

## EDITORIAL



# The evolutionary significance of post-transcriptional gene regulation

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Understanding the molecular mechanisms that give rise to phenotypic diversity is a major goal in evolutionary biology. Ever since King and Wilson (1975) hypothesised that changes in gene regulation may be important for species' evolution, research has explored the various ways in which regulation of gene expression may have mediated evolutionary transitions and innovations. So far, most studies have focused on changes in mRNA abundance, i.e., total gene expression. However, the regulation of genes is not restricted to their transcript levels but includes a wide range of post-transcriptional mechanisms that affect transcript levels, functions, and/or transcript structures. These include the control of transcript levels through miRNAs (Filipowicz et al. 2008), RNA modifications (Zhao et al. 2017), and/or changes in transcript structure through alternative splicing (Verta and Jacobs 2022). In contrast to gene transcript level variation, the role of post-transcriptional mechanisms in mediating phenotypic diversity and evolutionary dynamics remain relatively unknown, yet recent work has argued that post-transcriptional processes play a potentially important role in adaptation (Verta and Jacobs 2022; Singh and Ahi 2022; Wright et al. 2022).

Alternative splicing (AS), a mechanism that can change the composition of mature transcripts through the inclusion/exclusion of exons and introns and changes in exon length, is one of the best studied post-transcriptional mechanisms (Verta and Jacobs 2022; Singh and Ahi 2022; Wright et al. 2022). Despite being discovered nearly 50 years ago (Berget et al. 1977; Chow et al. 1977), it wasn't until a revolution in sequencing technologies that the transcriptome-wide contribution of AS for evolution could be thoroughly investigated. Diversity in AS scales positively with organismal complexity (Chen et al. 2014), lending support to the hypothesis that AS contributes to evolutionary divergence and innovation over macroevolutionary timescales. Recent technological and analytical developments have further accelerated the investigation of post-transcriptional mechanisms in an expanded range of divergence-time contrasts from different organisms, including recently diverged ecotypes. Thus, the evolution of post-transcriptional differences can now be studied over short evolutionary timescales and in natural systems in which divergent ecological selection is assumed to be at its greatest, giving us more power to identify patterns of adaptive relevance. Furthermore, application of functional genomics tools in a rapidly growing breadth of non-model species can potentially lead to the discovery of previously unknown post-transcriptional mechanisms of great evolutionary relevance. One such novel mechanism, small mitochondrially highly expressed RNAs (smithRNAs) is highlighted in this issue.

Stemming from the symposium "*Beyond transcription: the role of post-transcriptional regulation in adaptation and evolution*" organised as part of the Congress of the European Society for

Evolutionary Biology in Prague August 2022, this collection brings forth exciting articles that investigate evolutionary patterns in post-transcriptional mechanisms (Fig. 1), mainly in AS, and further our understanding of the evolutionary significance of post-transcriptional mechanisms.

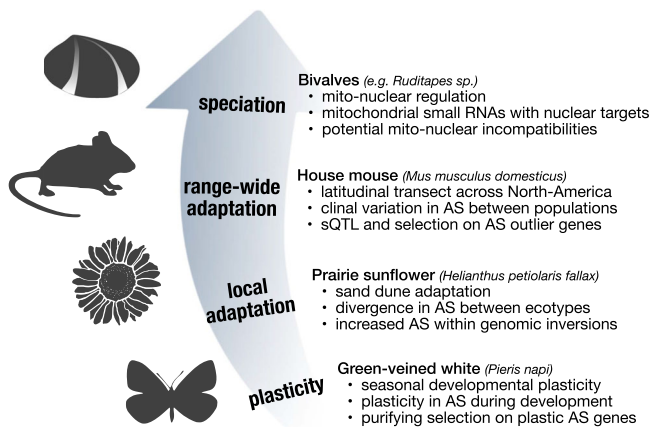
In this issue, Innes et al. (2023) show that AS plays a potentially important role in mediating fine-scaled adaptation of wild sunflowers to locally restricted sand dune environments alongside divergence in total transcript levels. While AS influenced a more restricted set of genes compared to differential transcript abundance, both mechanisms of regulatory divergence contributed to separate ecotype transcriptomes. Interestingly, the authors documented increased splicing and transcript abundance differences within inversion haploblocks separating locally adapted ecotypes, indicating that genome structural variation may mediate local adaptation by changes in gene regulation, including the isoform diversity being expressed through AS. Further investigation of splicing differences led the authors to identify phenotypes that may be involved in sand dune adaptation in sunflowers. As an example, genes controlling for seed development, abiotic stress response, growth strategies, and root structure seem to be prime candidates for mediating local adaptation through AS.

The evolutionary significance of AS in adaptation across large geographical distances is exemplified by findings reported in this issue by Manahan and Nachman (2023), who identified splicing variation associated with local adaptation in wild house mice. By combining RNA-seq, exome-capture, and existing data on targets of natural selection in a latitudinal transect of mice populations, the authors found strong candidate genes for which clinal variation in AS associated with splicing-Quantitative Trait Loci (sQTL) and genomic signatures of selection. Strikingly, none of the identified candidate genes for local adaptation through AS overlapped with genes known to contribute to adaptation through mRNA abundance, clearly demonstrating the unique roles of AS and total transcript levels in microevolution in this species. The top candidate genes had functions associated with growth, body size, and metabolism, which are phenotypes known to differ in the latitudinal gradient. In wild house mice, AS thus seems to provide an alternative mechanism for local adaptation that plays a role that is complementary to overall transcript levels.

