blackwell Publishing, Oxford, UK

Puritz JB, Hollenbeck CM, Gold JR (2014) dDocent: a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. PeerJ 2:e431

Pusey B, Kennard MJ, Arthington AH (2004) Freshwater fishes of north-eastern Australia. CSIRO Publishing, Melbourne, VIC, Australia

Pusey BJ, Arthington AH, Read MG (1995) Species richness and spatial variation in fish assemblage structure in two rivers of the Wet Tropics of northern Queensland, Australia. Environ Biol Fishes 42:181–199

Raj A, Stephens M, Pritchard JK (2014) fastSTRUCTURE: variational inference of population structure in large SNP data sets. Genetics 197:573–589

Ralls K, Ballou JD, Dudash MR, Eldridge MD, Fenster CB, Lacy RC et al. (2018) Call for a paradigm shift in the genetic management of fragmented populations. Conserv Lett 11:e12412

RC Team (2019) R: A language and environment for statistical computing. https://www.R-project.org/. R Foundation for Statistical Computing, Vienna, Austria

Reed JM (1992) A system for ranking conservation priorities for Neotropical migrant birds based on relative susceptibility to extinction. Ecology and Conservation of Neotropical Migrant Landbirds. Smithsonian Institution Press, Washington, DC, USA

Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R (2015) A practical guide to environmental association analysis in landscape genomics. Mol Ecol 24:4348–4370

Rohlf, F (2017) Program TpsDig, version 2.31. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, NY, USA

Russell D, Ryan T, McDougall A, Kistle S, Aland G (2003) Species diversity and spatial variation in fish assemblage structure of streams in connected tropical catchments in northern Australia with reference to the occurrence of translocated and exotic species. Mar Freshw Res 54:813–824

Sandoval-Castillo J, Gates K, Brauer CJ, Smith S, Bernatchez L, Beheregaray LB (2020) Adaptation of plasticity to projected maximum temperatures and across climatically defined bioregions. Proc Natl Acad Sci USA 117:17112–17121

Sandoval-Castillo J, Robinson NA, Hart AM, Strain LW, Beheregaray LB (2018) Seascape genomics reveals adaptive divergence in a connected and commercially important mollusc, the greenlip abalone (Haliotis laevigata), along a longitudinal environmental gradient. Mol Ecol 27:1603–1620

Schluter D (2001) Ecology and the origin of species. Trends Ecol Evol 16:372–380

Shuai F, Yu S, Lek S, Li X (2018) Habitat effects on intra-species variation in functional morphology: evidence from freshwater fish. Ecol Evol 8:10902–10913

Siepielski AM, Morrissey MB, Buoro M, Carlson SM, Caruso CM, Clegg SM et al. (2017) Precipitation drives global variation in natural selection. Science 355:959–962

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236:787–792

Smith S, Bernatchez L, Beheregaray LB (2013) RNA-seq analysis reveals extensive transcriptional plasticity to temperature stress in a freshwater fish species. BMC Genomics 14:1–12

Smith S, Brauer CJ, Sasaki M, Unmack PJ, Guillot G, Laporte M et al. (2020) Latitudinal variation in climate-associated genes imperils range edge populations. Mol Ecol 29:4337–4349

Smith TB, Wayne RK, Girman DJ, Bruford MW (1997) A role for ecotones in generating rainforest biodiversity. Science 276:1855–1857

Smith TB, Thomassen HA, Freedman AH, Sehgal RN, Buermann W, Saatchi S et al. (2011) Patterns of divergence in the olive sunbird Cyanomitra olivacea (Aves: Nectariniidae) across the African rainforest–savanna ecotone. Biol J Linn Soc 103:821–835

Stein JL, Hutchison MF, Stein JA (2011) National Environmental Stream Attributes v1.1.3. http://pid.geoscience.gov.au/dataset/ga/73045 Geoscience Australia, Canberra. Accessed June, 2017

Storfer A, Murphy M, Evans J, Goldberg C, Robinson S, Spear S et al. (2007) Putting the 'landscape' in landscape genetics. Heredity 98:128

Sunnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). Mol Biol Evol 13:510–524

Susuki K, Raphael AR, Ogawa Y, Stankewich MC, Peles E, Talbot WS et al. (2011) Schwann cell spectrins modulate peripheral nerve myelination. Proc Natl Acad Sci USA 108:8009–8014

