Liao et al. *Zool. Res.* 2025, 46(3): 527−537
https://doi.org/10.24272/j.issn.2095-8137.2024.383
https://cstr.cn/32248.14.zr.2024.383

ZR | Zoological Research

Article Open Access

RNA editing generates mRNA isoforms with distinct stabilities that may expand the thermal tolerance of mRNA and proteins in *Mytilus* species

Ming-Ling Liao[1,2,*], Ya-Jie Zhu[1], Xiao-Lu Zhu[1,3], George N. Somero[4], Yun-Wei Dong[1,2]

[1] *Key Laboratory of Mariculture of Ministry of Education*, *Fisheries College*, *Ocean University of China*, *Qingdao*, *Shandong* 266003, *China*
[2] *Shandong Key Laboratory of Green Mariculture and Smart Fishery*, *Fisheries College*, *Ocean University of China*, *Qingdao*, *Shandong* 266003, *China*
[3] *Academy of the Future Ocean*, *Ocean University of China*, *Qingdao*, *Shandong* 266100, *China*
[4] *Department of Biology*, *Hopkins Marine Station*, *Stanford University*, *Pacific Grove*, *CA* 93950, *USA*

ABSTRACT

Ectothermic organisms may expand their thermal tolerance by producing multiple protein isoforms with differing thermal sensitivities. While such isoforms commonly originate from allelic variation at a single locus (allozymes) or from gene duplication that gives rise to paralogs with distinct thermal responses, this study investigated mRNA editing as an alternative, post-transcriptional mechanism for generating mRNA variants. Cytosolic malate dehydrogenase (cMDH) was examined in foot tissue of two congeners of the marine mussel genus *Mytilus*, which occupy different thermal environments. Multiple editing events were detected within the mRNA coding region in both species. Editing sites were species-specific, with no shared positions identified. In *M. coruscus*, editing occurred at 117, 123, 135, 190, 195, 204, 279, and 444, while in *M. galloprovincialis*, editing was detected at 216 and 597. Each species exhibited multiple edited mRNA variants, and these isoforms were associated with differential protein expression. These findings suggest that mRNA editing may contribute an additional layer of molecular variation. The generation of diverse mRNA isoforms from a single DNA coding sequence may enhance enzymatic flexibility across temperature ranges, supporting eurythermal physiological performance and mitigating thermal stress. Moreover, the presence of multiple edited transcripts within individual organisms raises important caveats about the limitations of approaches that deduce amino acid sequences or estimate adaptive variation solely from genomic data.

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright ©2025 Editorial Office of Zoological Research, Kunming Institute of Zoology, Chinese Academy of Sciences

Keywords: RNA editing; mRNA stability; mRNA isoform; Temperature adaptation

INTRODUCTION

For over five decades, evolutionary and ecological studies have examined the extent and significance of genetic variation in natural populations (Charlesworth et al., 2016; Hubby & Lewontin, 1966; Singh et al., 1975, 1976; Somero & Soulé, 1974; Watt & Dean, 2000). One theme in this research concerns the capacity of protein isoforms to confer physiological resilience across fluctuating environmental conditions, particularly in the context of temperature. It has been hypothesized that ectothermic organisms may achieve broader thermal tolerance through the expression of protein isoforms with distinct thermal optima, thereby enabling cellular function across a wider range of temperatures than would be possible with a single protein variant (Powers et al., 1993). Several mechanisms underlying the generation of thermally adaptive isoforms have been identified, including multiple genes that encode temperature-adaptive protein paralogs and are differentially regulated in response to temperature shifts (Baldwin & Hochachka, 1970; Goldspink, 1995; Watabe, 2002), allelic variation producing allozymes with varying thermal sensitivities (Place & Powers, 1984; Watt, 1983; Watt & Dean, 2000), conformational variants arising from a single amino acid sequence that exhibit temperature-dependent folding behaviors (Fields & Somero, 1997; Fields et al., 2002), and temperature-induced alternate splicing of mRNA transcripts (Healy & Schulte, 2019). While each of these mechanisms contributes to thermal adaptation to some extent, current evidence does not support a dominant or proteome-wide role for any single pathway.

Two recent advances in the study of temperature effects on mRNAs have expanded our understanding of macromolecular

Received: 21 December 2024; Accepted: 14 January 2025; Online: 15 January 2025

Foundation items: This study was supported by the National Key Research and Development Program of China (2022-24), National Natural Science Foundation of China (42025604, 42376102), and Fundamental Research Funds for the Central Universities

*Corresponding author, E-mail: liaoml@ouc.edu.cn

isoform diversity, highlighting post-transcriptional mechanisms that may contribute to thermal adaptation across both the proteome and transcriptome. One such mechanism involves environmentally responsive RNA editing, which has emerged as a potential mediator of acclimation to environmental change (Buchumenski et al., 2017; Rieder et al., 2015; Stocker et al., 2013). Temperature-dependent mRNA editing introduces adaptive protein isoforms through nucleotide substitutions that alter coding sequences (Birk et al., 2023; Garrett & Rosenthal, 2012a, 2012b; Rangan & Reck-Peterson, 2023; Rosenthal, 2015; Shoshan et al., 2021). RNA editing via adenosine deamination, catalyzed by adenosine deaminases acting on RNA (ADARs), has been shown to recode protein sequences, particularly in ion channel proteins, thereby modulating their thermal sensitivity in an adaptive manner. For instance, ADAR-mediated editing that substitutes isoleucine with valine in the Na^+/K^+-ATPase of octopuses enhances ion transport, while the same substitution in a potassium channel increases its closing rate. These modifications correspond with the thermal environments of the source organisms, suggesting a role in environmental adaptation (Colina et al., 2010; Garrett & Rosenthal, 2012a). The underlying mechanism involves the conversion of adenosine (A) to inosine (I), which is interpreted as guanosine (G) during translation, and is thus conventionally referred to as A-to-G editing. In addition to recoding, A-to-I editing can also alter the stability of RNA secondary structures. Another RNA deamination process, catalyzed by cytidine deaminases acting on RNA (CDARs), converts cytidine (C) to uracil (U) and has been implicated in stress-responsive RNA editing in animals, particularly under hypoxic or inflammatory conditions in leukocytes (Licht & Jantsch, 2016). While C-to-U editing occurs at high frequencies in land plants, particularly in organellar transcripts, and displays temperature sensitivity (Chu & Wei, 2020; Zhang et al., 2020), its occurrence in metazoans is relatively infrequent. To date, the potential role of CDAR-mediated editing in thermal adaptation in animals remains largely unexplored.

A second, more recent discovery that broadens our understanding of temperature-isoform diversity highlights the role of RNA structural adaptation. Specifically, the thermal stability of mRNA secondary structures has been shown to correlate strongly with the evolutionary thermal environments of organisms (Liao et al., 2021). Comparative analyses of orthologous mRNAs across species reveal a significant association between mRNA structural stability and species-specific adaptation temperatures. Given the multiple regulatory and functional roles of mRNA secondary structures (Mortimer et al., 2014), this temperature-related variation in stability of secondary structures is viewed as a relationship similar to that found for orthologous proteins, wherein stabilities of higher order structures correlate positively with temperature of adaptation (Becskei & Rahaman, 2022; Dong et al., 2018, 2022; Liao et al., 2019; Somero et al., 2017). Collectively, these two lines of evidence on temperature-mRNA relationships raise a number of interesting questions regarding temperature-specific isoforms at both the transcript and protein levels. In particular, the occurrence of mRNA editing suggests that cells may generate diverse transcript and protein isoforms with distinct thermal sensitivities, as reflected by differences in structural stability. Such thermally tuned isoform pools may expand the biochemical flexibility of ectothermic organisms, enhancing their ability to maintain cellular function under fluctuating environmental temperatures.

Previous studies have demonstrated that the majority of RNA editing events in animals involve ADAR and CDAR activities (Alon et al., 2015; Zhang et al., 2016). Extensive studies in cephalopods have revealed a striking predominance of A-to-I editing, accounting for 84%–93% of all detected editing events in squid (*Doryteuthis pealeii*), cuttlefish (*Sepia officianalis*), and octopuses (*Octopus vulgaris* and *O. bimaculoides*). In contrast, transcriptomic analyses of the nautiloid *Nautilus pompilius* and the gastropod *Aplysia californica* reported no enrichment for A-to-G mismatches, but readily apparent G-to-A, C-to-T, and T-to-C mismatches (Liscovitch-Brauer et al., 2017). These atypical patterns were interpreted as likely resulting from background noise, although the precise origins remain unresolved. Recent studies have also documented a wide array of noncanonical mRNA editing events, collectively termed “alternative mRNA editing”, including A-to-C, A-to-T, C-to-A, C-to-G, G-to-A, G-to-C, G-to-T, T-to-A, T-to-C, and T-to-G substitutions (Christofi & Zaravinos, 2019; Huang et al., 2022; Ruchika et al., 2021). Such editing events have been implicated in environmental stress responses. For example, a higher frequency of RNA editing sites has been detected in drought-tolerant compared to drought-susceptible genotypes of wheat (*Triticum aestivum* L.) (Rasool et al., 2022). Despite these findings, the underlying molecular mechanisms governing these atypical editing events in nuclear-encoded RNAs remains largely unknown.

