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EDITORIAL

Ecology of Gene Expression

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ABSTRACT

The fundamental importance of the expression of genes has long been recognised in biology, but understanding its role in ecology and evolution has only recently begun to gain traction. This Special Issue highlights recent developments in this field, with 43 papers focusing on transcriptional variation in ecological processes, responses to environmental gradients or stressors, and as an important phenotype affecting other measurable traits, including fitness. This issue also highlights the rapid advancements in methods that will continue to improve our understanding of this molecular phenotype. In this Editorial, we highlight the diversity of authors in these papers and how they contribute to an improved understanding of molecular ecology. We hope that this discussion will inspire and drive progress towards equity, diversity and inclusion in ecology and evolutionary biology. Finally, we suggest some recommendations for Molecular Ecologists to advance the research area of ecology of gene expression.

1 | Introduction

The central dogma of biology is the transcription of the genetic code into the translation and production of proteins that make up the planet's biodiversity. While the genetic code provides the blueprint for this process, it is gene expression, or the product of transcription (RNA), that remains central to the regulation of all gene products in biology. While the importance of this process has long been appreciated (Britten and Davidson 1969), the molecular ecology of gene expression and understanding of its role in ecology and evolution is arguably still in its infancy. Most

genomes contain thousands of genes, but the genetic architecture of these genes is still poorly described. Most genes have been annotated in only a few organisms, tissues, and environments, with the characterisation and role of gene pathways (where many genes interact to produce a protein and ultimately phenotype) only beginning to be elucidated. Yet, the regulation of gene expression is fundamental to genetic divergence, adaptation, and speciation (Cutter 2023; Mack and Nachman 2017; Triant et al. 2021). Furthermore, plasticity is becoming recognised as a central mechanism through which organisms may persist in a new or changing environment rather than an 'alternative to

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adaptation'. All of these processes point to the 'ecology of gene expression' as paramount to the field of Molecular Ecology and the motivation for this special issue.

This special issue brings together 43 papers that highlight the role of gene expression and transcriptional variation as a 'molecular phenotype' and explores the recent ecological and evolutionary insights gained that would otherwise not be evident from the study of classic behavioural, morphological, or physiological traits. As evidenced by the sheer number of submissions we received, empirical studies increasingly cover a wide taxonomic breadth and are addressing emerging hypotheses and debates about ecology and evolution. Studies of gene expression are frequently providing novel insights into ecological and evolutionary processes, as transcription profiles and abundance have the ability to uncover hidden phenotypes and relationships among traits not readily measurable via traditional approaches. The issue highlights rapid advances in the methods used to characterise gene expression profiles and regulatory mechanisms to gene expression. Improvements in RNA sequencing and genomics continue to revolutionise the field, with emerging advances in the measurement (e.g., traditional RNA Sequencing, long-read direct nanopore RNA/cDNA sequencing, full-length transcripts and isoforms, gene editing, cell-specific expression, epigenetic profiling, miRNA, environmental RNA, non-coding RNAs, etc.), application and analysis of gene expression contributing to a wide range of interesting questions in Molecular Ecology. Below we highlight how the diversity of contributed manuscripts advances our understanding of the ecology of gene expression.

2 | Linking Ecological Processes to a Role for Gene Expression

Several studies elegantly linked classical ecological questions about species interactions, such as competition, herbivory, parasitism and predation, with patterns of gene expression. In this issue, Monteiro et al. (2024) linked variation in melanin synthesis genes to colour morphs in the pumpkin toadlet, a trait directly related to predation risk. Similarly, Wong et al. (2024) identified a loss of function mutation and differential expression to cause flower colour morphs in a bee-pollinated orchid species, Glossodia major, which allows the flowers to better attract their pollinators, using a combination of transcriptomics, functional analysis, metabolite profiling and comparative spectral analysis. In a more direct manipulation, Garrigós et al. (2023) revealed that mosquitoes respond differently to two Plasmodium species, contributing to the hypothesis that these differential gene expression responses may influence the virulence and transmissibility of protozoan disease. The study by Chen et al. (2024) showed that transcriptional plasticity is critical in shaping host range differences among herbivores. They highlight differences in transcriptomic variability between two polyphagous spider mites, Tetranychus urticae (generalist) and T. truncatus (specialist). T. urticae exhibited higher transcriptional plasticity, particularly in detoxification and metabolic pathways, enabling broader host adaptation. Moreover, both species showed enriched xenobiotic metabolism on new hosts, but T. truncatus displayed limited plasticity. Yoon et al. (2025) investigated the case of novel host plant colonisation in Lycaeides melissa butterflies

