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Caution in Interpreting Regulatory RNA Associations With Eusocial Evolution in Bees

Petr Heneberg *

Third Faculty of Medicine, Charles University, Prague, Czech Republic

*Corresponding author: E-mail: petr.heneberg@lf3.cuni.cz.

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Abstract

Recent advances in transcriptomics and regulatory genomics have expanded interest in the potential roles of alternative splicing and noncoding RNAs in the evolution of eusociality in bees. A recently published review by Brenman-Suttner and Zayed synthesized this literature, highlighting numerous associations between the expression patterns of regulatory RNA and caste, task, and developmental states. However, there is a distinction between molecular associations and demonstrated causal roles in evolutionary processes, particularly for complex social traits. Drawing on established principles from evolutionary genomics and regulatory biology, we discuss the limitations of correlational transcriptomic data and highlight ongoing debates over the functional inference of circular RNAs and long noncoding RNAs. Clarifying these interpretive boundaries is essential for aligning conclusions with current evidence and for guiding future experimental research on the molecular basis of social evolution.

Key words: Hymenoptera, evolution, regulatory RNA, circular RNA, eusociality, long noncoding RNA.

Significance

Studies of regulatory RNAs have generated enthusiasm for molecular explanations of social evolution in bees. Many reported links between regulatory RNA expression and eusocial traits are based on association rather than functional validation. Distinguishing correlation from causation is essential for accurate evolutionary inference. This letter clarifies the interpretive boundaries for the use of regulatory genomic data and supports the careful use of molecular evidence within the established framework of evolutionary theory. Doing so will help guide future experimental work aimed at testing causal mechanisms underlying social complexity.

Dear editor,

We read with interest the recently published review by Brenman-Suttner and Zayed entitled "The evolution of bees: insights from 'omic studies" in *Genome Biology and Evolution* (Brenman-Suttner and Zayed 2026). The article provides a broad synthesis of genomic, transcriptomic, and environmental 'omics approaches relevant to bee evolution and sociogenomics. Its integration of phylogenetics, regulatory genomics, and ecological applications represents a valuable overview of rapidly expanding research areas. In this letter, we focus on the interpretation of evidence linking regulatory processes, such as alternative splicing, long noncoding RNAs, microRNAs, and circular RNAs, to eusocial traits in bees. In several sections of the review, regulatory RNA classes are described as having facilitated, enabled, or contributed to the emergence and elaboration of caste differentiation, reproductive division of labor, and behavioral

specialization. Even when qualified, this phrasing may be read as implying evolutionary causality.

Currently, most evidence linking regulatory RNAs to eusocial phenotypes in bees is derived from transcriptomic and comparative genomic studies that report differential expression, enrichment, or co-expression patterns across castes, behavioral states, developmental stages, or infection contexts (Price et al. 2018; Thölken et al. 2019). These approaches are informative and hypothesis-generating, making them well-suited for identifying candidate regulatory elements associated with phenotypic plasticity. However, such data alone do not establish necessity or sufficiency. Expression differences may arise as downstream consequences of developmental state, physiological condition, or demographic structure rather than as drivers of social evolution.

This distinction is well established in evolutionary genomics. Regulatory variation can accompany phenotypic divergence without being causally responsible for evolutionary transitions, particularly in complex traits influenced by developmental, ecological, and demographic factors (Lynch 2007; Wray 2007). Longstanding critiques of adaptationist inference caution against attributing evolutionary significance to every molecular difference observed between phenotypic states without independent functional or fitness-relevant validation (Gould and Lewontin 1979). To clarify this distinction, Table S1 summarizes studies cited in the review and classifies the type of evidence they provide with respect to causal inference. Across various regulatory mechanisms, most studies documented associations between the expression patterns of regulatory RNA and social or physiological traits. Only a limited subset included functional perturbation, and those experiments generally addressed specific physiological processes, such as ovary activation or egg laying, rather than evolutionary transitions related to eusociality.

Related concerns arise in the interpretation of circular RNA mechanisms. The review frequently frames circRNA function using microRNA sponging or competing endogenous RNA models. In most bee studies, such interpretations are based on predicted interactions and expression correlations rather than direct experimental validation (Thölken et al. 2019; Zhu et al. 2022). Quantitative analyses in other systems have demonstrated that effective microRNA sequestration requires stringent stoichiometric relationships and appropriate subcellular localization, conditions that are not commonly assessed in transcriptome-wide surveys (Denzler et al. 2014). Syntheses of circRNA biology emphasize that mechanistic generalizations should be approached with caution, as many circRNAs remain functionally uncharacterized (Olesen and Kristensen 2021; Liu and Chen 2022).

Similar considerations apply to long noncoding RNAs. Comparative studies indicate that many lncRNAs are weakly conserved, expressed at low levels, or exhibit context-dependent effects, with only a subset demonstrating clear organismal phenotypes following perturbation (Ulitsky 2016; Kopp and Mendell 2018). It has also been proposed that a substantial fraction of lncRNAs originates from transcriptional noise or lineage-specific regulatory experimentation rather than representing conserved functional elements (Palazzo and Koonin 2020). These observations do not preclude functional importance in specific cases, but they suggest the need for careful calibration of claims regarding broad evolutionary roles.

Similar limits apply to alternative splicing. Differences in isoform usage across castes or reproductive states are well-documented in bees and other social insects (Price et al. 2018). These patterns are consistent with a role for splicing in phenotypic plasticity. Demonstrating that alternative splicing events are necessary or sufficient for the origin or maintenance of eusocial traits requires experimental manipulation and evidence that splicing variation precedes, rather than follows, phenotypic divergence (Prud'homme et al. 2007; Wray 2007).

These observations do not detract from the contribution of the reviewed article. The cited literature is appropriate for documenting associations between regulatory processes and social phenotypes in bees. The primary issue concerns interpretation rather than the selection of evidence. A clearer distinction between molecular features associated with eusocial traits and those features demonstrated to causally influence these traits would strengthen interpretability and align conclusions with established standards of inference in evolutionary genomics. We offer these comments as part of an ongoing scientific dialogue on integrating regulatory genomics into explanations of social evolution. Incorporating experimental manipulation, replication across lineages, and fitness-relevant measures will be essential for clarifying the causal roles of regulatory RNAs in eusociality in future studies.

Supplementary Material

Supplementary material is available at *Genome Biology and Evolution* online.

Author Contributions

P.H. conceived and wrote the critique and performed the methodological analysis.

Conflict of Interest

The author declares no conflict of interest.

Data Availability

No new data were generated or analyzed in support of this research.

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