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From Genes to Function: Transcriptomic Analysis in Insect Studies

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Abstract
In recent times, the new wave of molecular biology has completely changed the way gene expression research is conducted. The value of such investigations is exemplified by recent discoveries of potential genes and pathways involved in development, morphology and biology of insects. Researchers anticipate that transcriptome data will continue to improve our knowledge of insect biology and insect tripartite interactions, particularly on non-model species. Furthermore, phenotype-biased gene expression raises a number of significant unsolved problems. These inquiries offer a variety of directions for further investigation into the study of eukaryotic organisms in general and insects in particular. Therefore, we anticipate a bright future in which the molecular foundation of phenotypic variation will be investigated through the expanding use of transcriptomics (RNA seq).

Introduction
With the recent sequencing revolution, an era of data collection and analysis has begun like nothing unparalleled before. Overall, the omics revolution has catapulted the study of gene function across all biological systems. Hundreds of published genomes are only making the genomics data more accessible and more valuable for comparative studies. Genome sequences are used to study the evolutionary aspects and genetic structure of insects. But to analyse the complete potential of a gene in temporal and spatial manner requires the transcriptome of the insect. When there are multiple isoforms are formed from a gene due to alternative splicing, to find out which one is more common and when, transcriptome is essential. The bioinformatic analyses paired with transcriptome data help us identify different types of RNA, enhances, promoters, transcription factor binding sites, resistant genes and their functions and much more.

The Past till RNA seq: The earliest methods used for transcripts was northern blotting (Alwine *et al*., 1977) and its modification, endonuclease protection assays (Friedberg *et al*., 1990). Yet trancriptome profiling was only possible after two decades, thanks to expressed sequence tags (ESTs). Being first used on human cells, EST was later applied for insects in transcriptomics of *Toxoptera citricida* (brown citrus aphids) (Hunter *et al*., 2003) and *Bemisia tabaci* (whitefly) (Leshkowitz *et al*., 2006). Then the sequencing load problem in EST was overcome using Serial Analysis of Gene Expression (SAGE) (Velculescu *et al*., 1995) and Cap Analysis Gene Expression (CAGE) techniques (Shiraki *et al*., 2003). The extensive labour and economic costs involved in these techniques left a room for innovation to Stanford researchers who came up with first microarray to quantify parallelly and compare the transcript quantity between samples (Schena *et al*., 1995).

Still there existed the problem of low-level transcript generation, which got resolved in 2000s by the advent of cheap, highly parallel, high- throughput RNA sequencing that

detected rare transcripts in accurate manner (Zhao *et al*., 2014). These technologies basically sequence the cDNA from the mRNA. Further using softwares, the reads are mapped to reference genomes or assembled de novo if no reference is available. Obstacles like sequencing errors and repetitive regions or splice variants can hamper these pipelines (Geniza and Jaiswal, 2017). The end data is used for quantifying the number of transcripts per gene, differential gene expression between samples, finding new splice variants or transcription start sites, phylogenetic analysis etc.

The Present of RNA seq: The short read sequencing Illumina technology by Illumina company has been ruling since ages. As it yields 300 bp, the RNA transcripts are fragmented and then sequenced (Adams *et al*., 2000). But advanced technologies like PacBio and Oxford Nanopore sequencing have been promising long reads from single molecules, although at present higher error rate and consistency have been a concern (Garalde *et al*., 2018).
In insect sciences, transcriptomics has been aiding in unravelling several aspects like follows:

Development and morphology: In order to comparatively characterize the spinnerets and Filippi's glands of the silkworm for their specific functions, the mRNA was profiled by Wang *et al*. (2016). They found that filippi's gland is the major supporter of silk production in spinnerets via ion and amino acid transportation.

Forensic entomology: Blowfly that is used for solving crime cases, had ambiguity in identifying the age of larvae using only the DNA. But using transcriptomics, 15 promising life stage specific markers were designed that made accurate prediction of blowfly larvae age possible which is not dependent on any ecological factors (Zajac *et al*., 2018).

Phenotypic plasticity: Researchers have used transcriptomics to study this phenomenon in a wide range of arthropods, such as moths, mosquitoes, Drosophila spp., and mites (Kang *et al*., 2016; Shearer *et al*., 2016; Zhao *et al*., 2017; Deng *et al*., 2018). Cast differentiation in Honey bees was ruled out using RNA seq (Hartfelder *et al*., 2018), and the key genes have been identified in the development of soldier castes of subterranean termites (Wu *et al*., 2018). In case of the devastative and voracious pest, the swarming Locust, *Locusta migratoria*, the switching from solitary to swarming morph was studied and key regulatory genes were discovered using transcriptomics that would help in targeted management of locust in future (Wang *et al*., 2014).