by conducting a larval rearing experiment. They tested the effects of viral infection and host plant treatment (novel vs. native) on larval performance. Their results revealed that while both viral infection and host plant treatment influenced phenotypic traits, only host plant treatment significantly affected the differential expression of immune and detoxification genes.

Competition and priority effects are also important ecological mechanisms shaping the composition of communities, often involving indirect interactions between three or more species. Yet, the molecular mechanisms influencing these ecological dynamics are often overlooked. In this Special Issue, two studies investigated multi-species interactions. Transcriptomics of the tetillid sponge revealed that competition for space with a cnidarian at the same trophic level evoked a stronger stress response than competition with a macroalgae, which is in a different trophic level (Deshpande et al. 2023). An experimental study of the hummingbird-pollinated plant Diplacus (Mimulus) aurantiacus allowed researchers to examine priority effects and their relationship with gene expression (Chappell et al. 2024). In this plant-pollinator system, nectar yeast (in this case, Metschnikowia reukaufii) is known to experience strong priority effects when colonising flowers after other nectar yeasts like M. rancensis. Their results revealed that yeasts placed in nectar depleted of amino acids by a prior yeast exhibited a distinct transcriptional response compared to those in amino acid-rich nectar, emphasising that intraspecific genetic variation in the ability of nectar yeasts to respond to nutrient limitation and direct fungal competition underpins priority effects in this microbial system (Chappell et al. 2024). These studies highlight the crucial role of gene expression in modulating responses to ecological processes.

3 | Gene Expression Patterns Across Gradients of Environmental Stress

While gene expression diversity may facilitate adaptation to new or changing environments, without requiring underlying genetic change, its role in adaptive evolution and population divergence has been understudied, especially in non-model species. The importance of gene expression in the ecological invasion process was thoroughly reviewed by Vaughan and Dhami (2024), emphasising the importance of transcriptional variation to act as the first molecular change enabling species to persist in new and potentially stressful environments. In an empirical study of range expansion—a topic related to ecological invasion—Lee et al. (2024) studied Kellet's whelk, whose range is expanding along the North American Pacific coast. Using transcriptomic data from offspring collected from historical and expanded populations, they discovered several putatively adaptive loci associated with cold tolerance and metabolic stress, suggesting that genetic adaptation may play a critical role in helping marine species withstand colder temperatures as the whelks shift their ranges poleward. The impact of environmental and anthropogenic stressors on marine calanoid copepods was revealed by significant variation in metabolic and stress-response genes, with temperature correlated with upregulation of genes related to proteolytic activity and heat shock proteins (Semmouri et al. 2024). Interestingly, exposure to anthropogenic chemicals did not significantly affect expression of genes linked to fatty acid metabolism or other stress-related genes in this species,

emphasising the potential use and limitations of biomonitoring using gene expression.

In the Florida Keys, Gallery et al. (2024) studied the corals Montastraea cavernosa and Siderastrea siderea, which each consist of four genetically distinct lineages, distributed unevenly by depth and the nearshore-offshore gradient. Despite this genetic differentiation, responses of these cryptic lineages to natural environmental variation were remarkably similar at the genome-wide gene coexpression network level. Differences in gene expression were greater between habitats than among lineages. These findings challenge the hypothesis that regulatory evolution drives lineage specialisation (Dixon et al. 2018; Kenkel and Matz 2016) and suggest that other factors, such as algal symbionts, microbiomes, or spatially varying selection, may play a dominant role in shaping adaptation across these coral populations. In a comparison between penguin species, Paris et al. (2024) used gene expression data from multiple tissues from Emperor and King penguins to identify the crucial roles played by the muscle and liver in the adaptation to the extreme environments experienced by Emperor penguins in Antarctica.