To investigate the contribution of mRNA editing to the generation of mRNA and protein isoforms with different thermal sensitivities, two mesophilic mussel species (*Mytilus coruscus* and *M. galloprovincialis*) with distinct biogeographic distributions were selected. In China, *M. coruscus* is widely distributed along the warmer coastal waters of the Yellow Sea and East China Sea, particularly concentrated in the southern regions around Zhejiang Province (~N29°–31°) (Ye et al., 2012). In contrast, *M. galloprovincialis* is an invasive species originating from the Mediterranean Sea and eastern Atlantic, first recorded in Chinese waters during the 1950s. It has since established dominance in the cooler northern coastal regions (Han et al., 2024; Wang, 1997). Based on their thermal ecologies, *M. coruscus* and *M. galloprovincialis* were designated as heat-tolerant and heat-sensitive species, respectively.

To minimize the confounding influence of interspecific genomic divergence on RNA editing analyses, individuals from both species were collected from a shared locality. For each individual, paired genomic DNA (gDNA) and complementary DNA (cDNA) sequences of cytosolic malate dehydrogenase (cMDH) were obtained. The study focused on the cytosolic paralog of cMDH, a well-established model for investigating thermal adaptation of enzyme structure and function. Previous comparative studies have demonstrated that cMDH orthologs exhibit species-specific variation in protein thermostability and catalytic performance that reflects habitat temperature regimes, even among closely related congeners (Dahlhoff & Somero, 1991; Dong et al., 2018; Dong & Somero, 2009; Fields et al., 2006; Liao et al., 2017, 2019, 2021; Lin & Somero, 1995; Schwantes & Schwantes, 1982). Similarly, cMDH mRNA exhibits temperature-adaptive variation in secondary structure stability across mollusks, consistent with thermal adaptation (Liao et al., 2021).

By sequencing cMDH-encoding mRNA and their corresponding genomic DNA from individuals of both species

and calculating the thermal stabilities of each mRNA isoform, this study revealed extensive mRNA editing that produced multiple transcript isoforms within a single organism. These isoforms differed markedly in predicted thermal stability. We propose that such editing-mediated diversification of mRNA structure contributes to enhanced biochemical flexibility, providing a molecular mechanism by which ectothermic species expand their functional temperature range.

MATERIALS AND METHODS

Animal acclimation and treatment

Two mesophilic mussel species, *M. coruscus* and *M. galloprovincialis*, were selected for this study. Specimens of *M. coruscus* and *M. galloprovincialis* (~100 individuals per species) were collected from Dongji Island (N28.72°, E121.93°) and Qingdao (N36.06°, E120.34°), China, respectively. All specimens were transported live to the laboratory within 24 h of collection and acclimated under controlled indoor conditions for at least one week. Acclimation temperatures (~20 °C) were maintained to reflect the respective intertidal environments of each species, with mussels kept fully immersed. During the acclimation period, individuals were fed concentrated cultures of microalgae (*Isochrysis galbana* Parke) three times per week.

DNA/RNA extraction and sequencing and editing site detection

Foot muscle tissue was selected as the target organ for analysis of both cMDH enzyme and mRNA variants. Eight individuals per species were dissected, and foot tissue was immediately flash-frozen and stored in liquid nitrogen until further use. Genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) protocol (Ding et al., 2018). Total RNA was purified from the same tissue using TRIzol reagent (Invitrogen, USA), according to the manufacturer's instructions. First-strand cDNA synthesis was performed using PrimeScript™ RT reagent kits (TaKaRa, China). Amplification of cMDH sequences from both gDNA and cDNA was carried out using a high accuracy proofreading *Ex Taq*® polymerase (TaKaRa, China; error rate=1.93×10^{-3} mutation/kb) with pre-designed primers (Supplementary Table S1). Polymerase chain reaction (PCR) products from gDNA and cDNA were sent to commercial companies for Sanger sequencing (Sangon Biotech, China), and PacBio CCS high-throughput sequencing (Novogene, China), respectively.

Quality control of the high-throughput sequencing data followed procedures described in our previous study (Zhu et al., 2024). RNA editing sites were identified by aligning cMDH sequences to their corresponding gDNA sequences. Single nucleotide polymorphisms (SNPs) identified in heterozygous individuals were excluded from analysis to avoid confounding with editing events. A minimum threshold of five identical nucleotide substitutions at a given position was required for a site to be regarded as edited. Editing levels were quantified for each candidate site for each individual by dividing the number of mutated nucleotides by the total number of nucleotides at that position (Chu & Wei, 2020; Yablonovitch et al., 2017). Percentages of editing events were calculated independently for each editing type.

Codon adaptation index (CAI) analysis of mRNA

To evaluate the codon usage patterns of cMDH isoforms generated through RNA editing, the well-established CAI metric was employed as a quantitative measure of codon usage bias (Sharp & Li, 1986). CAI reflects the extent to which a transcript utilizes codons that are preferentially used in a reference set of highly expressed genes, with values approaching 1 indicating exclusive use of the most common codons for each amino acid (Allen et al., 2022). CAI scores were calculated using the R package *seqinr* (v.4.2.2).

Prediction and comparative analysis of mRNA secondary structures

Secondary structure predictions for individual cMDH transcripts were performed using the ViennaRNA package (v.2.4.17) (Lorenz et al., 2011). Thermodynamic stability of predicted structures was assessed by calculating the Gibbs free energy change associated with the formation of mRNA secondary structures (ΔG_{fold}). Comparisons of secondary structures before and after RNA editing were conducted using the RNAstructure package (v.6.2) based on the predicted secondary structures described above (Mathews et al., 1999). Structure predictions were performed with default parameters at a temperature of 37°C for all orthologs, a temperature at which RNA structure stability is best determined (Lu et al., 2006; Mathews et al., 2016). Calculations of ΔG_{fold} were performed with default temperatures of 37, 42.5, 47.5, 52.5, and 57.5°C for all orthologs. Additional methodological details can be found in our earlier study (Liao et al., 2021).

Statistical analyses

Differences in the temporal changes of residual enzymatic activity were assessed by comparing regression slopes between species using analysis of covariance (ANCOVA), followed by a least significant difference (LSD) multiple comparisons test (P=0.05). Interspecies differences in RNA editing metrics, including percentage of RNA editing sites, number of secondary structures, CAI, ΔG_{fold}, sensitivity score, and percentage of substitutions, were analyzed using an independent sample *t*-test (P=0.05) when normality assumptions were met. For non-normally distributed data, differences were analyzed using the Kruskal-Wallis test, followed by Dunn *post hoc* test (P=0.05) using R (v.4.2.2). Associations between ΔG_{fold} and the number of predicted secondary structural elements were analyzed using Spearman's rank correlation. All graphs were constructed using the R package *ggplot2* (Kyte & Doolittle, 1982).

RESULTS

Species-specific RNA editing profiles and editing levels

Multiple RNA editing events, including A-to-G, G-to-A, C-to-T, T-to-C, and T-to-A modifications, were identified in the cMDH transcripts of both mussel species (Table 1). Editing sites were species-specific, with no shared positions observed between *M. coruscus* and *M. galloprovincialis*. No nonsynonymous (recoding) substitutions were found in the cMDH orthologs of either species (Table 1). The frequency of synonymous substitutions was significantly higher in *M. coruscus* (0.75%) than in *M. galloprovincialis* (0.15%) (P<0.001; Figure 1A).

No significant differences in the mean value of RNA editing were detected between the *M. coruscus* and *M. galloprovincialis* cMDH orthologs (Figure 1B, mean±standard deviation (SD): 0.67±0.20 and 0.59±0.25, respectively, P>0.05). Notably, no editing events overlapped with functionally critical regions of the enzyme, such as active site

Table 1 RNA editing events of cytosolic malate dehydrogenases (cMDH) mRNA in *Mytilus coruscus* and *M. galloprovincialis*.

