

# Adaptation of plasticity to projected maximum temperatures and across climatically defined bioregions

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**Resilience to environmental stressors due to climate warming is influenced by local adaptations, including plastic responses. The recent literature has focused on genomic signatures of climatic adaptation, but little is known about how plastic capacity may be influenced by biogeographic and evolutionary processes. We investigate phenotypic plasticity as a target of climatic selection, hypothesizing that lineages that evolved in warmer climates will exhibit greater plastic adaptive resilience to upper thermal stress. This was experimentally tested by comparing transcriptomic responses within and among temperate, subtropical, and desert ecotypes of Australian rainbowfish subjected to contemporary and projected summer temperatures. Critical thermal maxima were estimated, and ecological niches delineated using bioclimatic modeling. A comparative phylogenetic expression variance and evolution model was used to assess plastic and evolved changes in gene expression. Although 82% of all expressed genes were found in the three ecotypes, they shared expression patterns in only 5 out of 236 genes that responded to the climate change experiment. A total of 532 genes showed signals of adaptive (i.e., genetic-based) plasticity due to ecotype-specific directional selection, and 23 of those responded to projected summer temperatures. Network analyses demonstrated centrality of these genes in thermal response pathways. The greatest adaptive resilience to upper thermal stress was shown by the subtropical ecotype, followed by the desert and temperate ecotypes. Our findings indicate that vulnerability to climate change will be highly influenced by biogeographic factors, emphasizing the value of integrative assessments of climatic adaptive traits for accurate estimation of population and ecosystem responses.**

climate change | ecological genomics | teleosts | thermal biology | climatic variability hypothesis

**C**haracterizing mechanisms underpinning variation in ecological adaptation can assist in identifying biogeographic patterns of vulnerability and resilience to environmental change. Climate change has promoted numerous range shifts and local extinctions due to exposure of populations to conditions outside their zones of tolerance (1–3). However, it is expected that some populations will be able to persist in situ if they are not already living at the edge of their tolerance limits or if they are able to acclimatize or adapt outside their current range of tolerance (4–7). Species' distributions are strongly influenced by thermal conditions in their native climates; it is expected that tolerance ranges and vulnerability to change will also be influenced by biogeographic factors (8–10). Exploring how molecular mechanisms influence upper thermal resilience is an important step for inferring responses to a warming environment (11). While plastic regulation of gene expression is expected to play an important role in adaptation, the effects of selection on plasticity are poorly understood and untangling them requires integrative approaches (12, 13).

Plasticity refers to a change in expressed phenotype as a function of the environment and occurs through direct effects of

the environment on allelic expression, as well as changes in interactions among loci (14, 15). Here, we focus on plasticity as the ability or tendency of an individual to up- or down-regulate genes in response to the environment and, particularly, on how plasticity might provide adaptive resilience to climate change. For many genes, this occurs primarily at the level of transcription, and a complexity of responses (i.e., adaptive, maladaptive, or neutral) has been documented (16, 17). For instance, plasticity can act as a buffer against environmental pressures (16, 18) and can be a target of selection if genotypes differ in environmental sensitivity (19). Alternatively, initial plastic responses could be nonadaptive under novel environmental conditions (20). In the context of climate, gene expression can inform about the functional pathways relevant for persistence under given conditions, as well as the likely targets of selection (11, 21). This is especially important where phenotypes of ecological relevance are not obvious and may be difficult to distinguish using traditional approaches (22, 23). Relatively few studies have attempted to find signals of selection acting on gene expression. Challenges include controlling for internal and external environmental variables

## Significance

**Adaptation to climate change is expected to be influenced by thermal conditions experienced by species during their evolutionary history. We studied plastic capacity as a target of climatic selection, hypothesizing that populations that evolved under warmer climates have greater plastic adaptive resilience to climate change. This was tested experimentally by comparing upper thermal tolerance and gene expression in fish populations from desert, temperate, and subtropical regions of Australia. Divergent adaptive plastic responses to future climates were found across different bioregions, including in key heat stress genes. The greatest adaptive resilience was shown by the subtropical ecotype, followed by the desert and temperate ecotypes. These results have implications for large-scale assessments of climate impacts and for predictions of species distribution changes.**

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Data deposition: The sequences for all the transcripts and ddRAD loci have been deposited to the Figshare repository, <https://doi.org/10.6084/m9.figshare.12110991>.

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influencing expression (24), as well as for the effect of genetic distance on the variation in transcription between lineages (25).

Climatically defined bioregions provide a scale at which environmental variation drives meaningful differences in evolutionary and ecological processes (26, 27). The ability of populations to persist in a warmer climate is predicted to vary geographically (6, 28–31), making climatic bioregions valuable systems for comparative studies of adaptation. For instance, the climatic variability hypothesis (CVH) predicts a positive relationship between breadth of thermal tolerance and the level of climatic variability experienced by organisms as latitude increases (32). Studies of climate change impacts are increasingly seeking to integrate spatial modeling (e.g., climatic envelopes) to uncover associations between landscape features and evolutionary processes such as temperature adaptation (33–35). While a majority of species distribution models are primarily correlative, there has been an urgent call for an increase in mechanistic approaches for predicting species' responses to climate change (36–39). Mechanistic approaches have the advantage of explaining the underlying processes associated with observed trends, allowing for findings to be interpreted more generally (40).

Freshwater fishes represent an important component of vertebrate diversity. They are arguably the most threatened group of vertebrates and, as ectotherms, are especially vulnerable to thermal changes (41). The subjects of this study are Australian rainbowfishes of *Melanotaenia* (family Melanotaeniidae), a freshwater genus with evolutionary origins in tropical southern New Guinea (42). *Melanotaenia* spp. of the “australis” clade (43) provide an ideal model system to study climatic-driven adaptive evolution and to address predictions from the CVH. The clade contains a minimum of eight largely allopatric species that recently radiated into tropical, subtropical, desert, and temperate regions of mainland Australia (43). They show adaptive phenotypic divergence due to selection linked to the hydrological environment (44, 45), as well as adaptive genomic divergence associated with hydroclimatic variation (46). In terms of gene expression, common garden experiments in a subtropical australis species (*Melanotaenia duboulayi*) have tested the effect of 2070-projected summer temperatures on short-term (47) and long-term (48) transcriptional responses. Both studies indicated capacity for plastic responses to future climates and identified candidate genes for thermal adaptation (47, 48). In addition, a transgenerational experiment revealed pedigree-based evidence for heritability of observed plastic responses (48).