Seasonal environmental change can be dramatic in some habitats and gene expression data is revealing the molecular mechanisms enabling organisms to inhabit drastically fluctuating environments. Organisms inhabiting highly seasonal environments must cope with a wide range of environmentally induced challenges, often requiring extensive phenotypic modifications to survive. The molecular basis and evolutionary outcomes of adaptation to seasonal environmental change have been difficult to pin down. Roberts et al. (2024) tackled this challenge by investigating the role of microRNA (miRNA) expression during winter diapause, a period of minimised cellular processes and low energetic expenditure, in the butterfly Pieris napi. They identified coordinated miRNA expression patterns that included two candidate miRNAs that may regulate diapause through the ecdysone pathway, providing new insights into the hormonal regulation of diapause. Berger et al. (2024) examined diapause in two of the most abundant large copepods in the Southern Ocean, Calanoides acutus and Calanus propinguus, known for their ecological success in surviving food deprivation in highly seasonal environments. Their starvation experiments revealed overlapping but species-specific transcriptomic responses in the copepods, with a conserved core set linked to RNA and protein metabolism. In this case, a phylotranscriptomics approach revealed selection on lipid storage genes highlighting molecular adaptations critical for starvation tolerance in these copepods. Lipid genes are also crucial in the timing of maturation in Atlantic salmon, with individuals with different vgll3 genotypes showing differential expression of 100s of genes, ultimately resulting in different seasonal dynamics in lipid profiles (Ahi et al. 2024).

Palma-Silva et al. (2024) analysed environmental variables and gene expression profiles of tropical ground-herb species in a waterlogging gradient of Amazonian riparian forests. The flooding gradient delineates distinct wetland forest types, shaping habitats and species characteristics. Seasonal flooding poses a significant challenge to organisms adapted to Amazonian riparian forest: such as Igapó, characterised by higher water column heights and longer flood durations compared to Terra Firme. Environmental variables, particularly flooding, were key drivers of population genetic differentiation and differential gene

expression in this ground herb species. By identifying genes associated with stress response pathways, this Amazonian riparian plant offers valuable insights into the molecular mechanisms underlying plant adaptation to the challenges posed by seasonally flooded neotropical ecosystems. In a similar study on rice from either wet or dry environments found an interaction between evolutionary history (i.e., varietal group) and recent environmental conditions shaping plasticity of gene expression (Hamann et al. 2024).

Not all extreme environmental fluctuations are predictable, however, and a study of free-ranging rhesus macaques reveals that a single massive perturbation—in this case, a hurricane—can cause changes in methylation at 1000s of genetic regions (Watowich et al. 2024), highlighting the potential for long-term changes to gene expression in response to the environment. Another unpredictable environmental stressor is viral infections; infection with the influenza A virus altered the expression of many genes in grey seal pups, including resulting in down-regulation of immune system genes. The patterns of regulatory changes to the immune response could be indicative of adaptation in the grey seals to resist morbidity and mortality as a result of infection, contributing to their status as a disease reservoir (McCosker et al. 2025)—and thus having impacts on other species in their ecological community.

A number of studies in the special issue focused on withinspecies variation, emphasising that the state of the field is importantly still in the process of characterising the ecology of gene expression within species, often in response to distinct environments (e.g., wet versus dry; Hamann et al. 2024; Palma-Silva et al. 2024), behaviour (early versus late maturation; Ahi et al. 2024; e.g., social versus asocial; Omufwoko et al. 2023; Stoldt et al. 2025), habitat (e.g., near shore versus offshore; Semmouri et al. 2024), life-history (novel versus native host plant use; Yoon et al. 2025), and temperature-associated trait variation (Han et al. 2024). Overall, these studies collectively advanced efforts to elucidate the role of gene regulation in ecological adaptation, such as facilitating adaptive gene expression within a lifetime (e.g., Ahi and Singh 2024), offering a deeper understanding of the processes driving evolutionary change in diverse ecological contexts.