Species	Position	Nucleotide substitution	Substitution type	Genomic position	Reference genome
M. coruscus	117	C > T	Synonymous mutation	CM029599.1: 33340279	GCA_017311375.1
	123	A > G		CM029599.1: 33340285	
	135	G > A		CM029599.1: 33340297	
	190	C > T		CM029599.1: 33340352	
	195	G > A		CM029599.1: 33340357	
	204	T > C		CM029599.1: 33339590	
	279	A > G		CM029599.1: 33339665	
	444	T > C		CM029599.1: 33338532	
M. galloprovincialis	216	T > A		CM075334.1: 29322688	GCA_037788925.1
	597	T > C		CM075334.1: 29320794	

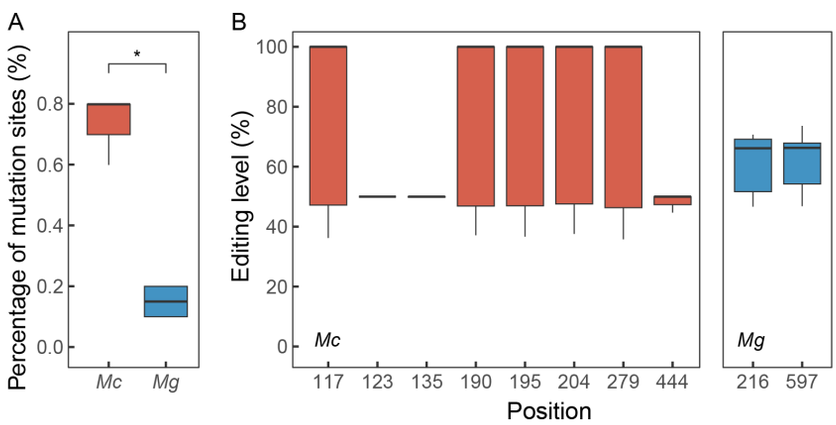

Figure 1 RNA editing in cytosolic malate dehydrogenase (cMDH) transcripts from two mesophilic mussel species

Mytilus coruscus (*Mc*) and *M. galloprovincialis* (*Mg*) (n=8 individuals per species). A: Percentages of editing sites in cMDH mRNAs. *: P<0.05. B: RNA editing level for each editing position.

residues, cofactor-binding motifs, or subunit interaction interfaces (Supplementary Figure S1).

Editing-generated isoforms of cMDH mRNA

RNA editing generated a diverse array of cMDH transcript variants in both species. After accounting for individual-level SNPs, 21 distinct mRNA isoforms were identified in *M. coruscus* and 22 mRNA isoforms were detected in *M. galloprovincialis* (Figure 2).

CAI of editing-derived cMDH isoforms

The CAI metric can serve as a proxy for translational efficiency and, by extension, potential protein expression levels, with relevance to thermal adaptation and stability (Venev & Zeldovich, 2018). A similar CAI pattern for the RNA editing-generated cMDH isoforms was found between the *M. coruscus* and *M. galloprovincialis* congeners (Figure 3). The calculations before and after editing were based on the gDNA and cDNA (mRNA) sequences, respectively. In both species, RNA editing resulted in a significant increase in CAI (P<0.05). Specifically, mean CAI values increased from 0.210 (gDNA) to 0.214 (cDNA) in *M. coruscus*, and from 0.203 (gDNA) to 0.229 (cDNA) in *M. galloprovincialis*.

Variations in ΔG_{fold} among editing-generated cMDH mRNA isoforms

RNA editing also produced multiple cMDH mRNA isoforms that differed in conformational stability, as quantified by Gibbs free energy of folding (ΔG_{fold}) (Figure 4). Across all assessed temperatures, *M. coruscus* exhibited significantly higher absolute ΔG_{fold} values than *M. galloprovincialis* (P<0.05). Notably, in *M. coruscus*, the percentage of isoforms with lower absolute ΔG_{fold} values increased with increasing temperature, while in *M. galloprovincialis*, the opposite trend was observed, with an increasing proportion of isoforms with higher ΔG_{fold} values at elevated temperatures (Figure 4A). Temperature-dependent trends were also reflected in the coefficient of variation (CV) of ΔG_{fold}. Across the range of tested temperatures, CVs generally decreased in both gDNA- and cDNA-derived cMDH sequences from both species (P=0.010, 0.014, 0.084, and 0.009 for *M. coruscus* gDNA and cDNA and *M. galloprovincialis* gDNA and cDNA, respectively) (Figure 4B). In *M. coruscus*, the regression slope describing the decline in CV with increasing temperature was significantly steeper for gDNA than for cDNA-derived sequences (P<0.001), with a similar pattern observed in *M. galloprovincialis* cMDH (P<0.001).

To assess the potential contribution of RNA editing to thermal performance, predicted ΔG_{fold} values for editing-derived mRNA isoforms were evaluated using a thermal adaptation regression framework based on LT_{50} values (temperature at which 50% lethality occurs) from 22 molluscan species (Liao et al., 2021). Using this reference "standard curve" (Figure 5, dashed lines), the extent to which RNA editing shifted the thermal optimum of mRNA structural stability was determined. Results revealed that editing generated isoform sets with distinct ΔG_{fold} distributions, corresponding to inferred increases in thermal optima of 0.79 °C for M. coruscus and 1.56 °C for M. galloprovincialis.

Differences in elements of secondary structures

RNA editing introduced measurable alterations in cMDH mRNA secondary structures, with differences observed between isoforms derived from gDNA and cDNA sequences (Figure 6). These structural changes were quantified using

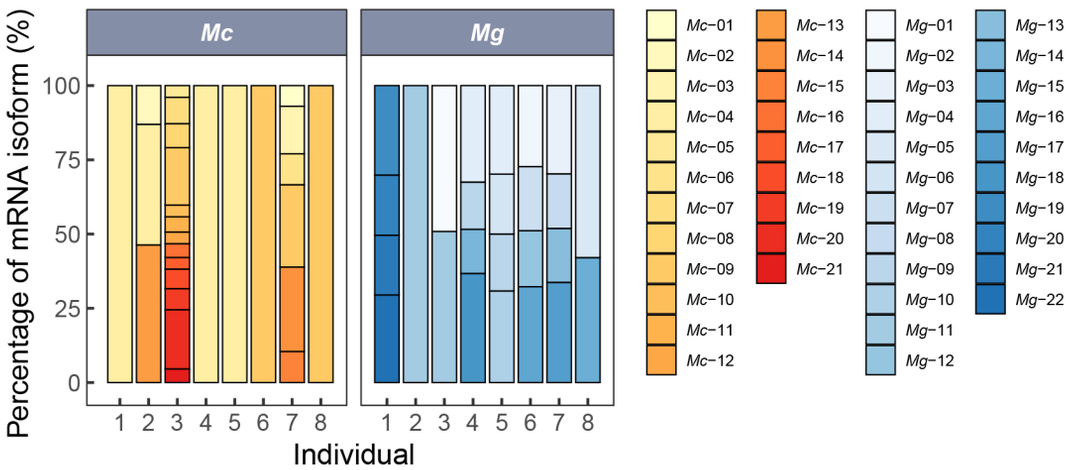

Figure 2 Percentages of cytosolic malate dehydrogenase (cMDH) mRNA isoforms generated by RNA editing in the coding sequence region

Mytilus coruscus (*Mc*) and *M. galloprovincialis* (*Mg*) (*n*=8). Color-coded bars on the right represent different mRNA isoforms shown in the left panel.

sensitivity scores, reflecting the degree of structural deviation between the pre- and post-editing isoforms, with higher scores denoting greater structural reconfiguration following editing. The sensitivity of cMDH secondary structure in *M. coruscus* was significantly higher than that in the congener *M. galloprovincialis* cMDH (P<0.001), indicating that RNA editing induced greater structural change in *M. coruscus* isoforms (Figure 6A). Structural element composition also differed before and after editing. In *M. coruscus*, the number of base-paired regions and internal loops increased significantly in edited cMDH transcripts relative to their unedited counterparts (P<0.05; Figure 6B). No significant structural changes were detected in *M. galloprovincialis* for any of the four examined secondary structure elements —base pairs, internal loops, multiloops, and hairpins (P>0.05). Correlations between structural element abundance and mRNA stability, as indexed by ΔG_{fold}, also revealed species-specific patterns. In *M. coruscus*, the number of internal loops was positively correlated with greater secondary structure stability (larger negative ΔG_{fold} values; P<0.001), while the number of hairpins showed a significant negative correlation with stability (P=0.017) (Figure 6C). In contrast, *M. galloprovincialis* displayed the inverse pattern: internal loop frequency negatively correlated with ΔG_{fold} (P=0.007), whereas hairpin number was positively correlated with stability (P=0.005).

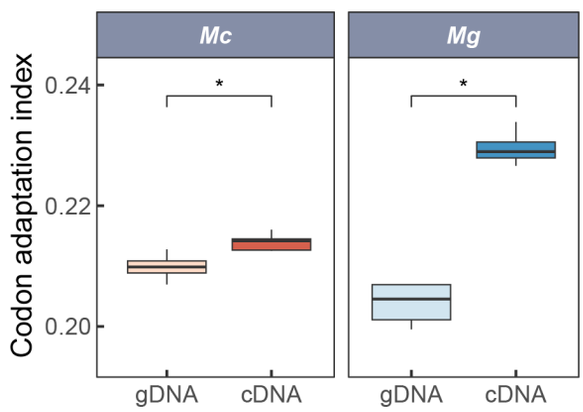

Figure 3 Changes in codon adaptation index (CAI) of cytosolic malate dehydrogenase (cMDH) before and after RNA editing

Mytilus coruscus (*Mc*) and *M. galloprovincialis* (*Mg*) (*n*=8). Calculations before and after editing are based on gDNA and cDNA sequences, respectively. *: P<0.05. Maximum value of the upper whisker represents the 1st quartile (Q1)+1.5×interquartile range (IQR), and minimum value of the lower whisker represents Q1–1.5×IQR (McGill et al., 1978).

