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Chapter- 26

Environmental DNA: an Emerging Sustainable Tool for Ecological Monitoring

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Abstract:

One can extract DNA from any environmental sample irrespective of the organism i.e., Soil, Water, Air. This DNA is identified as environmental DNA or eDNA. The application of the novel eDNA approaches, particularly NGS techniques, has evolved biodiversity surveys taking into account both the budget and the time. eDNA has revolutionized our thinking about biogeography. Results obtained from eDNA approaches have given some crucial insights into the study of ancient environments that are useful in the sustainable management of contemporary biodiversity in aquatic and terrestrial ecosystems. Advancements in eDNA technologies also enhance the knowledge of molecular ecology and make it possible to answer different ecological questions by using genetic methods.

Introduction:

Depletion in biodiversity is one of the most important concerning issues in the 21st century (Butchart et al., 2010). Anthropogenic disturbances are the main cause behind this worldwide depletion (Barnosky et al., 2011; Dirzo et al., 2014). Biodiversity loss has a great negative impact on human health and the sustainability of our planet (Diaz et al., 2014). Our knowledge about biodiversity is still incomplete or even undescribed for various taxa and geographical realms (Vié et al., 2009). Some International political agreements have also been made to pause the current loss in biodiversity (UNEP, 2011). However, all such efforts to save biodiversity exclusively depend on biological monitoring to acquire precise data on species distributions and population size on a particular ecological time scale. Physical identification can monitor species (viz., visual surveys, counting the number of similar species in a particular area) but this monitoring technique leads to some confusion due to the phenotypic plasticity and close similarity in related species. Thus, there are some species data flaws with errors (Daan, 2001; Sharfuddin et al., 2023). Moreover, different traditional techniques are supposed to be invasive to the studying species or ecosystem (Jones, 1992). Furthermore, morphological identification strongly depends on taxonomic expertise, which is seldom unavailable (Hopkins and

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Freckleton, 2002; Wheeler et al., 2004). All these limitations of traditional biodiversity monitoring techniques demand an alternative approach, one of them is eDNA technology. eDNA technology has a strong potential to combat many of these challenges associated with biodiversity monitoring (Baird and Hajibabaei, 2012; Kelly et al., 2014).

eDNA is used to refer to DNA extracted from environmental samples (Barnes and Turner, 2016a). eDNA can also originate from skin, saliva, mucus, sperm, secretions, eggs, faeces, urine, blood, roots, leaves, fruit, pollen and decayed bodies of larger organisms including entire microorganisms (Bohmann et al., 2017; Barnes and Turner, 2016b). Hence, eDNA is a mix of nuclear, mitochondrial and chloroplast DNA from various organisms (Taberlet et al., 2012). It enables the detection of any life-stage species and from both sexes. eDNA can be sampled from dead organisms before decomposition.

Scientists have highlighted the fact that eDNA derives not just from microorganisms but from a wide range of plants as well as vertebrates. Many ancient flora and fauna have left their extrachromosomal DNA traces in the sediments instead of fossilization (Pal et al., 2017; Bashar et al., 2022). DNA traces from woolly mammoth and moa birds (both are extinct) were found in sediments from Siberia and New Zealand (Willerslev et al., 2003). Modern plant DNA can be recovered from the surface soil. At the same time, another team has successfully sequenced DNA from extinct giant ground sloth and other Pleistocene animals from a dry cave in the Southwest US (Hofreiter M et al., 2003). Furthermore, it is shown that eDNA data and other proxies such as pollen, macrofossils, living mammals and plants seem to complement each other demonstrating a range of species that is wider than achieved by using the methods separately (Pawłowska et al., 2014). Hence, eDNA should be regarded as a supplementary, not a replacement, method of analysis of more orthodox environmental proxies. In this, we provided a simple description of eDNA so as to remove the distinction between various forms of DNA in fact, in contrast to the community DNA (Deiner et al., 2017). Also the separation of eDNA and community DNA is very fundamental as the eDNA might be from a different location or predator faeces or from the past presence and the community DNA points to organism presence at a certain time and location (Creer et al., 2016; Deiner et al., 2017). This chapter assumes for simplicity that eDNA is collectively regarded as including many sectors of DNA biodiversity research that involve faecal analysis and bulk samples when they apply to biodiversity research and ecosystem analysis.