4 | Regulatory Mechanisms Mediating the Ecology of Gene Expression

Several studies in this Special Issue provided valuable insights into how genetic and regulatory changes shape phenotypic diversity across a range of populations and species. Rodríguez-Ramírez et al. (2023) characterised regulatory mechanisms underlying the *Eda* locus, a major effect region associated with the loss of lateral plate armour and changes in the sensory lateral line of freshwater threespine stickleback fishes. They discovered that the freshwater *Eda* haplotype influences gene expression and alternative splicing in pathways related to bone development, neuronal processes, and immunity, highlighting a potentially important role for alternative splicing as a key mechanism mediating adaptive phenotypes under rapid environmental change. In contrast, Yoon et al. (2025) found that the genetic architecture of gene expression in *Lycaeides melissa* butterflies

is highly polygenic, with a genome-wide distribution of genes showing significant differential expression in response to host plant treatment. Two other studies also focused on the role of genetic variants in determining gene expression profiles. In Eurasian perch, *cis*-expression quantitative trait loci (QTL) were found in regions of the genome that also were candidate loci underpinning local adaptation to humic environments (Ozerov et al. 2025). Costa et al. (2024) went a step further and found that many eQTLs were differentially methylated in rhesus macaques, providing important insight into the link between genotype and phenotype.

The role of selection on gene regulation is an important component of the ecology of gene expression, but much about this topic remains unknown. Broad et al. (2024) investigated this question in adaptive trait variation among contrasting coastal ecotypes of the Australian wildflower *Senecio lautus*. Using a hybrid population from two ecotypes with differing vegetative heights and gravitropic behaviours, differentially expressed genes were discovered to be linked to plant hormones with corresponding gene networks. This finding was used to develop a model for hormonal control of gravitropism, particularly auxin movement and accessibility, contributing to ecotypic adaptation. These findings underline the importance of intertwined signalling pathways in adaptive evolution and the origin of species.

Pleiotropic interactions are predicted to constrain gene expression evolution (Mank et al. 2008), and cis-regulatory changes are thought to have fewer pleiotropic impacts (Signor and Nuzhdin 2018). Kautt et al. (2024) revealed that the majority of differential expression between two species of *Peromyscus* mice was driven by cis-regulatory changes, reinforcing the role of pleiotropy in shaping patterns of gene expression evolution. Similarly, Tosto et al. (2024) found evidence that pleiotropic constraints might be preventing genes from evolving sex-specific expression, further highlighting the importance of considering the multiple functions that a single gene might serve.

Hybridisation is an important mechanism that can alter gene expression, contribute to reproductive isolation and, at the same time, drive evolutionary novelty (Runemark et al. 2024). In this Special Issue, a review and an empirical study both explored the mechanisms behind these changes, particularly focusing on how altered gene expression influences hybrid fitness and adaptation. Runemark et al. (2024) highlighted the importance of altered gene expression—transgressive expression of hybrids—in reproductive isolation and evolutionary novelty, while Choi et al. (2024) investigated the role of homoeologue expression bias in the hybridisation of New Zealand stick insects. Together, these studies provide a deeper understanding of the complex relationship between hybridisation, gene expression and evolutionary processes.

5 | Differences in Expression Associated With Behaviours and Mating Systems

Gene expression is a fundamental mechanism enabling individuals to perform different behaviours. Two studies used a comparative approach in *Peromyscus* deer mice to reveal how sexual selection strengths and variation in mating systems has shaped gene expression. One study compared a monogamous species (P. californicus) to two polygynandrous species (P. boylii and P. maniculatus), looking across four tissues (Voss and Nachman 2024). This comparison identified genes with differential expression associated with mating system. These 'mating system genes' were most strongly differentially expressed in the seminal vesicles and included key seminal fluid proteins, suggesting that sexual selection might be shaping aspects of sperm motility, sperm-egg binding and copulatory plug formation. The other study compared only two species (P. maniculatus and P. polionotus) and their hybrid offspring and investigated expression in 10 brain subregions (Kautt et al. 2024). Importantly, each subregion seemed to have a conserved network of genes, with different genes showing species-level differential expression (and to a lesser extent, differences between sexes) within each subregion.