DISCUSSION

The *Mytilus* species complex serves as a robust model for investigating how genotypic variation underlies phenotypic divergence in marine organisms (Lockwood & Somero, 2012; Popovic & Riginos, 2020; Sun et al., 2017; Tan et al., 2023a). In mussels, polymorphisms within quantitative trait loci are associated with specific traits, such as shell size and body weight, providing valuable genetic markers for selective breeding programs (Bai et al., 2015; Nguyen et al., 2014; Takeuchi, 2017). In addition to their economic relevance, mussels also function as ecological engineers, modifying benthic habitats and promoting environmental heterogeneity, thereby supporting local biodiversity (Tan et al., 2023b; Ye et al., 2012). RNA editing represents a post- transcriptional mechanism that dynamically alters primary RNA transcripts in response to environmental conditions, including temperature variation (Nishikura, 2010). Recent evidence indicates that this process occurs in mussels and may contribute to adaptive plasticity in thermal physiology (Tan et al., 2023b; Zhu et al., 2024).

The present study demonstrated that editing of cMDH mRNA transcripts generated multiple mRNA isoforms that differed in thermal stability. This post-transcriptional diversification may enable ectothermic species to maintain enzyme function across a broader range of body temperatures by fine-tuning the structural properties of both mRNAs and proteins. In cephalopods, temperature-induced RNA editing has been linked to amino acid recoding events that may serve as adaptive responses to environmental thermal fluctuations (Birk et al., 2023; Rangan & Reck-Peterson, 2023; Riemondy et al., 2018; Rosenthal & Eisenberg, 2023). Differences in stability affect not only the half-life of proteins but also influence how well the protein is able to carry out its tasks, particularly in enzymes where conformational flexibility is critical for substrate and cofactor binding (Dong et al., 2018; Liao et al., 2019). In contrast, no nonsynonymous substitutions were detected in the *Mytilus* cMDH orthologs examined in this study. This absence of recoding is consistent

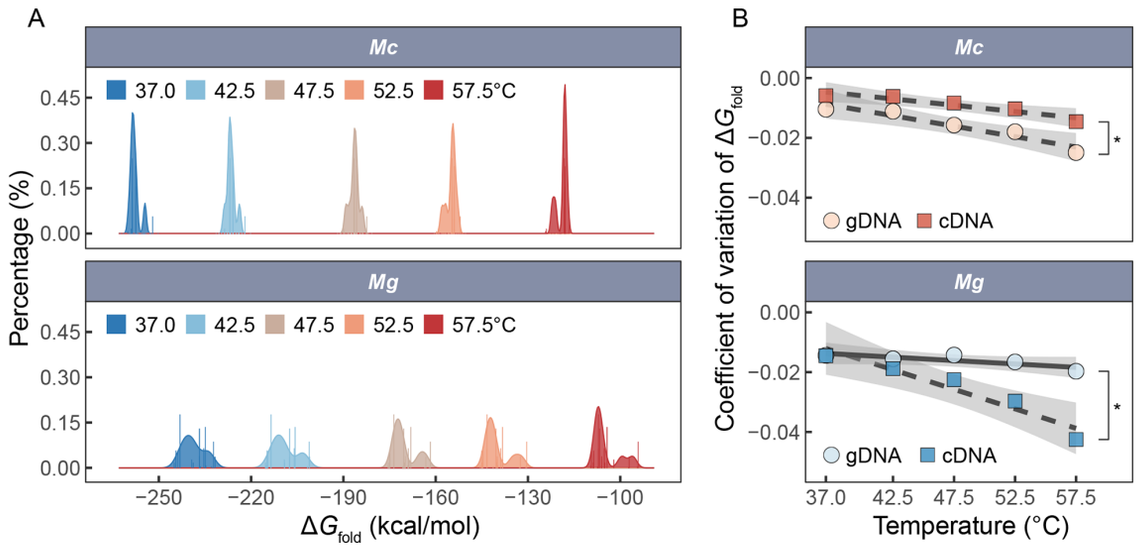

Figure 4 Pattern of ΔG_{fold} among RNA editing-derived isoforms of cytosolic malate dehydrogenase (cMDH) mRNA in two mussel species across different temperatures

Mytilus coruscus (*Mc*) and *M. galloprovincialis* (*Mg*) (n=8). A: Frequency distribution of ΔG_{fold} values for edited cMDH mRNA variants (cDNA) at different temperatures. Thin vertical lines represent frequency (percentage) of observed ΔG_{fold} values. B: Coefficient of variation of ΔG_{fold} for each species before (gDNA) and after (cDNA) editing across different temperatures. *: P<0.05. Shaded areas represent Gaussian kernel-smoothed trends.

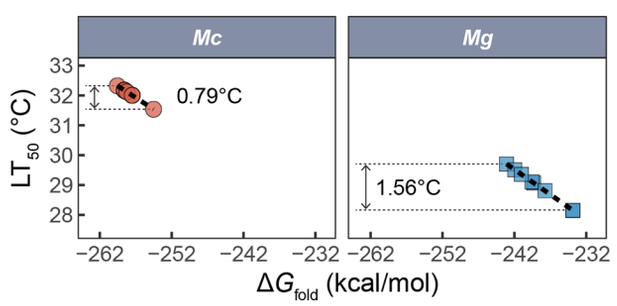

Figure 5 Relationship between ΔG_{fold} and thermal sensitivities of edited cMDH mRNA isoforms in two mussel species

Mytilus coruscus (*Mc*) and *M. galloprovincialis* (*Mg*). Estimated ranges of lethal temperature (LT_{50}) are indicated in the panels. LT_{50} values were inferred based on previously established regression from 22 marine mollusks (Liao et al., 2021) (y=–92.01x–189.81). Calculations of ΔG_{fold} were performed with default parameters at a temperature of 37°C.

with previous findings in marine mollusks, which showed that while the thermal stabilities of cMDH mRNA and protein tend to evolve in parallel, their evolutionary trajectories are independent (Liao et al., 2021).

The editing-generated cMDH mRNA isoforms exhibited a broad variation in secondary structure stabilities, as reflected by ΔG_{fold}. Notably, the two mussel species exhibited distinct temperature-dependent trends in structural adaptation. In the heat-sensitive *M. galloprovincialis*, increasing temperatures were associated with a higher proportion of isoforms possessing lower structural stability —indicative of greater conformational flexibility. In contrast, the heat-tolerant *M. coruscus* produced more thermodynamically stable isoforms under elevated temperatures. This divergence suggests that *M. galloprovincialis* may rely more heavily on RNA editing to generate structural flexibility, while *M. coruscus* favors stability to maintain transcript integrity across thermal gradients. This interpretation is supported by both the higher structural sensitivity scores and greater coefficients of variation in ΔG_{fold} observed in *M. galloprovincialis*, implying more extensive editing-induced structural remodeling (Figure 4 and 6A). Further, editing-induced changes in ΔG_{fold} were significantly correlated with changes in specific structural elements (Figure 6), particularly base-pairing in more thermally stable isoforms. Unlike proteins, which typically exhibit longer half-lives and higher thermal robustness, mRNAs are generally less stable and more transient (Buccitelli & Selbach, 2020; Wang et al., 2019). However, as with proteins, the functional performance of RNA can depend on reversible conformational changes. Localized shifts in the stability of specific secondary structures can exert profound effects on essential processes in protein synthesis. Consequently, the overall ΔG_{fold} of an entire transcript may obscure the functional importance of editing events that fine-tune discrete regions of the mRNA to support critical processes across varying thermal conditions (Becskei & Rahaman, 2022). These processes include mRNA turnover (Mauger et al., 2019), translation initiation and elongation (Chiaruttini & Guillier, 2020; Gu et al., 2010; Su et al., 2018), and splicing (Mortimer et al., 2014). By generating structurally diverse isoforms, RNA editing has the potential to broaden the thermal performance range of these post-transcriptional processes, offering enhanced functional flexibility relative to a single, unedited transcript. Furthermore, differential editing may facilitate partial temperature compensation across the protein synthesis machinery by generating isoforms that work more rapidly at low temperatures. In summary, editing-induced changes in mRNA structural stability may be important for maintaining transcriptomic and proteomic integrity under thermally dynamic conditions.