This work focuses on three closely related australis species, *Melanotaenia splendida tatei*, *M. fluviatilis*, and *M. duboulayi*. Their ranges show a striking concordance with three major contemporary climatic bioregions of the Australian continent (Fig. 1A), suggesting that their evolution has been influenced by selective pressures associated with climatic regimes. For this reason, we refer to them herein as climatic “ecotypes,” *sensu* Engelhard, Ellis, Payne, ter Hofstede, and Pinnegar (49). We used an experimental approach (Fig. 2) to compare short-term transcriptional responses to a projected future temperature in subtropical, temperate, and desert rainbowfish ecotypes. In addition, we delineated ecological niches and assessed physiological tolerance to thermal stress and warming for each ecotype. We hypothesize that ecotype resilience in future climates will be dependent on the biogeographic region in which a given ecotype has evolved. As such, we predict to find evidence for adaptation of plastic responses to temperature among ecotypes. To test this, we applied a comparative phylogenetic expression variance and evolution (EVE) model framework (Fig. 2) to detect transcriptional responses subject to ecotype-specific directional selection. This enabled us to explore how divergent selection on gene expression may have contributed to differences in thermal tolerance and to adaptive evolution in climatically defined ecotypes.

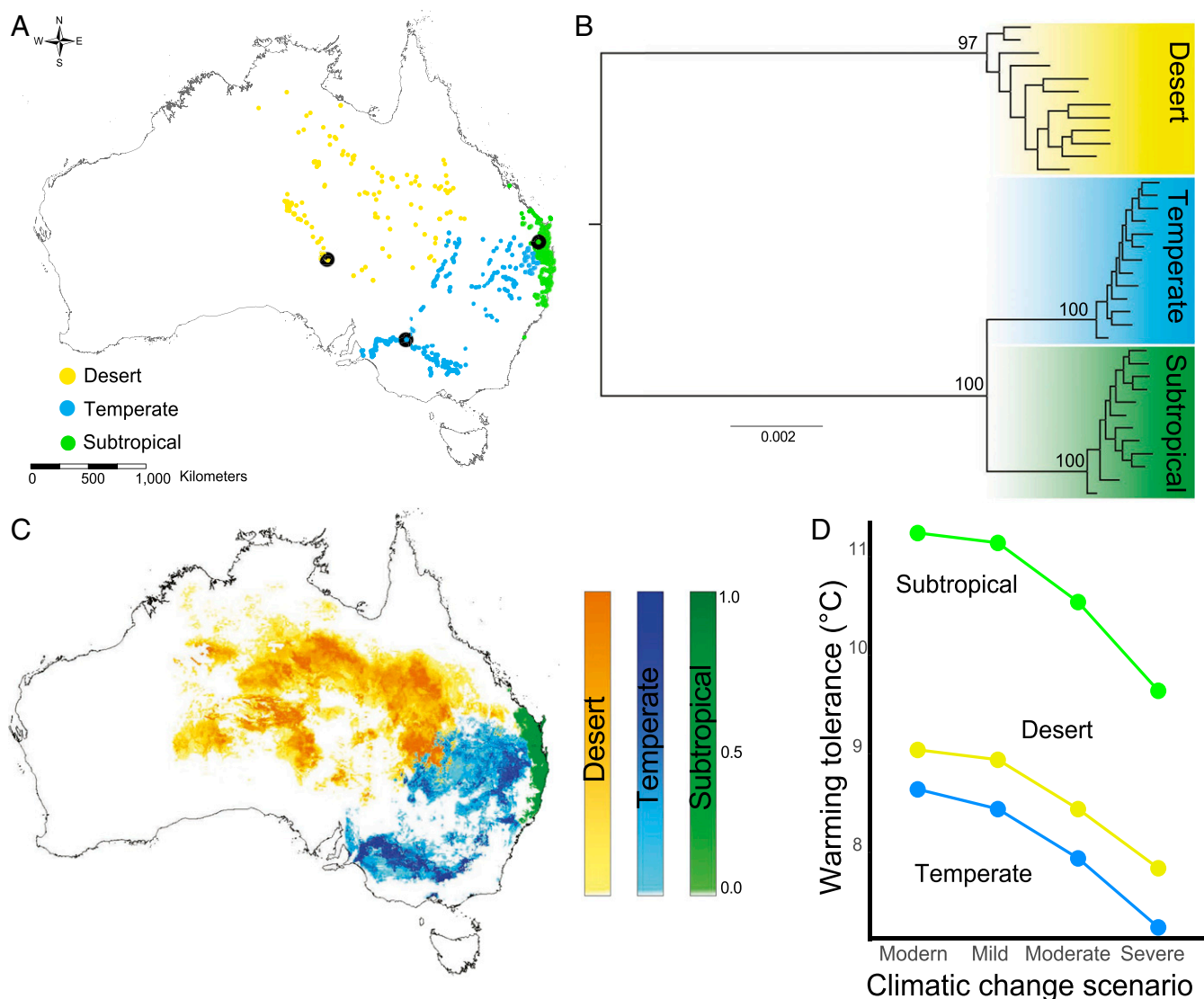
## Results

**Differential Gene Expression.** Sequencing and de novo assemblies produced transcriptomes with high percentages of gene completeness for the three rainbowfish ecotypes (*SI Appendix, Fig. S1 and Table S1*). Of the 34,815 identified unigenes, 82% (28,483) were present in all ecotypes (Fig. 3A). Comparison of gene expression profiles among ecotypes and between climate change treatments identified 2,409 differentially expressed (DE) unigenes. Expression profiles showed a strong phylogenetic pattern (i.e., transcription responses are most highly correlated among individuals within ecotypes), followed by high correlation between experimental and control groups within each ecotype (Fig. 3B; see also below). On the other hand, when gene expression was compared exclusively between climate change treatments, 236 unigenes were identified (Fig. 3C and D). Of these 236 unigenes responding plastically to climate change, 10 were shared by at least two ecotypes, with only five shared responses among all three ecotypes (Fig. 3D). In contrast, unique plastic responses to the projected summer temperature were observed for the temperate ecotype in 27 unigenes, the desert ecotype in 84 unigenes, and the subtropical ecotype in a much higher 109 unigenes. This indicates a strong phylogenetic effect on plastic gene expression but may also represent the effects of divergent selection and adaptation to different climatic ecoregions.