Instead of taking a comparative approach, Tosto et al. (2024) investigated gene expression in males and females of a polygynandrous pipefish species and compared expression patterns to estimates of the strength of and opportunity for sexual selection. They observed an overall bias towards increased expression in males in the gonads, livers, and gills of these fish, with most sex-biased genes exhibiting high tissue specificity, indicative of pleiotropic constraints hindering the evolution of sex-biased genes, which is consistent with Kautt et al. (2024). Both sexes experienced similar opportunities for sexual selection, which could be reflected in the overall high levels of sex-biased expression. Gamete fusion genes in particular were highly differentially expressed between the sexes in the gonads, reinforcing the finding from Voss and Nachman (2024) that reproductive proteins are likely targets of sex-specific selection.

Sociality involves a variety of behaviours, and research by Bolton et al. (2024), Campbell et al. (2024), and Omufwoko et al. (2023) highlights how gene expression interacts with systemic processes such as immunity, levels of circulating hormones and reproductive status. Bolton et al. (2024) measured behaviour and endocrine profiles of male individuals of an Amazonian bird over 3 years and found gene expression was highly associated with social status, testosterone phenotype and cooperative behaviour. Interestingly, testosterone and cooperative behaviours were related and reflected in the gene expression profiles, suggesting that male aggressive and sexual behaviours share regulatory mechanisms. Another study investigated the link between social behaviours and expression through repeated sampling of meerkat individuals (Campbell et al. 2024). That research showed that the expression landscape changed drastically in female individuals who shifted from subordinate to dominant status, a pattern that was associated with a change in immune response. In an experimental study on sweat bees, Omufwoko et al. (2023) revealed that spring soil temperatures are predictive of adult social structure, although adult transcriptomic profiles were reflective of reproductive status (queen versus worker) rather than social environment. Surprisingly, the social structure of the nest also influenced pupal transcriptomic profiles—suggestive of less flexibility than previously assumed in this system (Omufwoko et al. 2023).

Behaviours can be described as 'movement' at a variety of scales, from short-term and small-scale responses to environmental

cues such as light or gravity through to long-distance migrations that require physiological and behavioural changes coordinated to respond to complex sets of cues. A thorough review of migratory behaviours, focused primarily on birds, outlines how cues such as temperature can trigger cascades of tissuespecific changes in gene expression that ultimately are involved in migration timing (Sur and Sharma 2024). They highlight the importance of understanding sex differences in expression and movement behaviours, a point that is reinforced by the surprising finding of sex-biased opsin gene expression patterns that directly influence short-term phototactic behaviours in corn-borer moths (Huang et al. 2024). Huang et al. (2024) neatly demonstrated how the expression of three opsin genes influences phototactic behaviour, primarily in the eyes and brains, through a targeted approach focused on three opsin genes, including a knockdown experiment.

Shifting focus to plant behaviours, Broad et al. (2024) identified differential gene expression between *Senecio* families exhibiting gravitropic versus agravitropic behaviours. The genes showing differential expression included many related to hormone pathways, highlighting the important role of hormones in modulating a wide variety of behaviours across many organisms—providing a nice linkage between this plant behaviour and animal movement behaviours (Sur and Sharma 2024), as well as social status and cooperative behaviours (Broad et al. 2024).

6 | Experimental Manipulations of the Environment Change Gene Expression

Several studies explore how organisms alter gene expression in response to environmental variation. Collectively, these findings demonstrate that organisms in the wild actively regulate gene expression to adapt and survive under suboptimal or changing conditions. This research underscores the utility of gene expression analyses in assessing molecular responses to climate change. The studies also reveal that environmental stress elicits diverse gene expression responses. They highlight how adaptive molecular responses can vary depending on historical exposure to environmental conditions, interspecific interactions and selective pressures. Together, these findings illustrate the complex interplay between environmental variation and gene expression in shaping species' adaptive capacity.

Semmouri et al. (2024) investigated the effects of temperature, nutrient levels, salinity, turbidity, photosynthetic pigments and chemical pollutants on population density and gene expression in wild-caught copepods, finding that temperature and turbidity significantly predicted population densities, along with an interactive effect of chemical pollutants and chlorophyll concentrations. However, only a few variables, such as temperature and salinity, influenced gene expression in field-collected adults, with significant variation observed in metabolic and stress-response genes, while anthropogenic chemicals did not induce significant changes in gene expression. This study highlighted the potential of field gene expression analyses for biomonitoring. Similarly, Ren et al. (2024) examined mosquito-fish responses to high ($\rm CT_{max}$) and low ($\rm CT_{min}$) temperatures, demonstrating that differential splicing had a larger role than

gene expression changes in coping with acute thermal stress. Further emphasising environmental influences on gene expression, Huo et al. (2024) studied the sea cucumber *Apostichopus japonicus* under hypoxia, showing that altered translational efficiency was key to its response, providing insights into regulatory mechanisms enabling marine invertebrates to survive oxygen deficiencies and informing potential mitigation strategies for hypoxia's harmful effects.