When environments fluctuate in a predictable manner, species may adapt through an increase in phenotypic plasticity (Leung et al. 2020). Growing evidence indicates that AS might play an important role in mediating adaptive phenotypic plasticity (Marden 2006; Grantham and Brisson 2018; Verta and Jacobs 2022), but direct evidence for selection on plasticity in AS has been lacking. As *cis*-regulatory sites controlling for AS are often closely linked to the expressed gene, selection for plasticity in AS should lead to noticeable signatures in genetic diversity in gene loci. In this issue, Steward et al. (2024) tested this exciting hypothesis by using a unique system of

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**Fig. 1** The articles in this issue highlight the role of post-transcriptional mechanisms such as alternative splicing (AS) and small RNAs in mediating phenotypic plasticity (Steward et al. 2024), adaptation (Innes et al. 2023, Manahan and Nachman 2023), and speciation (Plazzi et al. 2023). The study questions span evolutionary processes from the micro- to the macro-level. Main topics and findings of the articles are outlined for each study organism.

developmental plasticity in seasonal diapause in the butterfly *Pieris napi*. The authors characterised AS and total transcript abundance through a time-series of development with and without diapause. The alternative developmental trajectories showed unique patterns of AS and transcript abundance changes, which indicated plasticity in both types of transcriptional programs. Strikingly, plasticity in AS was associated with a reduced genetic diversity in gene loci, indicating that purifying selection was acting to maintain plastic AS in seasonal diapause. These results highlight the multifaceted role that AS plays not only in local adaptation, but also in mediating plasticity in key developmental transitions.

Post-transcriptional regulation is not only limited to the alternative splicing of genes but involves a myriad of other processes that influence the stability or function of gene products. Short non-coding RNA molecules, such as small interfering RNA (siRNAs) or microRNAs (miRNAs), have been shown to play key roles in the post-transcriptional regulation of transcript and protein abundance by controlling the translation and stability of mRNAs (Filipowicz et al. 2008). To date, most work has focused on the interaction between nuclear-encoded miRNAs and mRNAs. However, a recently discovered class of miRNA-like elements, smithRNAs, are transcribed in mitochondria but regulate nuclear genes (Pozzi et al. 2017). This further expands the catalogue of potential post-transcriptional mechanisms. In this issue, Plazzi et al. (2023) review the literature around short non-coding RNA and estimate the probability of de novo evolved smithRNAs to find a suitable target gene with a likelihood above one in a hundred million. The authors hypothesise that the high likelihood of finding a suitable target gene without too many mismatches in the 3'mRNA region, combined with the high potential of smithRNAs to originate from mitochondrial RNAs, suggests an important role for smithRNAs in the evolution of mito-nuclear interaction in animals. Furthermore, the divergence of smithRNA subsets between species could lead to mito-nuclear incompatibilities and thus contribute to speciation, although this has not been empirically tested. Thus, this study highlights our ever-growing understanding of the evolution and functional role of post-transcriptional processes, and their potential contribution to adaptation and speciation.

The articles in this issue greatly add to our understanding of post-transcriptional mechanisms in adaptation and speciation.

This collection further suggests that AS and transcript levels can play both complementary and contrasting roles in local adaptation (Jacobs and Elmer 2021) by affecting either the same or different sets of genes (Manahan and Nachman 2023; Innes et al. 2023). This highlights a greater need for large-scale comparative studies of AS to better understand its broader role in adaptation. Furthermore, Steward et al. (2024) highlight a key role of purifying selection in maintaining plasticity in AS, suggesting that adaptive patterns in AS are not only driven by divergent selection. Lastly, Plazzi et al. (2023) highlight the need to expand the focus of post-transcriptional mechanisms in adaptation past AS, and also focus more on the role of short non-coding RNAs, those of nuclear and mitochondrial origin, in adaptation and speciation.

While this collection of articles enhances our understanding of post-transcriptional mechanisms in the context of adaptation, it also highlights that there is still a long way to go to fully unravel the complex contributions and mechanisms of post-transcriptional processes in plasticity, adaptive evolution, and speciation. It is reasonable to expect that striking adaptive patterns in post-transcriptional mechanisms as highlighted in this collection are the exception rather than the rule. Significantly more research is needed to evaluate the relative contributions of drift and selection in creating population-level patterns in the frequencies of post-transcriptional phenotypes such as AS. What are the population genetic parameters that allow adaptive AS to evolve? Is there a common set of gene functions across species that are susceptible for such evolution? How does the evolution of post-transcriptional mechanisms interact with genome evolution such as structural variation? Are these patterns similar across the tree of life? We are entering an exciting era where such questions can be addressed with the full diversity of study systems and approaches, which is certainly bound to renew our views about the mechanisms of evolutionary change.

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## COMPETING INTERESTS

The authors declare no competing interests.