**Divergent Selection on Gene Expression.** The phylogenetic tree provided strong support for reciprocal monophyly of each ecotype (Fig. 1B). This tree, which is consistent with previous studies (42) that indicated a sister relationship between the temperate (*M. fluviatilis*) and the subtropical (*M. duboulayi*) ecotypes, was used as the input phylogeny for the EVE analysis. Of the 34,815 unigenes assessed with EVE, 532 showed plasticity due to ecotype-specific directional selection (false discovery rate [FDR], 10%). These were genes that showed greater expression variance among rather than within ecotypes after controlling for phylogenetic effects. The dendrogram of expression level of these 532 genes was consistent with phylogenetic patterns (*SI Appendix, Fig. S2*). Twenty-three of these genes were also identified as responding to the climate change experiment (Fig. 4A). Only 1 of these 23 EVE candidate genes was DE between treatments in all ecotypes. This suggests that the plastic responses for these 23 genes are under divergent selection for resilience to thermal stress among ecotypes, with the greatest differences between desert and the other two ecotypes.

## Functional Annotation, Enrichment Analysis, and Network Analyses.

From a total of 25,315 protein hits, 24,276 (96%) were assigned to 293,781 gene ontology (GO) terms (*SI Appendix, Table S1*). Enrichment analysis of GO terms assigned to the 236 DE unigenes between treatments (Fig. 3C) found terms for five molecular functions (MFs), 13 biological processes (BPs), and five cellular components (CCs) ( $P < 0.01$ ; *SI Appendix, Table S2*). The same enrichment analysis using the 23 EVE candidates identified between treatments (Fig. 4A) found three MF, four BP, and two CC terms ( $P < 0.01$ ; *SI Appendix, Table S3*). The protein network analysis identified six genes with very high degree of interaction, all of which were heat shock proteins (Fig. 4B and *SI Appendix, Table S4*). These hub genes included the only EVE candidate that is DE between treatments in all ecotypes. In addition, 16 candidates for shared plasticity found to be DE between treatments in two or more ecotypes, as well as all 23 EVE candidates identified between treatments, showed higher average node degrees compared with other DE genes (*SI Appendix, Table S5*). This suggests an important role of these genes in plastic and adaptive thermal stress responses of rainbowfish, respectively.



**Fig. 1.** (A) Map of Australia showing spatially and taxonomically validated records that depict the range of the three *Melanotaenia* ecotypes. The black circles show sampling localities for the transcriptomic and physiological experiments. (B) Maximum-likelihood tree depicting evolutionary relationships among 36 individuals of the three ecotypes based on ddRAD sequences of 1,799 loci and 156,581 bp. Numbers above nodes denote bootstrap support values. (C) Ecotype niche model based on 1,279 unique distribution records and nine bioclimatic variables generated in MaxEnt 3.4.1. Color tone indicates habitat suitability of each ecotype. (D) Physiological sensitivity of ecotypes across their ranges estimated by the warming tolerance quantity (41). Modern days are based on monthly average maximum temperature (BIOCLIM 2010 to 2018 data) and climatic projections are based on a global climate model (BCC-CSM2-MR) and on three shared socioeconomic pathways (SSPs) (mild, SSP126; moderate, SSP245; severe, SSP585).

**Empirical Thermal Tolerance ( $CT_{MAX}$ ).** Upper thermal tolerance was significantly different among ecotypes ( $P = <0.001$ ; *SI Appendix, Table S5* and Fig. 5) with the highest  $CT_{MAX}$  shown by the subtropical ecotype (38.0 °C; CI = 37 to 38.6 °C), followed by the desert (37.2 °C; CI = 36.1 to 37.6 °C) and finally the temperate ecotype (34.9 °C; CI = 33.1 to 36.5 °C). Interestingly, these estimates of  $CT_{MAX}$  across ecotypes were correlated with the number of DE genes between climate change treatments displayed by each ecotype ( $r = 0.998$ ; Fig. 5).

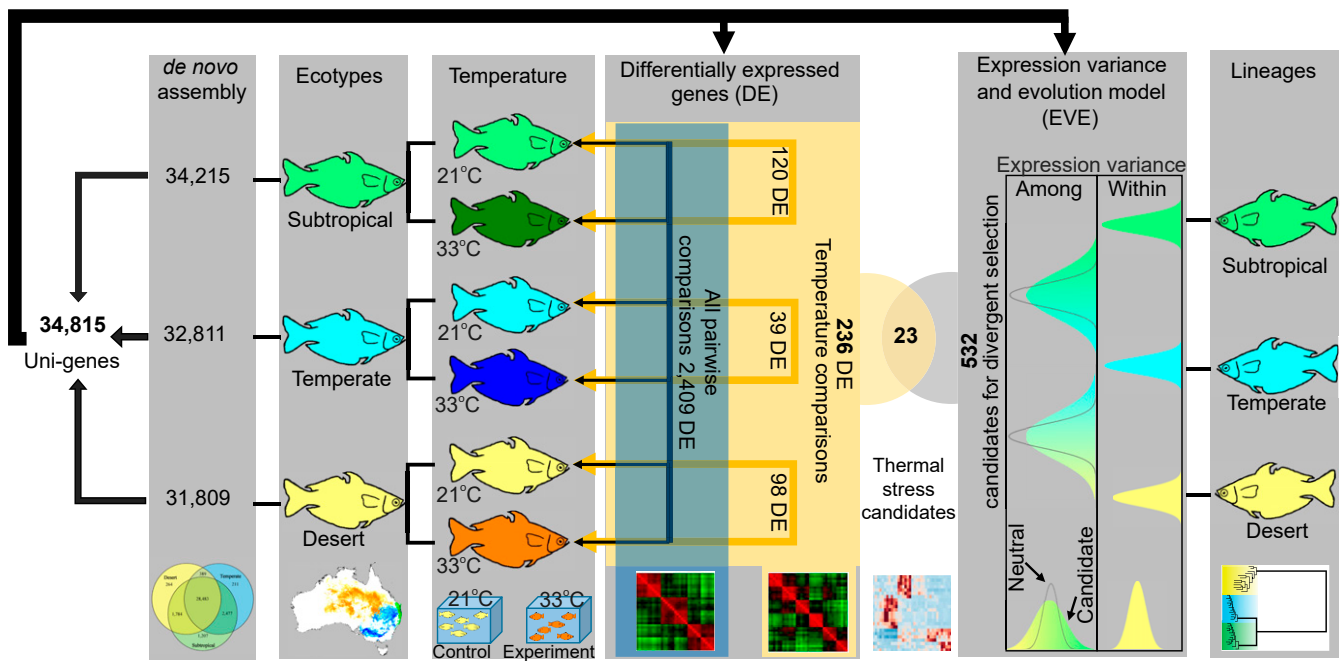
**Ecological Niche Modeling and Warming Tolerance.** Nine BIOCLIM variables were retained for analysis after correcting for data dimensionality and redundancy ( $|r| > 0.8$ ) (*SI Appendix, Fig. S3* and *Table S6*). The species distribution modeling (SDM) analyses revealed that the three ecotypes have markedly divergent bioclimatic niches (Fig. 1C), with near zero or zero niche overlap ( $P = <0.001$ ; *SI Appendix, Table S7*). The ecotypes also showed