Han et al. (2024) examines the genetic and transcriptomic basis of phenotypic plasticity in flowering time in *Arabidopsis thaliana* accessions from southern and northern regions in Sweden. Under two controlled greenhouse conditions (10°C and 16°C), northern accessions exhibited advanced flowering in response to lower temperatures, while southern accessions showed delayed flowering. This divergence in flowering time responses was linked to the isothermality of their native ranges. They also observed rewiring of gene co-expression networks, with the expression of genes associated with the mean flowering time and its plastic variation. The findings provide insights into the role of transcriptome plasticity in ecological adaptation and rapid environmental responses.

Omufwoko et al. (2023) used the socially flexible sweat bee Lasioglossum baleicum, which produces both solitary and eusocial nests, to demonstrate that soil temperatures strongly predict nest social structure, with temperature interacting with development to influence adult behaviour and physiology. Their findings revealed substantial differences in the transcriptomic profiles of stage-matched pupae from warmer, social-biased sites compared to cooler, solitary-biased sites. Similarly, Stoldt et al. (2025) showed that the social environment affects the molecular physiology of social insects such as the ant Temnothoraxrugatulus. By studying plastic changes in brain transcriptomes in queens and workers, they found that gene expression in queens' brains was largely influenced by the interaction between social environment and queen morph, highlighting how physiology depends on morph and role within the colony. Research in this Special Issue has also emphasised the influence of historical exposure to environmental variation on current gene expression responses to environmental changes. For example, Bonzi et al. (2024) investigated transgenerational thermal acclimation in the reef fish Acanthochromis polyacanthus by exposing two generations to elevated temperatures. Their results showed that parental temperature had a greater impact on adaptive gene expression than the individual's own temperature experience, underscoring the importance of parental environmental history in shaping responses in reef fish.

Studies have also emphasised how testing for selection on differentially expressed genes in response to environmental variation can deepen our understanding of adaptive evolution. Berger et al. (2024) conducted starvation experiments on two Southern Ocean copepods, revealing that starvation-response genes are under strong purifying selection at the sequence level and stabilising selection at the expression level, consistent with their critical biological functions. Their study provides insight into the molecular adaptations of high-latitude zooplankton to variable food conditions through gene expression data. Similarly, Huang et al. (2024) combined gene expression analysis and RNA interference of opsin genes in the nocturnal moth *Ostrinia furnacalis*

to explore the molecular relationship between phototactic behaviour and insect vision. They found that opsin genes regulate phototactic behaviour with sex-specific gene expression and are likely under selection in nocturnal Lepidoptera, contributing to adaptive responses to darkness. Cui et al. (2024) investigated salt tolerance in *Suaeda salsa* L., a halophyte vegetable, using transcriptomics and showed that high salinity significantly upregulated salt-resistance genes compared to low salinity. Their results highlight that salt tolerance in halophytes involves gene expression changes likely under selection. Finally, Hamann et al. (2024) studied different rice accessions to identify genomewide selection on gene expression plasticity, showing how molecular plasticity and adaptive evolution interact to shape drought tolerance in plants, with selection on plasticity mediating adaptation to drought.

