THE GENOMIC ERA OF INSECT TAXONOMY: BIOTECHNOLOGICAL ADVANCEMENTS

Abstract

Insects, characterized by their high diversity and adaptability, play a pivotal role in insect biotechnology, addressing challenges such as identification and pest control. The integration of DNA barcoding, specifically targeting the COI gene, expedites biodiversity documentation and overcomes traditional taxonomy challenges. Biotechnological methods, including PCR and DNA sequencing, revolutionize molecular systematics, allowing for comprehensive gene analysis and a deeper understanding of insect phylogeny. Moreover, Next-generation sequencing (NGS) has transformed molecular taxonomy by enabling rapid and cost-effective whole-genome and transcriptome sequencing, facilitating an in-depth exploration of genetic diversity and evolutionary relationships in insects. Biotechnological progress has also resulted in sophisticated software tools employing computational algorithms for phylogenetic analysis. Further, metagenomics has also enhanced insect phylogenies, providing a comprehensive understanding of their evolutionary history by integrating molecular data with morphological, ecological, and behavioural information. This approach also allows for the study of insect-associated microbial communities, offering insights into co-evolution and ecological relationships. The development of gene editing tools, such as CRISPR-Cas9, has enabled the study of specific gene functions in insect phylogeny, influencing evolutionary processes through expression manipulation or mutations. Insect biotechnology, utilizing insects for agriculture, industry, and human welfare, significantly impacts bio-insecticide efficacy, medicine synthesis, and the production of various chemicals and enzymes, advancing across agricultural,

Authors

Nishi Pandya
Department of Zoology
Faculty of Science
The Maharaja Sayajirao University of Baroda, Vadodara, India.

Parth Pandya
Department of Biomedical and Life Sciences
School of Science
Navrachana University
Vadodara, India.

Pragna Parikh
Department of Zoology
Faculty of Science
The Maharaja Sayajirao University of Baroda, Vadodara, India.
php59@yahoo.co.in

medical, and industrial sectors. Thus, genetically modified (GM) insects, created by introducing new genes offer potential benefits in population suppression and replacement strategies for pest control, providing targeted solutions while minimizing harm to beneficial insects and reducing reliance on harmful insecticides.

Keywords: Genomic Era, Taxonomy, DNA Barcoding.

I. INTRODUCTION

Biotechnology has long been ingrained in human civilization (Montagu, 2019). The potential of biotechnology has increased dramatically with the introduction of recombinant DNA, sparking the curiosity of both scientists and laymen (Glick and Patten, 2022). Biotechnology is a set of techniques by which humans transform living organisms or utilize them as tools (Lellis et al., 2019). Modern biotechnology makes use of molecular biology methods to comprehend and work with the fundamental constituents of living things. However, biotechnology in the past has included the selective breeding of organisms to improve their nutritional value. It has long been a practice of using living organisms and their by-products to produce goods that benefit humans or other animal species, such as food, drinks, and medication (Siad and Bouzid, 2023). They also play a significant role in the conservation of biodiversity by enabling the vegetative multiplication of several species, enabling the synthesis of a huge number of plants by utilizing small fragments of the stock plant in a fairly short period, and assisting the recovery of virus-free plants (Niazian, 2019). Additionally, they may be used in the production of somatic hybrids, organelles, cytoplasm transfer, genetic modification, and cryopreservation (Bhatia, 2015).

II. DNA BARCODING

In terms of diversity and adaptability, insects are the most successful species on Earth. An emerging field of biotechnology with many claims is insect biotechnology, which uses insect resources. Numerous topics in the insect research field, including insect identification, insect genetic relationships, and insect (pest) control, have been studied using biotechnological methods. There is a need for methods to: (1) partially automate the initial screening and separation of insect species and (2) develop complementary and collaborative methods to improve the performance of current practices for the delineation and identification of closely related and cryptic species. This is required because taxonomic expertise for diverse and complex insect groups is scarce. Integrated taxonomy combines many data types for species identification and has gained widespread acceptance in contemporary taxonomy (Miraldo et al., 2013; Miraldo et al., 2014). Molecular techniques are widely used as part of integrative taxonomy, including DNA barcoding, using additional mitochondrial DNA regions, and nuclear genes (Alex Smith et al., 2013; Germain et al., 2013; Yang et al., 2014). Numerous taxonomic studies have integrated ecological observations into analysis, including host selection, geographical distribution, biochemical characteristics, ecological niche modeling, cross-breeding analysis, and sound production (Famah Sourassou et al., 2012; Miraldo et al., 2014). Aphrodes (Hemiptera: Cicadellidae), a genus of leafhoppers, has four obscure species, which were confirmed by Bluemel et al. (2014) as behaviorally, genetically, and morphologically distinct. Over the past 200 years, traditional taxonomy has been crucial for the identification of more than 1.4 million insect species worldwide. However, the pace at which we are progressing in this area is insufficient to fully document the biota before it becomes extinct. Therefore, novel technologies led by DNA barcoding have gained momentum in documenting biodiversity rapidly and economically. India is a mega-diverse country that contributes substantially to achieving the United Nations' Sustainable Development Goals (SDGs) and their targets. However, this review highlights the current scenario of DNA barcoding in India, where only 3.97% of the known species of insects have been collected, which is capable of leaving behind us in documenting our rich biodiversity. Currently, a growing interest in using image technologies for classifying species has led to