Swofford DL, Sullivan J (2003) Phylogeny inference based on parsimony and other methods using PAUP. Phylogenetic Handb: A Practical Approach DNA Protein Phylogeny, cáp 7:160–206

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526

Tay HG, Ng YW, Manser E (2010) A vertebrate-specific Chp-PAK-PIX pathway maintains E-cadherin at adherens junctions during zebrafish epiboly. PLoS ONE 5:e10125

Termignoni-García F, Jaramillo-Correa JP, Chablé-Santos J, Liu M, Shultz AJ, Edwards SV et al. (2017) Genomic footprints of adaptation in a cooperatively breeding tropical bird across a vegetation gradient. Mol Ecol 26:4483–4496

Terrain NRM. (2016) Catchment Profiles, Wet Tropics Plan for People and Country. https://www.wettropicsplan.org.au/regional-themes/water/catchment-profiles/ accessed July 2020

Thomaz AT, Christie MR, Knowles LL (2016) The architecture of river networks can drive the evolutionary dynamics of aquatic populations. Evolution 70:731–739

Turner IM (2001) The ecology of trees in the tropical rain forest. Cambridge University Press, Cambridge, UK

Van der Auwera G, D O'Connor B (2020) Genomics in the Cloud: Using Docker, GATK, and WDL in Terra. O'Reilly Media, Inc, Sebastopol, Canada

de Villemereuil P, Frichot É, Bazin É, François O, Gaggiotti OE (2014) Genome scan methods against more complex models: when and how much should we trust them? Mol Ecol 23:2006–2019

Voas MG, Lyons DA, Naylor SG, Arana N, Rasband MN, Talbot WS (2007) αII-spectrin is essential for assembly of the nodes of Ranvier in myelinated axons. Curr Biol 17:562–568

Vuilleumier BS (1971) Pleistocene changes in the fauna and flora of South America. Science 173:771–780

Wainwright PC (1996) Ecological explanation through functional morphology: the feeding biology of sunfishes. Ecology 77:1336–1343

Waldvogel A-M, Schreiber D, Pfenninger M, Feldmeyer B (2020) Climate change genomics calls for standardized data reporting. Front Ecol Evol 8:242

Wallace A (1854) On the monkeys of the Amazon. Ann Mag Nat Hist 14:451–454

Weber LC, VanDerWal J, Schmidt S, McDonald WJ, Shoo LP (2014) Patterns of rain forest plant endemism in subtropical Australia relate to stable mesic refugia and species dispersal limitations. J Biogeogr 41:222–238

Weir JT, Schluter D (2007) The latitudinal gradient in recent speciation and extinction rates of birds and mammals. Science 315:1574–1576

Wellenreuther M, Hansson B (2016) Detecting polygenic evolution: problems, pitfalls, and promises. TRENDS Genet 32:155–164

Wideman JG, Novick A, Muñoz-Gómez SA, Doolittle WF (2019) Neutral evolution of cellular phenotypes. Curr Opin Genet Dev 58–59:87–94

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191

Wilson KA, Auerbach NA, Sam K, Magini AG, Moss ASL, Langhans SD et al. (2016) Conservation research is not happening where it is most needed. PLoS Biol 14:e1002413

Wootton RJ (2012) Ecology of teleost fishes. Springer Science & Business Media, London, UK

Zelditch ML, Swiderski DL, Sheets HD (2012) Geometric morphometrics for biologists: a primer. Elsevier Academic Press, London, UK

Zhang J (2018) Neutral theory and phenotypic evolution. Mol Biol Evol 35:1327–1331

Zhen Y, Harrigan RJ, Ruegg KC, Anderson EC, Ng TC, Lao S et al. (2017) Genomic divergence across ecological gradients in the Central African rainforest songbird (Andropadus virens). Mol Ecol 26:4966–4977

AUTHOR CONTRIBUTIONS

LBB conceived the study. KG generated the data with contributions from all other authors. KG analysed the data with contributions from JS-C, CJB, ML and LBB. KG drafted the article. All authors contributed to data interpretation and critically revised the article.

COMPETING INTERESTS

The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to Luciano B. Beheregaray.

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