7 | Modern Approaches in the Estimation and Analysis of Gene Expression

The shift towards reference-guided approaches for differential gene count estimation is evident across the papers compiled in this Special Issue. Freedman and Sackton (2024), in their comprehensive review, highlight how advancements in genomic technologies—such as affordable reference genome sequencing and single-cell RNA sequencing—are overcoming limitations of de novo assemblies, including missing expression data from rare cell types and confounding effects (Freedman and Sackton 2024). Similarly, Hoedjes et al. (2024) emphasise the advantages of reference-guided differential gene expression analysis of non-model organisms. One study used a combined approach, in which a de novo transcriptome merged with a reference-guided approach (McCosker et al. 2025), yielding an improved set of quality metrics. Over two thirds of the 35 empirical transcriptomic studies used a reference genome, with 10 studies relying on de novo transcriptome assemblies. Of those that presented new de novo assemblies, the assembly completeness was evaluated using BUSCO scores, which ranged from 85.5% to 96.8% (Berger et al. 2024; Deshpande et al. 2023; Monteiro et al. 2024; Palma-Silva et al. 2024; Semmouri et al. 2024; Tosto et al. 2024; Voss and Nachman 2024; Wong et al. 2024). These scores indicate that de novo transcriptome assembly remains a valid approach for transcriptomic studies of organisms lacking a reference genome.

For those species lacking a reference genome, using a closely related species' reference could be an alternative to generating a de novo transcriptome, as Garrigós et al. (2023) did. However, a technical paper demonstrated that reference genes validated for European Apis mellifera workers are unsuitable for African A. m. scutellata, even under standardised conditions (Buttstedt et al. 2023). This underscores the critical importance of subspecies-specific revalidation to ensure the accuracy and reliability of gene expression analyses, especially for species with a broad distribution range.

The extension of RNA-seq analyses beyond snapshots of differentially expressed genes was also highlighted in the main themes of the reviews contributed by Freedman and Sackton (2024), Hoedjes et al. (2024) and Vaughan and Dhami (2024), and is reflected by the empirical papers compiled in the special issue;

studies are more often incorporating analyses that aim to yield mechanistic understanding. Frequently, weighted correlation network analysis (WCGNA; Langfelder and Horvath 2008) was used to identify and compare changes in expression of gene modules or describe gene networks (Ahi et al. 2024; Berger et al. 2024; Broad et al. 2024; Chen et al. 2024; Gallery et al. 2024; Han et al. 2024; Lee et al. 2024; Paris et al. 2024; Rodríguez-Ramírez et al. 2023; Semmouri et al. 2024; Voss and Nachman 2024), with others using different forms of clustering (Kautt et al. 2024; Roberts et al. 2024) or a focus on gene families (Cui et al. 2024). Papers that did not statistically interrogate gene clustering included analyses or approaches to determine mechanisms—for example testing functions of mutations and differential expression using transgenic organisms (Wong et al. 2024), incorporation of data on methylation patterns (Costa et al. 2024; Ren et al. 2024; Watowich et al. 2024) and genetic variants (eQTLs; Costa et al. 2024; Ozerov et al. 2025), investigating microRNAs (Ren et al. 2024), and comparing patterns of translation to transcription (Huo et al. 2024). Still others measured quantitative traits or fitness parameters of the organisms they studied, such as immune response (Campbell et al. 2024; McCosker et al. 2025) or effects of viral infection (Yoon et al. 2025), body size and mating success (Tosto et al. 2024), and structural colour traits at the cellular level (Monteiro et al. 2024).

Studies of gene expression are also rapidly integrating epigenomic and transcriptomic data to understand the molecular mechanisms contributing to shifts in phenotype. Such studies, across a variety of systems, have shown that epigenetic modifications (i.e., methylation) and transcriptional regulation are central to environmental and physiological adaptation. In this issue, we see the utility and importance of these integrated datasets for understanding responses to acute environmental stressors, including hypoxia (Huo et al. 2024), temperature (Ren et al. 2024) and natural disaster (hurricane) (Watowich et al. 2024). These studies all found that extreme events shape gene expression and regulatory mechanisms, although the molecular pathways affected were unique. Studies in this issue also demonstrated how coordinated shifts in methylation and gene expression accompany aging (Ahi and Singh 2024; Watowich et al. 2024), yielding important new insights into the molecular mechanisms associated with significant physiological shifts. Together, this set of studies emphasised that adaptation involves complex and sometimes non-overlapping contributions from gene expression, splicing, methylation and translational regulation, depending on the species and stressor.