the use of integrated taxonomic analysis, which can significantly accelerate the assessment of biodiversity and identification of novel species, characteristics, and connections between species (Nansen and Elliot, 2015; Nansen and Elliot, 2016).

Biotechnology plays a crucial role in advancing insect phylogeny and molecular taxonomy by providing powerful tools and techniques for analyzing genetic data. When morphological characteristics cannot be distinguished between species, DNA barcoding is used for identification (e.g., field-collected mosquito specimens) based on the pattern of nucleotide organization in a DNA fragment of the known and undiscovered species (Kumar et al., 2007). In light of the present biodiversity issue, several researchers have suggested using DNA barcoding in taxonomy to rapidly describe species (Ball and Armstrong, 2006). In DNA barcoding, a brief, standardized DNA sequence (in insects, a 720 base pair (bp) fragment of the mitochondrial cytochrome c oxidase (COI component I (gene)) was employed to identify and classify unidentified specimens as well as to aid in the discovery of new species. Hard-core taxonomists and graduate molecular biologists alike accept this tool on a global scale.

Recently, mtDNA studies have become more popular for determining the genetic variations and structure of populations. It plays an important role in the genetic makeup of populations and variation due to its rapid rate of evolution, maternal inheritance, lack of intermolecular genetic recombination. Mitochondrial markers are more prone to genetic drift than are nuclear markers (Filipova et al., 2011). COI, a protein-coding gene in mtDNA, is highly competent for species identification, and its universal primer (720-bp) can be directly sequenced for all animal phyla that were formerly designed for marine invertebrates (Folmer et al., 1994). As it is a protein-coding area, their alignment technique is not difficult, and errors can be identified by determining whether the resultant sequence can be translated. Hence, it is now commonly employed as a genetic marker for population genetic investigations, especially intraspecific analysis, because of its rapid evolution, high polymorphism, simplicity in amplification, and information-rich sequencing (Xu et al., 2011).

DNA barcoding is used by both specialists and non-specialists as a tool for species identification. Biotechnology has enabled the development and application of DNA barcoding as a rapid and accurate method of species identification. By analyzing short and standardized DNA regions, researchers can quickly identify insect species and assess their relationships within a broader phylogenetic context. Before being deposited in BOLD, species must be taxonomically characterized to meet the CBOL objectives, which aids in the early resolution of analytical, technological, and fundamental problems. Precise taxonomic identification of a wide range of species can be aided by additional access to a public reference database of taxa. DNA barcoding is a 'formidable technology' that can be used in conjunction with taxonomy, molecular phylogenetics, and population genetics to accelerate the discovery and description of new species (Hajibabaei et al., 2007).

According to Miller (2007), the advent of DNA barcodes is a taxonomic renaissance. Barcoding has helped to speed up identification with more accessibility to the possible extent, even in the field, via an internet-content search engine such as Google. Taxonomists are the primary "developers" of these databases, because they curate data by connecting names to DNA sequences based on the analysis of voucher specimens and creating reference sequences that serve as the foundation for DNA barcoding. However, it is acknowledged as a barrier in

barcoding logistics (Borisenko et al., 2009); taxonomists usually do not receive compensation or are given the credit they deserve for their labor. Even though sequencing of every living species would be a monumental and nearly impossible undertaking, scientists are progressively utilizing and expanding the data pertaining to these markers, and major databases are expanding tremendously. In the GenBank database, DNA barcoding alone (COI) attained 2.5 million sequences in 2018 (Porter and Hajibabaei, 2018), whereas BOLD attained 11 million COI sequences in 2022 (BOLD, 2022), representing approximately 385,000 described species, and serving as a valuable scientific data source. The two primary publicly accessible databases for DNA barcoding, BOLD and GenBank, are significantly different in that BOLD can be regarded as a sequence curation tool, whereas GenBank is a library (Meiklejohn et al., 2019).