different physiological sensitivities based on the warming tolerance quantity, with the subtropical ecotype being the more tolerant, the desert being intermediate, and the temperate the less tolerant (Fig. 1D). This pattern was observed for modern as well as for the three projected climates (Fig. 1D) and remained unaffected if warming tolerance is estimated using the maximum temperature of the warmest month (*SI Appendix, Fig. S4*).

## Discussion

We compared transcriptional plasticity to projected summer temperatures and physiological tolerance in three climatic ecotypes of Australian rainbowfish: temperate, desert, and subtropical. These ecotypes showed divergent bioclimatic niches and different physiological sensitivities to upper thermal stress and to environmental warming. Within ecotypes, individuals exhibited very similar changes in both the direction and the magnitude of expressed genes. However, gene response mechanisms to projected





**Fig. 2.** Experimental design and pipeline of the comparative transcriptomic section. Wild-caught individuals from each *Melanotaenia* ecotype (subtropical, temperate, and desert) were subjected to control (21 °C) and experimental (33 °C) treatments. Their transcriptomes were assembled de novo. Expression profiles of 34,815 unigenes were used to identify 2,409 differentially expressed (DE) genes in all pairwise comparisons (blue shading, 15 comparisons), and 236 genes DE between temperature treatments for the same ecotype (orange shading, three comparisons). In parallel, an expression variance and evolution (EVE) model was used to identify genes for which expression plasticity is under divergent selection. Under a neutral model, the ratio of the expression variance between vs. within lineages (i.e., ecotypes) is the same for all genes, compared to a higher relative ratio for genes under divergent selection. This resulted in 532 EVE candidates for divergent selection on expression between ecotypes. Twenty-three genes (overlapping gray and orange area) that were DE between temperature treatments for the same ecotype and also identified by EVE were considered candidates for adaptation to projected upper thermal stress.

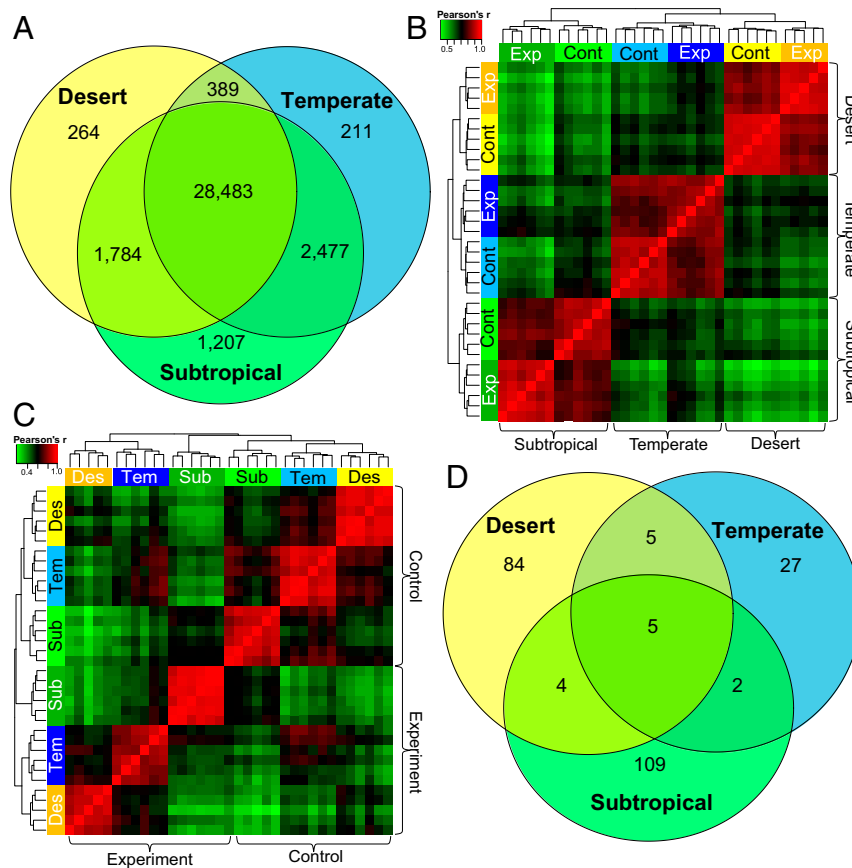
thermal stress differed remarkably among ecotypes. Interestingly, both plastic responses and estimates of physiological tolerance (i.e.,  $CT_{MAX}$  results and environmental warming tolerance) varied in a biogeographically determined manner. Subtropical rainbowfish showed both the highest transcription response and tolerance among ecotypes, temperate rainbowfish showed the lowest responses and tolerance, and desert rainbowfish showed intermediate transcriptional responses and physiological tolerance.