8 | Diversity of Authors

The fields of molecular ecology and evolution exhibit a lack of diversity among scientists from the Global South and those who identify as people of colour (POC), with black and Indigenous people being particularly underrepresented. The complex reasons behind this disparity are only now beginning to be understood (Nakamura et al. 2023; O'Brien et al. 2020; Tseng et al. 2020). Historically, these disciplines developed alongside European colonisation of other continents and often provided justification for it (Graves 2001). Prominent figures in the modern synthesis of evolutionary biology endorsed various forms of biological determinism and eugenics, either actively or passively

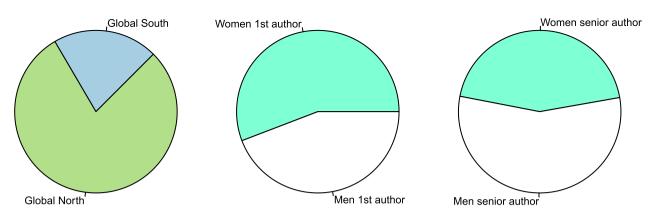


FIGURE 1 | The Special Issue includes contributions led primarily by researchers from the Global North and a relatively even balance of women and men. 21% of papers had a first author from the Global South, 55% had a female first author and 44% had a female last author. [Colour figure can be viewed at wileyonlinelibrary.com]

(Weindling 2012). This has likely influenced mainstream evolutionary biology, making it less accessible for POC and individuals from the Global South. Persistent systemic barriers continue to affect the recruitment and retention of POC and Global South scientists, a challenge that is particularly severe for black women and POC members of the LGBTQ+ community, who face discrimination at the intersections of race, gender and sexual orientation (Miriti 2020).

For this Special Issue, Molecular Ecology sent out an open call for editors, with a specific goal of selecting co-editors with shared goals to reduce barriers for underrepresented authors in publishing. In this issue, we specifically strived to increase the representation of women, POC, and Global South scientists by advertising broadly, actively reaching out to our networks to spread the word, and directly identifying members of underrepresented groups in molecular ecology and evolutionary biology. This approach resulted in approximately 21% of the manuscripts published in this issue being led by people based in the Global South (Figure 1; note that this did not count scientists who may identify as being from the Global South but are based in the Global North). Given that 85% of the world's population resides in the Global South, there is much room for improvement in increasing their representation in our field of research. Gender statistics were comparatively better, with 55.8% of manuscripts with women as first authors. However, women in senior author roles decreased to 44% (Figure 1). This suggests that while the representation of gender diversity may have improved at early career stages, it leaks away at advanced career stages, particularly at the transition from postdoc to professorship (e.g., Greska 2023). We hope this Special Issue will inspire and drive progress towards equity, diversity and inclusion in science.

9 | Future Outlook for the Field

Despite gene expression's role in the central dogma, including the tremendous ability of the environment and molecular mechanisms to modify the resulting phenotype, the ecology of gene expression is only just beginning to find its place in Molecular Ecology. As this special issue illustrates, the field is moving from 'laundry lists' of differentially expressed genes to sophisticated experiments and analyses that can reveal the ecological significance of gene expression. In addition to advances in molecular

ecology, this Special Issue points to advances in the approaches to isolate and analyse RNA and associated molecular tools to carry out such studies, many of which have reduced in cost (especially compared to proteomics, which remains expensive). Based on these findings, we suggest some recommendations for Molecular Ecologists to advance the ecology of gene expression:

- Consider RNA, especially functional transcriptomics and putative epigenetic factors in the experimental design of molecular ecology studies, such as population genomics and phylogenetics.
- Integrate gene network and pathway analyses into experimental designs.
- 3. Increase sample sizes.
- 4. Acknowledge the current limitations of ecological annotations of genes (e.g., Landry and Aubin-Horth 2007) and carry out process-oriented experiments that determine how a genome or a population of genomes interacts with its environment across ecological and evolutionary timescales.
- 5. Integrate methods (e.g., CRISPR) that allow for controlled experiments in an ecological context.
- 6. Leverage published datasets to conduct metanalyses across systems to find broad molecular patterns underlying ecological and evolutionary transitions.
- 7. Advancements in incorporating gene regulation into evolutionar theory.
- 8. Enable and promote scientists from groups that are underrepresented in molecular ecology (including the global south) to contribute to and lead cutting-edge research.

Author Contributions

All authors conceived of the manuscript, contributed to writing the first draft and provided substantive edits.

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