DNA barcodes support a variety of scientific fields (such as conservation biology and evolutionary biology), assist in recognizing, detecting, and tracing the dispersal of patented organisms in biotechnology, and ensure the retention of intellectual property rights for bioresources (such as truffles, Rastogi et al., 2007). This allows for the rapid retrieval of molecular information. However, there is a significant disparity in morphological tools; therefore, it can take time along with, in some cases, complete mystification (for example, earthworms, Huang et al., 2007). Despite its limitations, DNA barcoding has been shown to be successful in distinguishing between species and taxa and in detecting cryptic species. By utilizing genotypic differences, repeated use of pesticides has altered and developed genetic resistance.

The study of molecular systematics is changing drastically. With the availability of molecular data, there has been a conceptual shift in how these data are handled within a taxonomic or systematic framework. Since the development of next-generation sequencing technologies, most phylogenetic datasets for insects have included hundreds or thousands of genes, as opposed to one or two major gene sequences in the past. Biotechnological methods such as Polymerase Chain Reaction (PCR) and DNA sequencing are instrumental in generating molecular data from insects. These techniques allow for the identification of DNA sequences from specific genes or genomic regions that serve as markers for phylogenetic analysis.

III. HIGH THROUGHPUT SEQUENCING

For almost three decades, targeted nucleotide sequencing has dominated the field of insect taxonomy. First-generation sequencing or Sanger sequencing was the first widely used application of this method (Sanger et al., 1977). Using conserved primer areas and first-generation sequencing technology, this approach amplifies specific DNA sections, which are then individually sequenced (Faircloth et al., 2012). Next-generation sequencing (NGS), sometimes referred to as massively parallel sequencing or high-throughput sequencing, has gained popularity over the past five years as a replacement for conventional Sanger sequencing. Platform selection, marker creation, NGS generation, assembly, ortholog identification, gene alignment, and analysis constitute the overall workflow of an NGS project. In a single sequencing run, NGS simultaneously reads libraries of tagged DNA templates and produces millions of base pairs of data, which are subsequently analyzed for quality control, assembly, and analysis in accordance with the research objective. Compared with Sanger sequencing, the time commitment and cost per data fragment have been

significantly reduced, allowing access to enormous amounts of genomic data distributed throughout the genome, even for non-model organisms (Ekblom and Galindo, 2011; Carstens et al., 2012).

Three key methods have emerged as a result of the phylogenetic emphasis on taxonomy: whole-genome sequencing (WGS), mitochondrial genome sequencing, and genomic partitioning. As the name suggests, WGS results in the entire genome sequencing of an organism and yields a large amount of data. Despite recent technological advancements, high-quality WGS is still nontrivial, expensive, and time-consuming (Ekblom and Wolf, 2014; Xia et al., 2014). A fraction of the complete genome is sampled and carried out using PCR-based NGS platforms (Lemmon and Lemmon, 2013). Whole-mitochondria sequencing has advanced beyond WGS in insects and has become a significant source of data for phylogenomic analysis (Cameron, 2014). Targeted amplicon sequencing, reduced-representation libraries, hybrid enrichment, transcriptome sequencing, and proteomics are the five main partitioning techniques that can be used. Lemmon and Lemmon (2013) explain an excellent evaluation of the platforms and approaches encompassed by NGS in the context of systematics and taxonomy.

The effectiveness of molecular markers and their open-access databases depends on the accurate mapping of names to gene sequences (Schoch et al., 2020). Public databases and the use of DNA markers in taxonomy have facilitated the fusion of experimental sciences with natural history practices (Strasser, 2008). Recent genome-scale investigations based on next-generation sequencing (NGS) have revolutionized taxonomy by enabling highly accurate differentiation of species and reconstructing phylogenies with great support. These methods assist in lowering the cost of sequencing for each sample in the study of species with large genomes. The diversity in insect genome size is astounding, with some of the shortest (chironomid midges, 68 Mbp) and largest (16,560 Mbp) among all metazoans. For insects with smaller genomes (1,000 Mbp), sequencing the entire genome and using bioinformatics techniques to create a dataset for phylogenomics may actually be more effective in terms of up-front locus development time, wet laboratory time, computation time, and overall cost (Cornette et al., 2015).