A notable outcome of this study was the predominance of C-to-T and T-to-C editing events relative to A-to-G and G-to-A substitutions across the full cMDH mRNA sequences. This suggests that C-to-T and T-to-C editing may have exerted a greater influence on mRNA structural variation and stability in these mussel species. C-to-U editing, a well-characterized RNA modification, has been observed in specific transcripts,

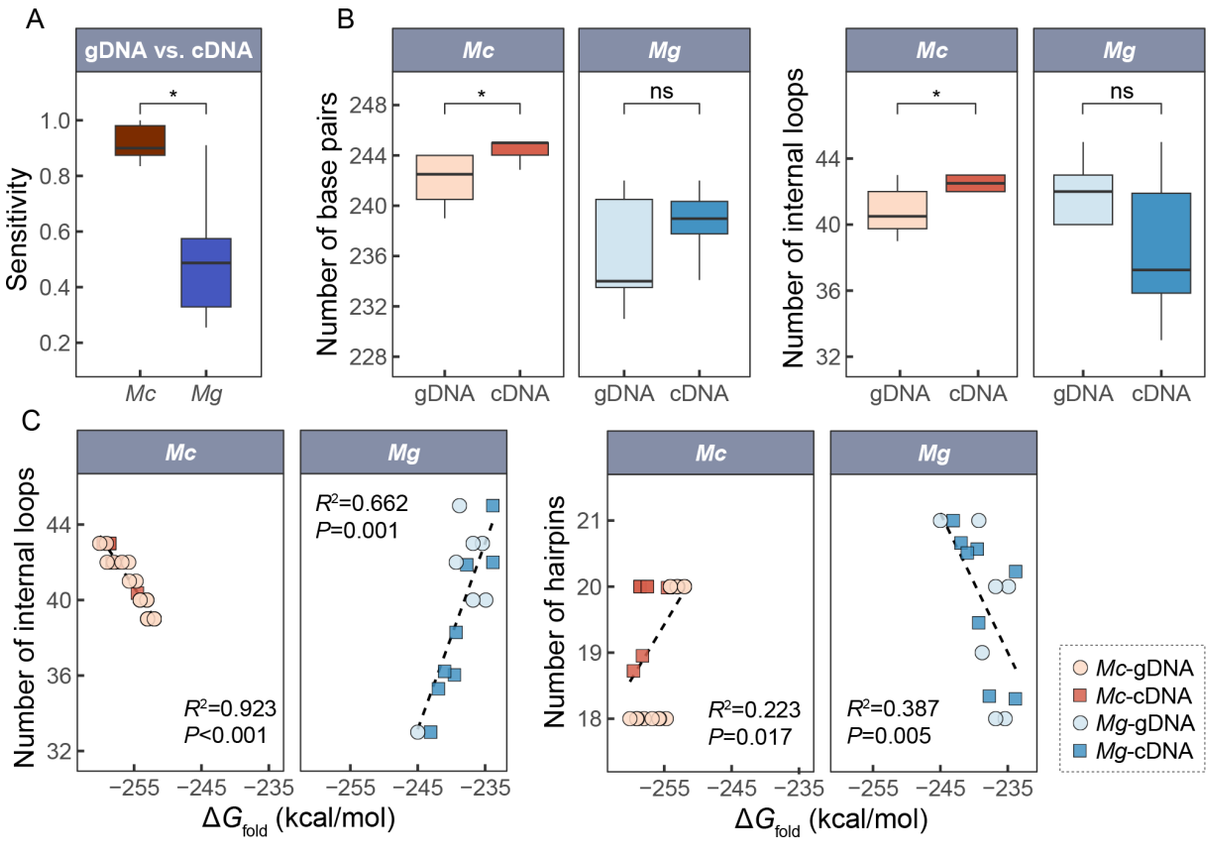

Figure 6 Characteristics of cMDH mRNA secondary structures before and after RNA editing in two mussel species

Mytilus coruscus (*Mc*) and *M. galloprovincialis* (*Mg*). A: Differences in secondary structures between mRNA isoforms before (gDNA) and after (cDNA) editing. Sensitivity scores were calculated based on edited structures (cDNA) compared to original structures (gDNA), with higher sensitivities indicating higher structural differences. B: Comparison of base pairs and internal loops in different mRNA isoforms before (gDNA) and after (cDNA) editing. C: Correlations between the number of internal loops and hairpins and ΔG_{fold} before (gDNA) and after (cDNA) editing. ns: Not significant; *: P<0.05. Maximum value of upper whisker represents the 1st quartile (Q1)+1.5×interquartile range (IQR), and minimum value of lower whisker represents Q1–1.5×IQR (McGill et al., 1978).

such as apolipoprotein-B48, where it generates truncated, functionally distinct protein isoforms (Powell et al., 1987). In *Arabidopsis thaliana*, C-to-U(T) editing in mitochondrial and chloroplast genes has been shown to decrease significantly under both heat and cold stress, a response attributed not to RNA structural sensitivity but to the alleviation of decreased translation rate at edited codons (Chu & Wei, 2020). Furthermore, base modifications have been shown to alter RNA reactivity, base-pairing interactions, and higher-order structure (Harcourt et al., 2017). Although the relative contributions of different editing types to mRNA stability remain difficult to quantify, their distinct biochemical outcomes suggest opposing effects on stability. Specifically, A-to-G or T-to-C substitutions may increase the potential for stronger hydrogen bonding by inducing the formation of strong G–C pairs. Conversely, C-to-T or G-to-A substitutions disrupt existing or potential G–C pairs, likely weakening secondary structure stability. If this scenario is correct, C-to-T and G-to-A editing may promote the generation of more flexible, less stable mRNA isoforms better suited to function at lower temperatures. Conversely, A-to-G or T-to-C editing may reinforce hydrogen bonding to RNA secondary structures, leading to more thermally stable mRNA. Our previous validation study in *M. coruscus* using high-fidelity Sanger sequencing of paired gDNA and mRNA (Zhu et al., 2024) confirmed the full range of RNA editing types observed in the present study. Among these, rare editing types such as T-to-A substitutions—comprising nearly half of the detected events in *M. galloprovincialis*—require further investigation to clarify their potential contributions to mRNA structural dynamics and cMDH function.

In addition, the role of thermal acclimation in modulating RNA editing patterns warrants further study. Recent research in zebrafish (*Danio rerio*) revealed tissue-specific and temperature-dependent shifts in editing activity during acclimation, including modifications in 3’ untranslated regions (UTRs) that affected translational efficiency in a temperature-compensatory manner (Li et al., 2023).

The secondary structure of CDS may play a critical role in adaptive RNA editing, extending beyond localized motifs to influence mRNA stability and function across broader transcript regions. Notably, high protein expression levels have been shown to correlate with increased secondary structure density approximately 10 codons downstream of the initiation site, emphasizing the importance of temperature-dependent mRNA stability throughout the molecule (Mauger et al., 2019). Temperature-dependent changes in RNA secondary structure can facilitate thermosensor functions in different regions of mRNA. This concept is exemplified by bacterial RNA thermometers, where conformational shifts in mRNA govern the initiation of translation for heat-shock proteins and other stress-responsive factors (Kortmann & Narberhaus, 2012). These examples of acute effects of temperature on mRNA structure, stability, and function

suggest that similar thermal sensing mechanisms by RNAs may contribute to the responses of animals to changes in body temperature (Somero, 2018), and these thermosensitive functions may be modulated through mRNA editing, providing an additional layer of regulatory flexibility.

Editing in the CDS region, whether or not it leads to recoding, may have important functional effects. In this study, all editing events identified within the CDS region of *Mytilus* cMDH transcripts were synonymous substitutions, yet their functional significance should not be underestimated. Even in the absence of amino acid changes, synonymous editing may still induce proteomic alterations (Shen et al., 2022; Walsh et al., 2020). For example, synonymous substitutions can influence splicing by altering splice site recognition and exon inclusion (Zheng et al., 2014), thereby reshaping the coding potential of transcripts without changing their amino acid sequences. When such substitutions arise through temperature-dependent RNA editing, they may contribute to dynamic regulation of proteome composition under varying thermal conditions. Supporting this, previous research has reported extensive cold-induced alternative splicing across hundreds of genes in four teleost species (Healy & Schulte, 2019). Notably, many of these alternative spliced genes were also up-regulated at low temperatures, indicating that cold acclimation has significant quantitative and qualitative effects on the proteome.

Another phenomenon that arises in the context of synonymous substitutions introduced through mRNA editing is codon bias, which involves the preferential use of specific synonymous codons over others in the transcriptome (Figure 3) (Hanson & Coller, 2018). This phenomenon, often described in terms of “preferred” versus “non-preferred” codons (Zhou et al., 2016), has emerged as a critical determinant of post-transcriptional gene regulation. Increasing evidence indicates that codon bias/optimality in gene coding regions plays a major role in regulating transcriptional efficiency, splicing accuracy, and translation kinetics (Hanson & Coller, 2018; Zhao et al., 2021; Zhou et al., 2016). Synonymous substitutions introduced via mRNA editing may therefore exert widespread regulatory effects on the transcriptome and proteome. For example, incorporation of non-optimal codons at certain sites within the CDS region can slow translation to facilitate productive folding of nascent proteins (Hanson & Coller, 2018). Under elevated temperatures —conditions that compromise protein folding fidelity—mRNA editing that replaces an optimal codon with a sub-optimal codon could act in a temperature-compensatory manner by moderating translational speed.