Although all species mounted substantial plastic responses to 2070-projected summer temperatures, a striking result was that responses were ecotype-specific, with transcriptional changes for only five genes common to all three ecotypes. This is consistent with lineage-specific adaptation resulting from contrasting selective pressures across the climatically defined bioregions, but can also be associated with neutral mechanisms of evolution (25, 50, 51). For this reason, we incorporated a comparative phylogenetic model to control for the effects of neutral drift on gene expression (52). This approach identified a large suite of 532 candidates for divergent selection on gene expression between ecotypes, of which a subset of 23 genes also showed significant ecotype-specific response to thermal manipulation (Fig. 4). We consider these 23 genes as strong candidates for adaptive (i.e., genetic-based) plastic response to future increases in temperature. Network analyses demonstrated centrality of these genes in thermal response pathways, while also identifying several highly conserved hub genes. These genes appear to be of fundamental importance for modulating thermal response pathways and adaptive potential in the three ecotypes. Together, these results show that while integral expression responses can be conserved among ecotypes, the tendency for divergence in response to upper thermal stress is high. This divergence not only exceeds neutral expectations but corresponds to inferred ecotype differences in niche suitability and tolerance to environmental

warming across climatically defined bioregions, speaking to the importance of biogeographic history in considerations of climate-adaptive potential.

**Adaptive Mechanisms Contribute to Gene Expression Plasticity among Ecotypes.** Shifts in gene expression regulation have for a long time been hypothesized to contribute to adaptive diversity (53). However, the evolution of plastic responses by natural selection has been infrequently documented in empirical studies, particularly in natural populations (but see refs. 51 and 54–57). Although a diversity of mechanisms regulate gene expression (58), substantial empirical evidence supports heritability of expression responses (51, 59, 60), including for the subtropical Australian rainbowfish (*M. duboulayi*) (48). As such, plasticity is likely to be subject to the same broad evolutionary processes as other heritable traits. For instance, under directional selection, limited expression polymorphism is expected within ecotypes, while extensive divergence is expected between ecotypes (61). Under stabilizing selection, expression regulation is predicted to be highly consistent within and across ecotypes (16, 61). Meanwhile, under neutral evolution, patterns of gene expression are expected to correlate with evolutionary divergence (51), which we assessed using a phylogenetic-based approach. Our comparative analyses suggested that all of the above mechanisms have influenced gene expression responses to projected thermal limits in rainbowfishes. This fits with our understanding of thermal tolerance adaptation in ectotherms as highly complex, and involving multiple levels of biological organization (62, 63).

The majority of DE genes under 2070-projected temperature manipulation exhibited patterns of variation that could be associated with phylogenetic distance (Figs. 1B and 3B). This demonstrates that plastic responses to future climates can be



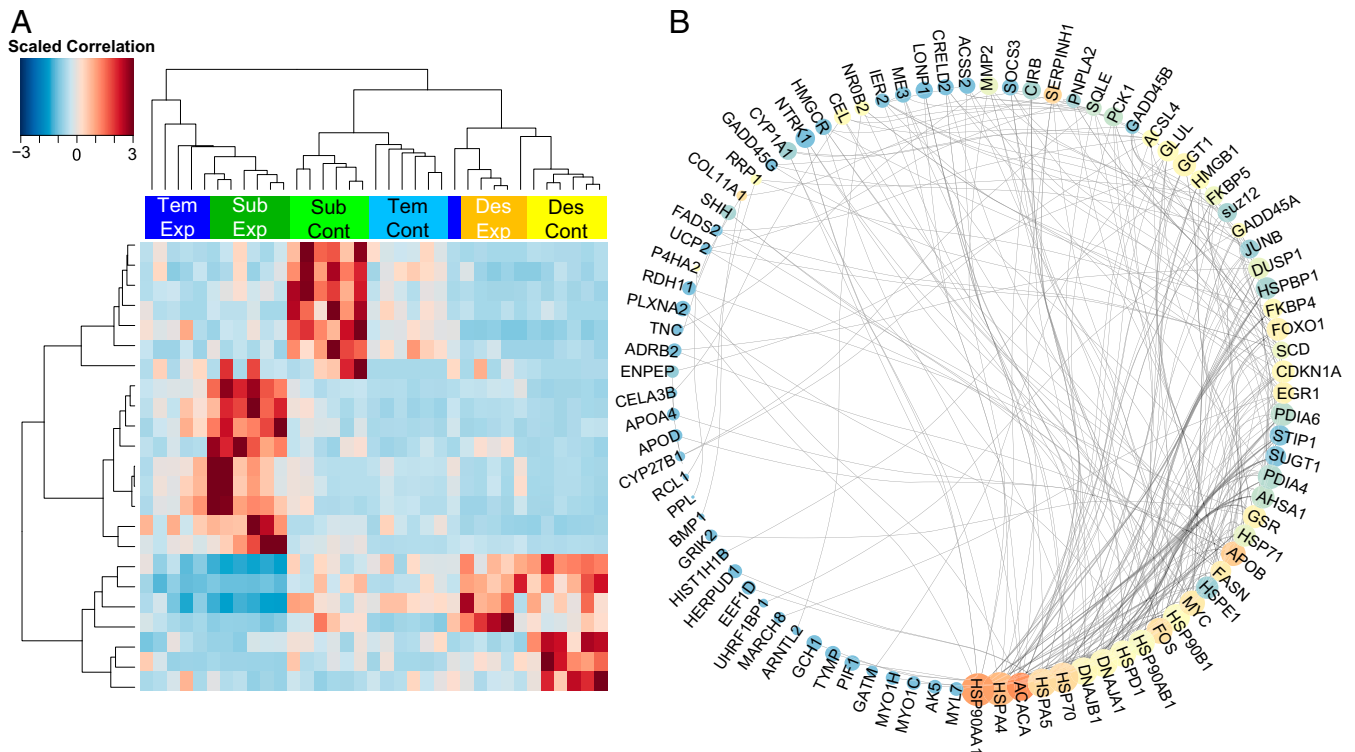
**Fig. 3.** (A) Venn diagram of unigenes identified in each ecotype of *Melanotaenia* as well as shared among ecotypes (based on a total of 34,815 unigenes). (B) Heatmap summarizing correlation among ecotypes in log<sub>2</sub> gene expression profiles. This analysis was based on 2,409 DE transcripts. The colored bars under the sample dendrograms represent the climate change experimental (Exp) and control (Cont) groups. (C) Heatmap summarizing correlation between treatments (control vs. experiment) in log<sub>2</sub> gene expression profiles. This analysis was based on 236 DE unigenes identified between control and experiment samples. Colored bars under the sample dendrograms represent the ecotypes, with climate change experimental groups represented by dark color variation and control groups represented by light color variation. (D) Venn diagram of DE unigenes shared between ecotypes.