Next-generation sequencing (NGS) technologies have revolutionized molecular taxonomy by enabling the rapid and cost-effective sequencing of entire genomes and transcriptomes. Collaboration among taxonomists, molecular biologists, and applied entomologists has suffered significantly as a result of this shift. NGS facilitates the exploration of genetic diversity within insect populations, and can reveal evolutionary relationships at a much deeper level. The ultimate goal of insect systematics is to make full genomes (nuclear and mitochondrial) available for investigation, even though next-generation sequencing has enabled a rapid rise in the number of studies employing entire mitochondrial genome sequences for phylogenetics in insects. In addition, biotechnology facilitates the comparison of entire genomes or specific genetic regions across different insect species. Comparative genomics helps to identify conserved genes, genomic rearrangements, and functional elements, shedding light on the evolutionary processes that shape insect diversity.

IV. PHYLOGENETIC ANALYSIS

Biotechnological advancements have led to the development of sophisticated software tools for phylogenetic analysis. These tools use computational algorithms to reconstruct evolutionary relationships among insects based on molecular sequence data, thereby providing insights into their evolutionary history and diversification. The importance of selecting appropriate software tools depends on several factors, including research objectives, dataset size, and the type of molecular data being analyzed (DNA, RNA, or protein). Techniques, such as RNA sequencing (RNA-seq) and mass spectrometry enable the study of gene expression patterns and protein profiles across different insect taxa. This information provides valuable insight into the functional aspects of insect evolution.

Molecular phylogeny, which aims to understand the evolutionary relationships among insects using molecular data, relies on various bioinformatic tools and software to analyze DNA or protein sequences. These are commonly used in various steps in the process, from sequence alignment to phylogenetic tree reconstruction. The software specifically used for constructing phylogenetic models includes 1) Molecular Evolutionary Genetics Analysis (MEGA), a comprehensive software that offers multiple algorithms for phylogenetic tree construction, including the maximum likelihood and neighbor-joining methods. 2) PhyML is a popular software tool for constructing phylogenetic trees using maximum likelihood (ML) methods. This allows researchers to estimate branch lengths and model parameters for the tree based on the likelihood of the observed sequence data. 3) Randomized Axelerated Maximum Likelihood (RAxML) is widely used for maximum likelihood-based phylogenetic tree inferences. It is particularly useful for large datasets owing to its efficiency and parallel-computing capabilities. 4) MrBayes, a Bayesian inference software commonly used for building phylogenetic trees, estimates posterior probabilities of phylogenetic trees using a Markov chain Monte Carlo (MCMC) approach. 5) BEAST (Bayesian Evolutionary Analysis Sampling Trees (BEAST), a software tool specifically designed for Bayesian phylogenetic analysis. This allows researchers to estimate the divergence times and other evolutionary parameters. 6) IQ-TREE is versatile software for phylogenetic analysis that includes maximum likelihood methods with ultrafast bootstrap approximation, model selection, and Bayesian analysis capabilities. 7) Garli (Genetic Algorithm for Rapid Likelihood Inference (Garli) is a software tool that uses a genetic algorithm to optimize the likelihood function for phylogenetic tree estimation. 8) FastTree is designed for rapid and large-scale phylogenetic tree construction using methods that approximate the maximum likelihood, making it useful for large datasets. 9) Phylogenetic Analysis Using Parsimony (PAUP*) tool uses parsimony methods to reconstruct phylogenetic trees based on character-state changes. 10) PHYML-MPI is a parallel version of PHYML that allows faster phylogenetic tree reconstruction by distributing computations across multiple processors or computer nodes.

V. METAGENOMICS

It enhances the resolution and accuracy of insect phylogenies and provide a more comprehensive understanding of their evolutionary history. Biotechnology enables the integration of molecular data with other types of data such as morphological, ecological, and behavioural data. In addition, metagenomics allows for the study of entire microbial communities associated with insects. Understanding insect microbiomes can offer insights into the co-evolution and ecological relationships between insects and their symbiotic

microbes. The term "meta-omics" currently refers to a defined group of techniques used to characterize communities of organisms, including meta-genomics, meta-transcriptomics, meta-proteomics, and metabolomics (Peršoh, 2015). The pool of genomes in a sample can be identified with the help of metagenomic methods; in reality, the metagenome is a complex of all the genomes of the species present in a given sample. Montero et al., (2016) used a short nucleotide fragment known as a barcode (e.g., 16S, 18S, ITS, COI) as a stand-in for identification and seeks to reconstruct the taxonomic structure of the biological communities in a specific sample. Both metagenomic and metabarcoding methods are helpful for determining the relative taxonomic abundance and the existence of particular genes in a sample as well as for qualitatively assessing the diversity of organisms present. However, these methods only reveal the presence of certain individuals (taxonomic reconstruction) and the possible activity of certain genes.