Codon usage not only governs the efficiency of protein synthesis but can also influence the conformational and functional properties of the resulting proteins. Translation of mRNAs with different synonymous codons has been shown to produce proteins with distinct conformations and *in vitro* stabilities (Buhr et al., 2016). For instance, lactate dehydrogenase-A from two *Gillichthys* goby species, although identical in amino acid sequence, was encoded by mRNAs with four synonymous codon differences, yielding enzymes with markedly different kinetic properties, thermal stabilities, and folding states when expressed *in vivo*. Notably, such differences were eliminated when the native proteins were denatured and renatured *in vitro* (Fields et al., 2002; Fields and Somero, 1997). These findings, previously unexplained, are consistent with the hypothesis that codon usage modulates co-translational folding *in vivo*—a mechanism that would not operate under *in vitro* renaturation conditions. Similar codon-dependent effects may underlie other cases of protein structural variants (Jacobson et al., 1970), implicating both genomic codon choice and RNA editing-driven codon substitutions as contributors to proteomic diversity.

Codon usage also affects mRNA stability, partly through its impact on translation elongation rates (Hanson & Coller, 2018; Mauger et al., 2019). Synonymous codon transitions have been shown to alter mRNA stability, highlighting another important role of mRNA editing in the context of thermal adaptation (Bartoszewski et al., 2010; Chamary & Hurst, 2005; Mishima & Tomari, 2016). These observations support the notion that a large number of temperature-adaptive responses may be mediated by codon usage effects, arising both from inherent codon preferences encoded in gDNA and from RNA editing-driven changes in synonymous codons within mRNAs.

Two broader implications of our findings warrant brief mention. First, the presence of multiple mRNA isoforms arising from RNA editing within a single individual challenges the reliability of protein sequence inference based solely on cDNA sequencing. When a family of edited mRNA isoforms coexists in the same cell, sequencing a single cDNA clone may provide an incomplete or misleading representation of the corresponding protein sequence. Even sequencing multiple cDNAs from the same individual cannot definitively identify the unedited genome-encoded transcript without parallel genomic data. This narrowly focused means of deducing protein sequences could also lead to the underestimation of protein polymorphism, as it neglects isoform variation arising from both genomic variation and RNA-level modifications.

Despite ongoing advances in sequencing techniques, current methods remain limited in their capacity to fully detect and recognize the functional impacts of RNA editing. High-throughput next-generation sequencing (NGS) has been widely applied to detect genome-wide single nucleotide variants (Metzker, 2010; Stasik et al., 2018); however, they often overlook transcriptomic diversity arising from post-transcriptional editing. While millions of reads (short sequences) can be sequenced using NGS, multiple mRNA isoforms due to post-transcriptional editing have largely been ignored due to limitations in sequencing technologies and bioinformatic approaches. Early genome array technologies using short oligonucleotide probes have been similarly limited, lacking the resolution to differentiate between alternatively edited transcripts or detect discrete post-transcriptional modifications (Piazzi et al., 2023). In addition, RNA editing detection using NGS often relies on indirect chemical conversion methods, in which modified RNA residues are made detectable through reverse transcriptase (RT) arrest or nucleotide misincorporation during cDNA synthesis (Motorin & Marchand, 2021). Most NGS-based analyses aim to elucidate a ‘general’ pattern by merging different isoforms into one ‘gene’ (transcript). Although long-read sequencing technologies such as Pacific Bioscience circular consensus sequencing and Oxford Nanopore direct RNA sequencing offer improved resolution and sensitivity, enabling detection of single nucleotide variants and RNA modifications at the level of individual molecules (Callahan et al., 2019; Kennedy et al., 2014; Leger et al., 2021), almost all sequences tend to be clustered into different operational taxonomic units (OTUs) based on a predefined similarity threshold (e.g., 97% identity). After clustering is applied, the representative sequence in

each OTU is selected based on the frequency of occurrence for annotation and taxonomic classification. However, this approach systematically excludes low-frequency variants, particularly when singleton filters are applied to remove OTUs represented by only a single read. Thus, variants in the same individuals are unlikely to be detected.

Second, this issue returns to the broader question posed at the onset of this study: the extent, origin, and functional significance of genetic polymorphism in natural populations. The identification of multiple cMDH mRNA isoforms generated through RNA editing highlights an additional source of genetic variation, whereby a single DNA CDS can give rise to a family of mRNA isoforms that may differ adaptively in thermal responses. Accordingly, much adaptively relevant variation in mRNAs may not be “genetic” in the strict sense when the term is limited to changes in DNA sequences. Variation introduced through RNA editing would therefore remain undetectable even with comprehensive DNA sequencing. Detecting such editing-driven variation remains a substantial challenge. Furthermore, protein polymorphism resulting from post-transcriptional mechanisms such as mRNA editing may account for considerable isoform diversity, yet it is unlikely to be captured by standard methods for detecting variation, including DNA sequencing and electrophoresis. In functional terms, the variation arising from mRNA editing may complement the proposed roles of other sources of variation, notably allelic variation, in providing ectothermic species with effective means of coping with fluctuations in body temperature.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS

Conceptualization: Y.W.D., G.N.S., and M.L.L. Investigation: M.L.L., X.L.Z., and Y.J.Z. Data curation: M.L.L. and X.L.Z., and Y.J.Z. Writing—original draft: Y.W.D., G.N.S., and M.L.L. Writing—review & editing: M.L.L., Y.W.D., and G.N.S. All authors read and approved the final version of the manuscript.

REFERENCES

Allen SR, Stewart RK, Rogers M, et al. 2022. Distinct responses to rare codons in select *Drosophila tissues*. *eLife*, **11**: e76893.

Alon S, Garrett SC, Levanon EY, et al. 2015. The majority of transcripts in the squid nervous system are extensively recoded by A-to-I RNA editing. *eLife*, **4**: e05198.

Bai ZY, Han XK, Luo M, et al. 2015. Constructing a microsatellite-based linkage map and identifying QTL for pearl quality traits in triangle pearl mussel (*Hyriopsis cumingii*). *Aquaculture*, **437**: 102–110.

Baldwin J, Hochachka PW. 1970. Functional significance of isoenzymes in thermal acclimatization. Acetylcholinesterase from trout brain. *Biochemical Journal*, **116**(5): 883–887.

Bartoszewski RA, Jablonsky M, Bartoszewska S, et al. 2010. A synonymous single nucleotide polymorphism in ΔF508 *CFTR* alters the secondary structure of the mRNA and the expression of the mutant protein. *Journal of Biological Chemistry*, **285**(37): 28741–28748.

Becskei A, Rahaman S. 2022. The life and death of RNA across temperatures. *Computational and Structural Biotechnology Journal*, **20**: 4325–4336.

Birk MA, Liscovitch-Brauer N, Dominguez MJ, et al. 2023. Temperature-dependent RNA editing in octopus extensively recodes the neural proteome. *Cell*, **186**(12): 2544–2555. e13.

Buccitelli C, Selbach M. 2020. mRNAs, proteins and the emerging principles of gene expression control. *Nature Reviews Genetics*, **21**(10): 630–644.

Buchumenski I, Bartok O, Ashwal-Fluss R, et al. 2017. Dynamic hyper-editing underlies temperature adaptation in *Drosophila*. *PLoS Genetics*, **13**(7): e1006931.

Buhr F, Jha S, Thommen M, et al. 2016. Synonymous codons direct cotranslational folding toward different protein conformations. *Molecular Cell*, **61**(3): 341–351.

Callahan BJ, Wong J, Heiner C, et al. 2019. High-throughput amplicon sequencing of the full-length 16S rRNA gene with single-nucleotide resolution. *Nucleic Acids Research*, **47**(18): e103–e103.

Chamary JV, Hurst LD. 2005. Evidence for selection on synonymous mutations affecting stability of mRNA secondary structure in mammals. *Genome Biology*, **6**(9): R75.

Charlesworth B, Charlesworth D, Coyne JA, et al. 2016. Hubby and Lewontin on protein variation in natural populations: when molecular genetics came to the rescue of population genetics. *Genetics*, **203**(4): 1497–1503.

Chiaruttini C, Guillier M. 2020. On the role of mRNA secondary structure in bacterial translation. *WIREs RNA*, **11**(3): e1579.

Christofi T, Zaravinos A. 2019. RNA editing in the forefront of epitranscriptomics and human health. *Journal of Translational Medicine*, **17**(1): 319.

Chu D, Wei L. 2020. Reduced C-to-U RNA editing rates might play a regulatory role in stress response of *Arabidopsis*. *Journal of Plant Physiology*, **244**: 153081.