highly constrained by demographic history. Nonetheless, we were able to reject neutral scenarios as the most parsimonious explanation for the variation in a large subset of DE genes. In the case of the EVE candidates for directional selection to thermal stress, there was very little expression variance within ecotypes, but high levels of divergence between ecotypes. We suggest that regulatory differences in these genes have helped to facilitate persistence of rainbowfish ecotypes within their respective thermal environments (Fig. 1C), bringing each closer to a local phenotypic optimum. While evidence of ecological selection on plasticity is rare, an example includes the gene expression divergence of a soil-dwelling hexapod (*Orchesella cincta*) in populations subsisting in contaminated mine sites (56). This was correlated with heavy metal tolerance, which resulted in a heritable increase in metal excretion efficiency. In fish, Brennan et al. (64) demonstrated a shift in salinity-specific expression responses in populations of killifish (*Fundulus heteroclitus*) adapted to habitats of contrasting salinity.

The center of the gene interaction network for the three rainbowfish ecotypes consisted largely of heat shock proteins that play an important role in thermal responses in a wide variety of taxa (65, 66). Patterns of plastic responses to temperature were most likely to be shared among ecotypes in these central “hub” genes (*SI Appendix, Table S4*). This indicates a conserved functional role, which may have been retained through purifying and possibly stabilizing selection. Hub genes influence the expression and activity of genes downstream in an expression

network and tend to be highly conserved in their expression between lineages (67). In genome-wide studies of model organisms, the deletion of hub genes is more likely to be deleterious than for nonhub genes. This can be due to either compromised network structure, or simply because they are more likely to be involved in essential interactions (68). However, the fact that the EVE candidates for divergent expression among ecotypes also exhibited greater average connectedness than other DE genes suggests the importance of these genes in the respective ecological adaptations they have likely facilitated. In fact, three EVE candidates were also identified as hub genes, and one of these shows plasticity in all ecotypes (HSP90AA1). A change in expression in one or a few hub genes could therefore translate to a completely different stress response pathway. Indeed, enrichment analyses indicate that functions as diverse as metabolism, immune response, oxidative stress response, DNA damage response, signal transduction, and other stress responses are contributing to local adaptation among ecotypes.

While the transcriptomic approaches used here directly address functional mechanisms for thermal response, we are not yet able to infer specific fitness effects of divergent expression patterns in warming climates. Despite this, the number of genes regulated in response to warming differed markedly between ecotypes, with the greatest number in the most heat-resilient subtropical ecotype (CT<sub>MAX</sub>, 38.0°), and the smallest number responding in the least heat-resilient temperate ecotype (CT<sub>MAX</sub>, 34.9°) (Fig. 5). This was consistent with range-wide



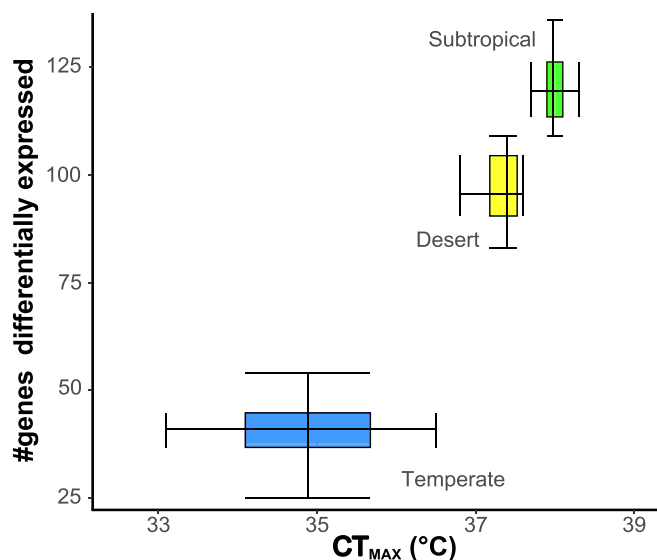
**Fig. 4.** (A) Hierarchical clusters of 23 transcripts identified as candidates for divergent selection on expression level and also showing significant differential expression between control and experiment. Color bars indicate the ecotype of the samples. DesCont, desert control (20 °C); DesExp, desert experimental (33 °C); SubCont, subtropical control (20 °C); SubExp, subtropical experimental (33 °C); TemCont, temperate control (20 °C); TemExp, temperate experimental (33 °C). (B) Protein interaction network containing 137 heat stress-associated proteins linked via 1,114 interactions. Size of node is proportional to its centrality in the network, color of node indicates the relative number of interactions it is directly involved in (blue, lower, to red, higher number of interactions), and both color and size of the node indicate relative importance of the protein in *Melanotaenia* heat stress response.

patterns of warming tolerance estimated for the ecotypes for both modern and projected climates (Fig. 1D). Similarly, previous work comparing montane and desert redband trout (*Oncorhynchus mykiss gairdneri*) found that the more resilient desert trout regulated a larger number of genes than the less resilient montane trout in response to acute warming conditions (69, 70). While the absolute number of transcripts regulated in a given condition can depend on many factors, including differences in constitutive expression or qualitative differences such as amino acid or regulatory changes (71), it is possible that a larger number of regulated genes may reflect a multifaceted [e.g., Chadwick et al. (72)] plastic response to environmental stressors. In rainbowfish, it is too early to say whether the observed increase in number of DE genes represents a more specialized adaptation to heat by the subtropical rainbowfish compared to desert and temperate ecotypes. However, the association between thermal tolerance and number of regulated transcripts does provide further evidence to support adaptive differences in the potential for expression-mediated phenotypic plasticity.