Host switching plasticity: Host related interactions and invasive pests is another important area complimented by RNA seq in recent times. To study the host switching and the drive behind this mechanism Rivera- Vega *et al*. (2017) conducted an RNA seq analysis on salivary glands of 5th instar of Cabbage looper, *Trichoplusia ni*, raised on either live cabbage (preferred host), live tomato (non-preferred), or artificial pinto bean diet (preferred). Based on the availability of plant genomes, assembly was carried out and it was found after BLAST that only 4% of total transcripts were differentially regulated between loopers reared on either artificial or cabbage diet, 18% of the transcriptome was differentially regulated between loopers reared on either cabbage or tomato, proving that the salivary gland transcriptome is highly plastic. These included transcripts related to immunity, digestion, and de toxification. Andino *et al*. (2016) using transcriptomics ruled out that *Varrao jacobsoni* had

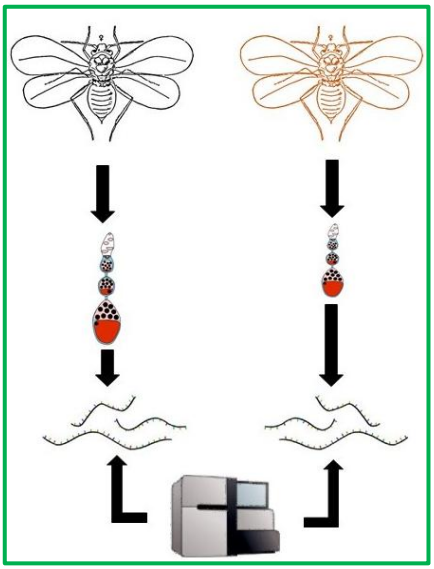

Fig: An overview of comparative transcriptomics of insect/biotypes/life stages/stress dependent insects

differentially expressed 246 genes when it attacked *Apis mellifera* compared to *A. cerana*.

Phylogenomics: Till trancriptomics, only the mitochondrial cytochrome oxidase subunit 1 (CO1) gene using sanger sequencing was used for DNA barcoding and further phylogenetic studies of insects since 1980 (Hebert *et al*., 2004; Smith *et al*., 2007). To solve this, 102 insect transcriptomes of insects from all the orders were sequenced for transcriptome and this vast amount of data was used to target out 1478 genes that gave phylogenetic tree of all the insects till then (Misof *et al*., 2014). Several theories on evolution of flight during the Devonian period and previous phylogenetic groups were supported by this data.

Caste differentiation in social insects: Researchers discovered that a transcriptional repressor, Kr-h1, regulated by juvenile hormone and ecdysone were crucial in suppressing the genes that were unsuitable for each caste in *Harpegnathos salator* ants (Gospocic *et al*.,2021). Differentially expressed genes in *Polistes dominula* between castes tend to be commonly connected with metabolism and are regularly biased towards workers, demonstrating the variety of responsibilities that workers play in societies (Standage *et al*., 2016). According to research, chemosensing is a crucial physiological activity that is distinct to a caste. OBP16 and CSP4, two chemosensory genes in A. mellifera, were found to be potential queen-rearing genes (Wu *et al*., 2019).

In recent years, applying single-cell transcriptomics to a range of insects from *Drosophila* spp. to *Bombyx mori* and 10 other species, has yielded many new insights on the evolution and development of insect nervous systems, offering a path forward for dissecting the neurological underpinnings underlying the diverse behaviours exhibited by insects (Liu and Li, 2024).

Conclusion

Although significant progress is being made in understanding the morphology, evolution, and development of insects, much more progress in knowledge is anticipated in the future due to technological advancements and innovation. Both today and in the near future, long molecule sequencing will make it possible to identify isoforms and improve read mapping. The RNA-seq bio informatic analysis procedures, which are currently a significant bottleneck in many of these investigations, will be shortened by these new technologies. With the development of single-cell RNA sequencing technologies, like 10X Genomics Chromium, RNA-seq resolution will continue to increase and unprecedented cell-type-specific transcriptome profiling will be possible without requiring labour-intensive hand dissections or preparatory work. Over time, these techniques will lead to a reduction in the amount of work required for sample preparation and bioinformatic pre-processing.

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