Colina C, Palavicini JP, Srikumar D, et al. 2010. Regulation of Na^+/K^+ ATPase transport velocity by RNA editing. *PLoS Biology*, **8**(11): e1000540.

Dahlhoff E, Somero GN. 1991. Pressure and temperature adaptation of cytosolic malate dehydrogenases of shallow and deep-living marine invertebrates: evidence for high body temperatures in hydrothermal vent animals. *Journal of Experimental Biology*, **159**(1): 473–487.

Ding MW, Wang ZK, Dong YW. 2018. Food availability on the shore: linking epilithic and planktonic microalgae to the food ingested by two intertidal gastropods. *Marine Environmental Research*, **136**: 71–77.

Dong YW, Liao ML, Han GD, et al. 2022. An integrated, multi-level analysis of thermal effects on intertidal molluscs for understanding species distribution patterns. *Biological Reviews*, **97**(2): 554–581.

Dong YW, Liao ML, Meng XL, et al. 2018. Structural flexibility and protein adaptation to temperature: molecular dynamics analysis of malate dehydrogenases of marine molluscs. *Proceedings of the National Academy of Sciences of the United States of America*, **115**(6): 1274–1279.

Dong YW, Somero GN. 2009. Temperature adaptation of cytosolic malate dehydrogenases of limpets (genus *Lottia*): differences in stability and function due to minor changes in sequence correlate with biogeographic and vertical distributions. *Journal of Experimental Biology*, **212**(2): 169–177.

Fields PA, Kim YS, Carpenter JF, et al. 2002. Temperature adaptation in *Gillichthys* (Teleost: Gobiidae) A_4-Lactate dehydrogenases: identical primary structures produce subtly different conformations. *Journal of Experimental Biology*, **205**(9): 1293–1303.

Fields PA, Rudomin EL, Somero GN. 2006. Temperature sensitivities of cytosolic malate dehydrogenases from native and invasive species of marine mussels (genus *Mytilus*): sequence-function linkages and correlations with biogeographic distribution. *Journal of Experimental Biology*, **209**(4): 656–667.

Fields PA, Somero GN. 1997. Amino acid sequence differences cannot fully explain interspecific variation in thermal sensitivities of gobiid fish A_4-lactate dehydrogenases (A_4-LDHs). *Journal of Experimental Biology*, **200**(13):

1839–1850.
Garrett SC, Rosenthal JJC. 2012a. RNA editing underlies temperature adaptation in K^+ channels from polar octopuses. *Science*, **335**(6070): 848–851.
Garrett SC, Rosenthal JJC. 2012b. A role for A-to-I RNA editing in temperature adaptation. *Physiology*, **27**(6): 362–369.
Goldspink G. 1995. Adaptation of fish to different environmental temperature by qualitative and quantitative changes in gene expression. *Journal of Thermal Biology*, **20**(1-2): 167–174.
Gu WJ, Zhou T, Wilke CO. 2010. A universal trend of reduced mRNA stability near the translation-initiation site in prokaryotes and eukaryotes. *PLoS Computational Biology*, **6**(2): e1000664.
Han GD, Ma DD, Du LN, et al. 2024. Chromosomal-scale genome assembly of the Mediterranean mussel *Mytilus galloprovincialis*. *Scientific Data*, **11**(1): 644.
Hanson G, Coller J. 2018. Codon optimality, bias and usage in translation and mRNA decay. *Nature Reviews Molecular Cell Biology*, **19**(1): 20–30.
Harcourt EM, Kietrys AM, Kool ET. 2017. Chemical and structural effects of base modifications in messenger RNA. *Nature*, **541**(7637): 339–346.
Healy TM, Schulte PM. 2019. Patterns of alternative splicing in response to cold acclimation in fish. *Journal of Experimental Biology*, **222**(5): jeb193516.
Huang KY, Kan SL, Shen TT, et al. 2022. A comprehensive evolutionary study of chloroplast RNA editing in gymnosperms: a novel type of G-to-A RNA editing is common in gymnosperms. *International Journal of Molecular Sciences*, **23**(18): 10844.
Hubby JL, Lewontin RC. 1966. A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in *Drosophila pseudoobscura*. *Genetics*, **54**(2): 577–594.
Jacobson KB, Murphy JB, Hartman FC. 1970. Isoenzymes of *Drosophila* alcohol dehydrogenase. *Journal of Biological Chemistry*, **245**(5): 1075–1083.
Kennedy SR, Schmitt MW, Fox EJ, et al. 2014. Detecting ultralow-frequency mutations by Duplex Sequencing. *Nature Protocols*, **9**(11): 2586–2606.
Kortmann J, Narberhaus F. 2012. Bacterial RNA thermometers: molecular zippers and switches. *Nature Reviews Microbiology*, **10**(4): 255–265.
Kyte J, Doolittle RF. 1982. A simple method for displaying the hydropathic character of a protein. *Journal of Molecular Biology*, **157**(1): 105–132.
Leger A, Amaral PP, Pandolfini L, et al. 2021. RNA modifications detection by comparative Nanopore direct RNA sequencing. *Nature Communications*, **12**(1): 7198.
Li WH, Bu MD, Hu RQ, et al. 2023. Tissue-specific temperature dependence of RNA editing levels in zebrafish. *BMC Biology*, **21**(1): 262.
Liao ML, Dong YW, Somero GN. 2021. Thermal adaptation of mRNA secondary structure: stability versus lability. *Proceedings of the National Academy of Sciences of the United States of America*, **118**(45): e2113324118.
Liao ML, Somero GN, Dong YW. 2019. Comparing mutagenesis and simulations as tools for identifying functionally important sequence changes for protein thermal adaptation. *Proceedings of the National Academy of Sciences of the United States of America*, **116**(2): 679–688.
Liao ML, Zhang S, Zhang GY, et al. 2017. Heat-resistant cytosolic malate dehydrogenases (cMDHs) of thermophilic intertidal snails (genus *Echinolittorina*): protein underpinnings of tolerance to body temperatures reaching 55°C. *Journal of Experimental Biology*, **220**(11): 2066–2075.
Licht K, Jantsch MF. 2016. Rapid and dynamic transcriptome regulation by RNA editing and RNA modifications. *Journal of Cell Biology*, **213**(1): 15–22.
Lin JJ, Somero GN. 1995. Thermal adaptation of cytoplasmic malate dehydrogenases of eastern Pacific barracuda (*Sphyraena* spp): the role of differential isoenzyme expression. *Journal of Experimental Biology*, **198**(2): 551–560.
Liscovitch-Brauer N, Alon S, Porath HT, et al. 2017. Trade-off between transcriptome plasticity and genome evolution in Cephalopods. *Cell*, **169**(2): 191–202. e11.
Lockwood BL, Somero GN. 2012. Functional determinants of temperature adaptation in enzymes of cold- versus warm-adapted mussels (Genus *Mytilus*). *Molecular Biology and Evolution*, **29**(10): 3061–3070.
Lorenz R, Bernhart SH, Höner zu Siederdissen C, et al. 2011. ViennaRNA package 2.0. *Algorithms for Molecular Biology*, **6**: 26.
Lu ZJ, Turner DH, Mathews DH. 2006. A set of nearest neighbor parameters for predicting the enthalpy change of RNA secondary structure formation. *Nucleic Acids Research*, **34**(17): 4912–4924.
Mathews DH, Sabina J, Zuker M, et al. 1999. Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *Journal of Molecular Biology*, **288**(5): 911–940.
Mathews DH, Turner DH, Watson RM. 2016. RNA secondary structure prediction. *Current Protocols in Nucleic Acid Chemistry*, **67**: 11.2. 1–11.2. 19.
Mauger DM, Cabral BJ, Presnyak V, et al. 2019. mRNA structure regulates protein expression through changes in functional half-life. *Proceedings of the National Academy of Sciences of the United States of America*, **116**(48): 24075–24083.
McGill R, Tukey JW, Larsen WA. 1978. Variations of box plots. *The American Statistician*, **32**(1): 12–16.
Metzker ML. 2010. Sequencing technologies—the next generation. *Nature Reviews Genetics*, **11**(1): 31–46.
Mishima Y, Tomari Y. 2016. Codon usage and 3'UTR length determine maternal mRNA stability in zebrafish. *Molecular Cell*, **61**(6): 874–885.
Mortimer SA, Kidwell MA, Doudna JA. 2014. Insights into RNA structure and function from genome-wide studies. *Nature Reviews Genetics*, **15**(7): 469–479.
Motorin Y, Marchand V. 2021. Analysis of RNA Modifications by second- and third-generation deep sequencing: 2020 Update. *Genes*, **12**(2): 278.
Nguyen TTT, Hayes BJ, Ingram BA. 2014. Genetic parameters and response to selection in blue mussel (*Mytilus galloprovincialis*) using a SNP-based pedigree. *Aquaculture*, **420–421**: 295–301.
Nishikura K. 2010. Functions and regulation of RNA editing by ADAR deaminases. *Annual Review of Biochemistry*, **79**(1): 321–349.
Piazzi M, Bavelloni A, Salucci S, et al. 2023. Alternative splicing, RNA editing, and the current limits of next generation sequencing. *Genes*, **14**(7): 1386.
Place AR, Powers DA. 1984. Purification and characterization of the lactate dehydrogenase ($LDH\text{-}B_4$) allozymes of *Fundulus heteroclitus*. *Journal of Biological Chemistry*, **259**(2): 1299–1308.
Popovic I, Riginos C. 2020. Comparative genomics reveals divergent thermal selection in warm- and cold-tolerant marine mussels. *Molecular Ecology*, **29**(3): 519–535.
Powell LM, Wallis SC, Pease RJ, et al. 1987. A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine. *Cell*, **50**(6): 831–840.
Powers DA, Smith M, Gonzalez-Villasenor I, et al. 1993. A multidisciplinary approach to the selectionist/neutralist controversy using the model teleost, *Fundulus heteroclitus*. *In*: Futuyma D, Antonovics J. Oxford Surveys in Evolutionary Biology. New York: Oxford University Press, 43–108.
Rangan KJ, Reck-Peterson SL. 2023. RNA recoding in cephalopods tailors microtubule motor protein function. *Cell*, **186**(12): 2531–2543. e11.
Rasool F, Ishtiaq I, Uzair M, et al. 2022. Genome-wide investigation and functional analysis of RNA editing sites in wheat. *PLoS One*, **17**(3): e0265270.
Rieder LE, Savva YA, Reyna MA, et al. 2015. Dynamic response of RNA editing to temperature in *Drosophila*. *BMC Biology*, **13**: 1.
Riemondy KA, Gillen AE, White EA, et al. 2018. Dynamic temperature-