**Physiological Thermal Tolerance Is Specific to Ecotype.** It is generally assumed that organisms are adapted to or have the ability to acclimate to the temperatures normally encountered in their habitat range (29). It has been proposed that organisms that evolved in warmer climates will have higher thermal tolerances than those in cool climates (73), and that those that evolved in variable climates will have greater acclimation capacities and tolerance ranges than those in more stable climates (32). We found that ecotypes show different climatic envelopes and environmental warming tolerance and differed significantly in

$CT_{MAX}$ . Although it is unclear how well  $CT_{MAX}$  predicts thermal tolerance in wild populations, a recent zebrafish study showed that tolerance to rapid warming correlates with tolerance to slow warming, indicating that  $CT_{MAX}$  is likely to be representative of resilience to longer episodes of warming, such as heat waves (74). Consistent with several studies assessing relationships between thermal tolerance and latitude (5, 10, 29, 41, 75, 76), and with Janzen's (32) CVH assumption that organisms adapt to the temperatures they normally encounter (29, 32), rainbowfish thermal tolerance increases with proximity to the equator. However,  $CT_{MAX}$  does not coincide entirely with average maximum summer temperatures (or average annual temperatures) in the climate of origin, with the hottest Australian temperatures found in the central deserts as opposed to the north-eastern subtropical region (SI Appendix, Table S5; ref. 77). Perhaps counterintuitively, this finding supports studies that emphasize the importance of temperature variability in relation to an organism's upper limits of thermal tolerance (5, 29, 76).

Although wider ranges of tolerance have been found at higher latitudes, these have been largely attributed to lower critical thermal minimums of temperate organisms (10, 29). Meanwhile, higher thermal tolerances have been observed in tropical regions, but with an inverse relationship to tolerance breadth (36). This has led to the use of the term "climate specialists" to describe tropical species, with an evolutionary trade-off suggested between upper thermal tolerance and the capacity to acclimate to a wide range of temperatures (36, 41, 78, 79). Due to this apparent trade-off, our findings may highlight an unforeseen risk for desert taxa, which are theoretically expected to show the greatest thermal plasticity. While the temperate ecotype showed



**Fig. 5.** Association between  $CT_{MAX}$  and number of genes differentially expressed in response to projected climate in three ecotypes of *Melanotaenia* ( $r = 0.998$ ). The box plots display the upper and lower quartiles, whiskers represent 95th and 5th percentiles, and their intersections represent the median.

the lowest transcription response and tolerance, the ability of the desert rainbowfish to adapt to extremely high temperatures may be compromised by the need to maintain a large window of tolerance. It is already common for ambient temperatures at the desert rainbowfish's sampling locations (77) to exceed its  $CT_{MAX}$ , although larger water bodies are unlikely to reach such extremes due to the fluctuation in diurnal temperatures ( $\sim 15^\circ\text{C}/\text{d}$ ) and slow rates of heat exchange between air and water (80). However, desert environments are predicted to experience more extreme heat waves and longer droughts under climate change scenarios (81). This is likely to not only increase the length of time in which organisms are exposed to thermal stress conditions, but decrease the overall volume of aquatic refugia, making them more susceptible to extreme ambient temperatures (82). In such circumstances, typical behavioral responses such as seeking shade or cool-water sites created by deeper water or inflowing tributaries may be unable to compensate for these effects (82, 83). The current poor understanding of desert ecosystems and the disparate approaches used to study climate change impacts in these regions (84) highlight the need for an integrated reassessment of dryland vulnerability to climate change.

**Conclusions and Perspectives.** Climate change is creating a discord between some organisms' physiologies and their environments. To predict the likelihood of range shifts, population declines, or local extinctions, it is useful to understand the distribution of adaptive diversity, including that of adaptive plasticity. However, despite extensive empirical studies about standing genetic variation and its effects on climate-related traits, the concept of adaptive plasticity remains relatively unaddressed. Our results supported the hypothesis that the capacity for plastic response to climate varies biogeographically, even within a closely related group. Moreover, by controlling for the effects of phylogeny, we showed that divergent selection on gene expression has contributed to observed differences in plastic capacity among ecotypes. By demonstrating immediate response mechanisms to upper thermal stress, as well as evidence for ecological selection on these mechanisms, our study emphasizes the key role of plasticity in both short- and long-term climatic adaptation. This has implications for broad biogeographic assessments of climate

impacts, as well as for more focused predictions of species distribution changes, which are only now beginning to account for intrataxonomic adaptive variation. This study represents a stride toward a more holistic understanding of climatic adaptive potential in natural populations.

## Materials and Methods

**Ecotype Range, Sampling, and Temperature Experiments.** Our study system includes the crimson spotted rainbowfish (*Melanotaenia duboulayi*)—a species with a subtropical distribution along coastal catchments of eastern Australia; the Murray River rainbowfish (*M. fluviatilis*)—a temperate species found in the inland Murray-Darling Basin; and the desert rainbowfish (*M. splendida tatei*)—a species found in arid and semiarid catchments of central Australia (Fig. 1 A and C). *Melanotaenia duboulayi* individuals were collected using bait traps and hand nets from the upper section of the Brisbane River, near Fernvale in Queensland (subtropical;  $27^\circ 26' 37.39''\text{S}$ ,  $152^\circ 40' 12.76''\text{E}$ ). *Melanotaenia fluviatilis* were collected from the midsection of the Murray River, close to Gol Gol in New South Wales (temperate;  $34^\circ 10' 50.3''\text{S}$ ,  $142^\circ 13' 16.8''\text{E}$ ) using a seine net. *Melanotaenia splendida tatei* were collected from Algebuckina Waterhole in South Australia (desert;  $27^\circ 51' 53.9''\text{S}$ ,  $135^\circ 53' 57.1''\text{E}$ ) using fyke nets. Between 42 and 60 individuals were collected at each locality, transported live to the Flinders University Animal House Facility and acclimatized at  $21^\circ\text{C}$  for at least 60 d prior to temperature experiments. Individuals from each species were maintained in single-sex aquaria ( $\sim 20$  fish/100 L) at  $21^\circ\text{C}$  under 12-h light/12-h dark and fed once daily with blood worms and fish pellets. To assess short-term responses to contemporary ( $21^\circ\text{C}$ ) and 2070-projected ( $33^\circ\text{C}$ ) average summer temperatures, individuals of each species were randomly assigned to an experimental or a control group ( $n = 6$  per group, per species). Temperature in these “climate change treatments” was increased by  $2^\circ\text{C}$  per d over 6 d toward the target of  $33^\circ\text{C}$ , and then maintained for 14 d. The  $33^\circ\text{C}$  is the projected average summer temperature for Australia's east coast in 2070 based on a high emission scenario (RCP8.5) of the International Panel on Climate Change (47, 85). Control groups were kept at  $21^\circ\text{C}$  for the duration of the experiment. Fish were euthanized following the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 2013 and immediately dissected to extract the liver. Sampling procedures took place between 9:00 AM and 11:00 AM. Only adult males of similar length were used to control for sex- and age-related effects on transcription responses. Liver tissue was incubated at  $4^\circ\text{C}$  for 12 h in RNAlater (Ambion) before storage at  $-80^\circ\text{C}$ . In addition to being a relatively homogenous tissue, liver was selected because metabolic conditioning and gene expression are known to respond to heat stress (47, 48).