Methods used in eDNA research:

DNA barcoding approach is used in eDNA research, in which the sequence of the mitochondrial cytochrome oxidase 1 (COI) gene is used as a marker. eDNA fragments are usually shorter (about 100bp) and sequences of mitochondrial, chloroplast or ribosomal RNA genes, aside from COI are used in the analysis (Diaz-Ferguson et al., 2014). For unknown taxa, target sequences are generally grouped by so-called molecular operational taxonomic units ($MTOU_S$).

Sampling:

Most environmental samples contain a very low number of endogenous DNA molecules along with some contamination. eDNA can be extracted from a variety of sources viz. ice and permafrost, lake sediments, stagnant water etc. (Pederson et al., 2015; Creer et al., 2016) so, contamination remains one of the greatest experimental challenges to DNA research. Due to the variable collection point sampling methods for eDNA studies are also variable. The method of sampling and the volume and the number of samples taken depends not only on the type of substrate but also on the specificity of the taxa of interest and the environmental heterogeneity (Ruppert et al., 2019; Creer et al., 2016). Negative control samples are also required to overcome the contamination problem. Samples are stored at –20°C, in 100% ethanol or a cell lysis buffer for further use (Pinakhina et al., 2020)

DNA extraction from environmental sample:

Unbiased extraction from environmental samples demands a great effort as it contains a high level of biological complexity. To be able to extract DNA from the samples with equal efficiency invariable seems as theory, because of the variety of sample types. None of the generic extraction methods yield uniform performance across all environments and taxonomic groups (Pont et al., 2018; Cowart et al., 2018; Garlapati et al., 2019). Despite the fact that a large number of commercial and custom extraction protocols were modified for handling different combinations of sample types and organisms. Some of them are generics and have been applied for the eDNA studies in lakes, ancient sediments, and ice (Cristescu et al., 2018; Barnes et al., 2014; Erickson et al., 2016; Seymour et al., 2018) but increasing the knowledge of extraction bias will be appreciated.

Primer Designing:

The most critical part of eDNA metabarcoding research is primer designing. Typically, COI for metazoa and Ribulose bisphosphate carboxylase large chain (rcbL) for plants are used as the standard choice but 12s and 16s ribosomal RNA are also observed to be used in different taxa (. A good primer for eDNA metabarcoding should be short enough to amplify the degraded DNA samples, identical within but variable between species, with highly conserved regions to amplify as many species as possible without compromising the primer specificity to the target group (Epp et al., 2012) The most common sequencing platform in present days eDNA metabarcoding is Illumina (Jarman et al., 2018). Third-generation sequencing technologies have as well been used.

Bioinformatic analysis:

The end of the eDNA research is a bioinformatic analysis of the resulting data. Due to technological development, a large amount of data has been produced which required several

programs for analysis as carriage provided by Alberdi et al. (2018). It consists of millions of reads which explain the genetic code of every strand of DNA that has been sequenced. These reads are aggregated in OTUs. OTUs were employed for the distinction of species/taxa via sequence similarity, but the traits of taxa such as ecological and physiological also need to be coupled with OTUs to get a key to identify them. Several programs are to enable this process, of inter-population, however, variation impedes them (Coissac et al., 2012; Cristescu, 2014; Deiner et al., 2017). OTU clustering is based on the similarity to a certain sequence and then grouping under similarity cutoffs, with 97–99% typically as a cutoff range.