sensitive A-to-I RNA editing in the brain of a heterothermic mammal during hibernation. *RNA*, **24**(11): 1481–1495.

Rosenthal JJC. 2015. The emerging role of RNA editing in plasticity. *Journal of Experimental Biology*, **218**(12): 1812–1821.

Rosenthal JJC, Eisenberg E. 2023. Extensive recoding of the neural proteome in Cephalopods by RNA editing. *Annual Review of Animal Biosciences*, **11**: 57–75.

Ruchika, Okudaira C, Sakari M, et al. 2021. Genome-wide identification of U-to-C RNA editing events for nuclear genes in *Arabidopsis thaliana*. *Cells*, **10**(3): 635.

Schwantes MLB, Schwantes AR. 1982. Adaptative features of ectothermic enzymes-I. Temperature effects on the malate dehydrogenase from a temperate fish *Leiostomus xanthurus*. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, **72**(1): 49–58.

Sharp PM, Li WH. 1986. An evolutionary perspective on synonymous codon usage in unicellular organisms. *Journal of Molecular Evolution*, **24**(1-2): 28–38.

Shen XK, Song SL, Li C, et al. 2022. Synonymous mutations in representative yeast genes are mostly strongly non-neutral. *Nature*, **606**(7915): 725–731.

Shoshan Y, Liscovitch-Brauer N, Rosenthal JJC, et al. 2021. Adaptive proteome diversification by nonsynonymous A-to-I RNA editing in coleoid cephalopods. *Molecular Biology and Evolution*, **38**(9): 3775–3788.

Singh RS, Hubby JL, Throckmorton LH. 1975. The study of genic variation by electrophoretic and heat denaturation techniques at the octanol dehydrogenase locus in members of the *Drosophila virilis* group. *Genetics*, **80**(3): 637–650.

Singh RS, Lewontin RC, Felton AA. 1976. Genetic heterogeneity within electrophoretic "alleles" of xanthine dehydrogenase in *Drosophila pseudoobscura*. *Genetics*, **84**(3): 609–629.

Somero GN. 2018. RNA thermosensors: how might animals exploit their regulatory potential?. *Journal of Experimental Biology*, **221**(4): jeb162842.

Somero GN, Lockwood BL, Tomanek L. 2017. Biochemical Adaptation: Response to Environmental Challenges from Life's Origins to the Anthropocene. Sunderland: Sinauer Associates.

Somero GN, Soulé M. 1974. Genetic variation in marine fishes as a test of the niche-variation hypothesis. *Nature*, **249**(5458): 670–672.

Stasik S, Schuster C, Ortlepp C, et al. 2018. An optimized targeted Next-Generation Sequencing approach for sensitive detection of single nucleotide variants. *Biomolecular Detection and Quantification*, **15**: 6–12.

Stocker J, Huang HW, Wang HM, et al. 2013. Reduction of RNA A-to-I editing in *Drosophila* acclimated to heat shock. *Kaohsiung Journal of Medical Sciences*, **29**(9): 478–483.

Su Z, Tang Y, Ritchey LE, et al. 2018. Genome-wide RNA structurome reprogramming by acute heat shock globally regulates mRNA abundance. *Proceedings of the National Academy of Sciences of the United States of America*, **115**(48): 12170–12175.

Sun J, Zhang Y, Xu T, et al. 2017. Adaptation to deep-sea chemosynthetic environments as revealed by mussel genomes. *Nature Ecology & Evolution*, **1**(5): 0121.

Takeuchi T. 2017. Molluscan genomics: implications for biology and aquaculture. *Current Molecular Biology Reports*, **3**(4): 297–305.

Tan Y, Ma CY, Li XX, et al. 2023a. Genome-wide sequencing identifies a thermal-tolerance related synonymous mutation in the mussel, *Mytilisepta virgata*. *Communications Biology*, **6**(1): 5.

Tan Y, Sun YX, Zhu YJ, et al. 2023b. The impacts of thermal heterogeneity across microhabitats on post-settlement selection of intertidal mussels. *iScience*, **26**(12): 108376.

Venev SV, Zeldovich KB. 2018. Thermophilic adaptation in prokaryotes is constrained by metabolic costs of proteostasis. *Molecular Biology and Evolution*, **35**(1): 211–224.

Walsh IM, Bowman MA, Soto Santarriaga IF, et al. 2020. Synonymous codon substitutions perturb cotranslational protein folding *in vivo* and impair cell fitness. *Proceedings of the National Academy of Sciences of the United States of America*, **117**(7): 3528–3534.

Wang DX, Eraslan B, Wieland T, et al. 2019. A deep proteome and transcriptome abundance atlas of 29 healthy human tissues. *Molecular Systems Biology*, **15**(2): e8503.

Wang ZR. 1997. Fauna Sinica. Mollusca, Bivalvia: Mytioida. Beijing: Science Press. (in Chinese)

Watabe S. 2002. Temperature plasticity of contractile proteins in fish muscle. *Journal of Experimental Biology*, **205**(15): 2231–2236.

Watt WB. 1983. Adaptation at specific loci. II. Demographic and biochemical elements in the maintenance of the Colias PGI polymorphism. *Genetics*, **103**(4): 691–724.

Watt WB, Dean AM. 2000. Molecular-functional studies of adaptive genetic variation in prokaryotes and eukaryotes. *Annual Review of Genetics*, **34**(1): 593–622.

Yablonovitch AL, Fu J, Li KX, et al. 2017. Regulation of gene expression and RNA editing in *Drosophila* adapting to divergent microclimates. *Nature Communications*, **8**(1): 1570.

Ye YY, Li JJ, Wu CW, et al. 2012. Genetic analysis of mussel (*Mytilus coruscus*) populations on the coast of East China Sea revealed by ISSR-PCR markers. *Biochemical Systematics and Ecology*, **45**: 1–6.

Zhang AD, Jiang XH, Zhang FP, et al. 2020. Dynamic response of RNA editing to temperature in grape by RNA deep sequencing. *Functional & Integrative Genomics*, **20**(3): 421–432.

Zhang LY, Yang CS, Varelas X, et al. 2016. Altered RNA editing in 3'UTR perturbs microRNA-mediated regulation of oncogenes and tumor-suppressors. *Scientific Reports*, **6**: 23226.

Zhao FZ, Zhou ZP, Dang YK, et al. 2021. Genome-wide role of codon usage on transcription and identification of potential regulators. *Proceedings of the National Academy of Sciences of the United States of America*, **118**(6): e2022590118.

Zheng SY, Kim H, Verhaak RGW. 2014. Silent mutations make some noise. *Cell*, **156**(6): 1129–1131.

Zhou ZP, Dang YK, Zhou M, et al. 2016. Codon usage is an important determinant of gene expression levels largely through its effects on transcription. *Proceedings of the National Academy of Sciences of the United States of America*, **113**(41): E6117–E6125.

Zhu XL, Liao ML, Zhu YJ, et al. 2024. Full-length PacBio amplicon sequencing to unveil RNA editing sites. *Current Bioinformatics*, **19**(5): 425–433.