**Transcript Quantification and Differential Expression Analysis.** Methodological details about RNA extraction, transcriptome sequencing, and assembly are provided in *SI Appendix*. For the differential expression tests between experimental groups and among ecotypes, reads for each sample were mapped back to the predicted protein coding regions using BOWTIE2 V2.2.7 (86), and then gene-level abundance was estimated with RSEM V1.2.19 (87). Read count estimations were cross-sample normalized using trimmed mean of M values (88). Normalized count data were then used as input in DESeq2 V1.10.1 (89). We used a conventional threshold (e.g., refs. 90 and 91) where transcripts with a minimum  $\log_2$  fold change of 2 between any two groups (i.e., experimental vs. control, ecotype vs. ecotype) were considered DE (FDR of 5%).

**Gene Expression Plasticity and Divergent Selection.** We implemented the EVE Model (52) to identify transcripts potentially under divergent selection for expression levels. Briefly, the model uses a phylogenetic tree and expression data to estimate a parameter  $\beta$  that represents the ratio of among-lineage expression divergence to within-lineage expression diversity. This ratio should be approximately constant over most genes if no divergent selection is acting between lineages. For each transcript ( $i$ ), the EVE model assesses the null hypothesis that independent transcript  $\beta_i$  is not significantly different to a shared  $\beta_s$  for all transcripts; if  $\beta_i$  is higher than  $\beta_s$ , the model assumes that transcript  $i$  is subject to lineage-specific directional selection on expression level. We considered transcripts to be under divergent selection when  $\beta_i$  was significantly higher than  $\beta_s$  (FDR of 10%).

To calculate the expected expression covariance between lineages under shared and independent evolutionary scenarios, we constructed a phylogenetic tree using genome-wide SNP (single-nucleotide polymorphism) data from 12 samples of each ecotype. These data were obtained using reduced-



representation sequencing (ddRAD) for studies of population genomics of the three ecotypes (46) (*SI Appendix, Table S8*). The software PyRAD V3.0.6 (92) was used to align the ddRAD sequences and RAXML V8.2.1 (93) used to perform a maximum-likelihood phylogenetic analysis, with the GTRGAMMA model and 1,000 bootstrap replicates. The final concatenated dataset for the 36 rainbowfishes was based on 1799 ddRAD loci and 156,581 bp. The consensus phylogenetic tree was used as the input phylogeny for the EVE analysis.

**GO Enrichment Analysis and Pathway Network Analysis.** Enrichment analysis on the DE genes and on the EVE candidate genes relative to all genes were performed using TOPGO v2.34 (94) based on both Fisher's classic and weight tests ( $P \leq 0.01$ ). To further understand the relative importance of candidate and shared plastic genes, a network analysis was conducted using CYTO-SCAPE V3.7 (95). First, a protein interaction network was created from the entire DE gene set by drawing edges between genes with physical and functional interactions reported for humans and with orthologous functions in zebrafish in the STRING database (96). The relative importance of a protein is correlated with its connectivity in an interactive network. We calculated the node degrees as an estimator of protein connectivity. We then identified highly connected genes (hubs) following ref. 97, as those with a node degree greater than or equal to the sum of the mean, plus twice the SD of the node degree distribution.

**Thermal Tolerance ( $CT_{MAX}$ ).** We determined the thermal tolerance of each ecotype via short-term  $CT_{MAX}$  experiments following Becker and Genoway (98). To control for sex- and age-related effects, we sampled 10 adult females of similar length from each ecotype from the same populations used for the transcriptomic experiments. Each fish was placed individually in a 5-L glass beaker containing 3 L of water at 21 °C. Temperature was increased at a rate of 0.33 °C/min using a digital water bath until the fish showed both motor disorganization and loss of equilibrium for a period of 1 min (98).  $CT_{MAX}$  for a given ecotype was obtained by averaging over 10 independent replicates. An ANOVA test was used to assess statistical differences in  $CT_{MAX}$  among ecotypes.

**Ecological Niche Modeling and Warming Tolerance.** The ecological niche for each ecotype was obtained via SDM using MaxEnt 3.4 (99). This capitalized on

extensive spatial records for the three ecotypes (Atlas of Living Australia; <https://www.ala.org.au/>) and on 19 BIOCLIM datasets (<https://www.worldclim.org/>) (35) that include variables known to influence hydroclimatic adaptation in Australian rainbowfish (46). Because BIOCLIM data are based on air temperature, we used a global standard regression model to calculate stream water temperature (100). Niche identity and overlap between ecotypes were assessed by calculating Schoener's D and Hellinger distance in ENMTools (101). Ecologically meaningful climatic envelopes were obtained with the Bayesian-based *Plateau* climate envelope function (102).

We estimated warming tolerance [*sensu* Deutsch et al. (41)] to assess physiological sensitivity of each ecotype across their ranges. This quantity approximates the average amount of environmental warming an ectotherm can tolerate before performance drops to fatal levels, and is defined as the difference between  $CT_{MAX}$  and the mean monthly maximum temperature experienced by an ecotype across its range (41). This was estimated for modern days based on monthly average maximum temperature (BIOCLIM 2010 to 2018 data) and for three projected warming scenarios based on the Beijing Climate Center Climate System Model (BCC-CSM2-MR) and three Shared Socioeconomic Pathways (SSPs) (mild, SSP126; moderate, SSP245; severe, SSP585). For comparison, we also estimated warming tolerance using the maximum temperature of the warmest month (*SI Appendix, Fig. S4*). Additional details for this section are in *SI Appendix*.

**Animal Ethical Approval.** Animal ethical approval was received from Flinders University Animal Welfare Committee (AWC) (AWC E342 and AWC E429).

**Data Availability.** Sequences for all of the transcripts and ddRAD loci have been uploaded to the Figshare repository at <https://doi.org/10.6084/m9.figshare.12110991>.

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