Additionally, tools like PICRUSt for bacteria or FUNGuild for fungi can be used to forecast the likely functional role of rebuilt communities (Jia et al., 2021; Guo et al., 2023). A metatranscriptomic technique can also be used to reconstruct taxa which will also help in revealing the genes expressed by a particular community (Tozkar et al., 2015). Therefore, in addition to knowing about the species inhabiting the sample, particularly the symbiotic organisms in the guts of insects, as well as investigating the variety of insect gut microbial communities, three main molecular methods have been used to find new genes and look into their genetic makeup. These approaches include Gene-targeting PCR, molecular fingerprinting methods like denaturing gradient gel electrophoresis (DGGE), and oligonucleotide probe-based hybridization methods like fluorescence in situ hybridization (FISH). However, meta-proteomics and metabolomics help in further understanding of the functions of microbial communities as the changes in gene expression are not always followed by phenotypical responses (Mallick et al., 2019; Bhosle et al., 2022).

VI. GENOME EDITING

In the modern era, the development of gene editing tools like CRISPR-Cas9 has opened novel opportunities for studying the functional significance of specific genes in insect phylogeny. The impact on evolutionary processes can be assessed by manipulating gene expression or by introducing mutations. Insect biotechnology uses insects and their derivatives in agriculture, industry, and human welfare. It significantly influences the effectiveness and affordability of bio insecticides. It is also utilized to synthesize medicines, microbial pesticides, and many other chemicals in addition to producing commercial enzymes like chitinases and cellulases. Lepidopteran cells in particular exhibit an aggressive approach to mammalian cells particularly when it comes to producing biotechnological products by post-translationally altering proteins. Within 34 years of complying with the introduction of genetic engineering of the Bacillus thuringiensis delta-endotoxin-producing gene against the pest of the cotton plant, insect biotechnology (also known as yellow biotechnology) has emerged. Insect biotechnology advances are essential to creating goods or services for human use in the agricultural (green biotechnology), medical (red biotechnology), and industrial (white biotechnology) sectors.

Genetically modified (GM) insects are produced by adding new genes to their DNA. There are numerous genes known to affect insects' biology and behaviour. These genes are known as transgenes when they are put into an insect's genome, and the insect is then referred

to as transgenic or genetically altered. Transgenes are introduced into the insect genome using short randomly inserted DNA sequences. They can be created using genetically modified strains with intricate arrangements of transgenes by inserting DNA containing the necessary genes into the eggs of insects, which is further detected by using marker genes to induce fluorescence, to distinguish them from unmodified varieties. In these GM insects, refractory genes offer resistance to a specific pathogen, preventing the spread of disease, while lethal genes cause insects to die or prevent them from reproducing.

For the deployment of GM insects, scientists have suggested two different strategies: population suppression and population replacement. GM insect population suppression is accomplished by developing GM insects that carry a fatal gene that, when mated with a wild insect gets silenced before being released and is passed on to the progeny, killing them. The insect population in the region would be wiped out if enough GM males were released to overwhelm the wild females. Due to the fact that harmful genes are intended to eradicate entire generations, the majority of suppression tactics are self-contained. Population replacement strategies use GM insects that have been modified to make them less capable of spreading illness and replace wild populations of insects permanently. In order to achieve this, a technique known as a "gene drive" can be used in addition to a genetic engineering system to give the insects the needed traits, which ensures the transmission to more than half of the progeny, thus overtime spreads throughout the population and eventually displaces the undesirable gene. Being a self-replicating process, fewer GM individuals are released in order to start the procedure of replacement.

The potential advantages of GM insect strategies are apparent as a tool to supplement current control techniques. Thus, GM insects have been suggested to have several distinctive advantages, specifically targeting a harmful insect pest while leaving beneficial insects unharmed, scientists have developed genetically modified insects that can take advantage of the natural tendency of insects to find and mate with each other. This method can effectively eliminate pest populations that are difficult to reach with conventional control methods, reducing the need for harmful insecticides and their residues in the environment. Furthermore, when used in disease control programs, GM insects can protect everyone in the release area, regardless of their socio-economic status, and require less community involvement, making them less vulnerable to the failure of individuals to participate in the program.

Overall, biotechnology has revolutionized insect phylogeny and molecular taxonomy by providing tools to study genetic diversity, evolutionary relationships, and functional aspects of insects. These advancements provide a deep understanding of insect evolution and the implications for biodiversity conservation, and pest management, as well as an understanding of the ecological roles of insects in ecosystems. Besides, biotechnology has also helped in modifying genomes which has an application in integrated pest management.

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