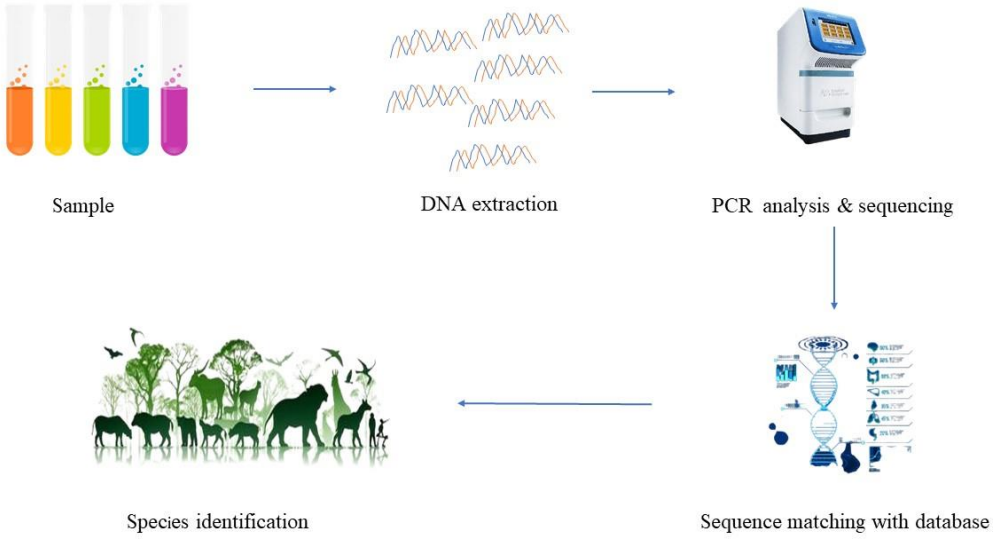

Figure 1. To identify species using environmental DNA (eDNA), samples are first collected from the environment, such as water, soil, or faeces. Subsequently, eDNA from organisms in each sample is extracted. The DNA sequences obtained are then multiplied through polymerase chain reaction (PCR) to ensure an adequate amount for analysis. Following amplification, the sequences are read on a sequencing machine, revealing the order of bases on the DNA strands. Finally, these sequences are compared and matched to known sequences in worldwide databases, facilitating the identification of the specific species present in the environmental samples.

Category of eDNA research:

eDNA research can be categorised into two main groups: targeted (species-specific) and multi-targeted (community) (Simmons et al., 2016).

Targeted (species-specific) eDNA research:

eDNA approach was highly successful in identifying a particular species, even in low abundance (Rees et al., 2014). With that in mind, specific primers were designed and only DNA of designated species was amplified to determine whether the species was present in the environment.

eDNA sample collected from aquatic habitats is rather homogenous and is usually perceived

to reflect the diversity of species residing in and around the sampled habitat (Cristescu et al., 2018). The DNA can also be used to detect terrestrial organisms from water samples, for example, the DNA fragments of terrestrial organisms enter aquatic systems when they drink water (Rodgers and Mock, 2015)) or move through water (Ushio et al., 2017). DNA from extinct and extant mammals, birds and plants has been detected in soil/sediments or dry cave sediments reported by Hofreiter and Rompler (2010). Species-specific monitoring offers further information concerning a species, save for this purpose, which is species detection. Hence, it improves the comprehension of ecological and evolutionary effects resulting from environmental alternations (Giguet-Covex et al., 2014).

Multitargeted (community) eDNA research:

eDNA research is equally applicable in community monitoring as individual species monitoring. Nowadays researchers started the diagnosis of other species that they have ever used general PCR primers paired with cloning and Sanger sequencing (Minamoto et al., 2012) or high-throughput sequencing (HTS; Thomsen et al., 2012). Community monitoring at multiple targets (or metagenomics, metagenetics, metasystematics, or metabarcoding) is sometimes called multi-targeted (community) monitoring, or passive monitoring (Taberlet et al., 2012; Simmons et al., 2016). Much economic and effort are to have also because this study of using eDNA makes surveys of many species by one activity. For instance, one piece of research employed HTS to discriminate several earthworm species in soil samples and conjectured that the same technique could be applied to characterization of other soil-dwelling taxa. The multi-species monitoring capacity of eDNA makes it a promising tool for conservation biology (Yoccoz, 2012).

Application:

eDNA technology is widely used in ecosystem and biodiversity monitoring. This approach is truly relevant in several different environments both ancient and modern, terrestrial and aquatic. Here are some major applications of eDNA research:

Species monitoring:

The most explored field of eDNA research is its application in species monitoring. In addition to monitoring the target species and the whole community, the research on eDNA is also used in invasive species monitoring and monitoring of rare and endangered species. Besides the multitude of works on fish and amphibians, methodologies have been proposed for the identification of invasive freshwater mollusc species from Europe (Clusa et al., 2017), the Burmese python in Florida, and also the algae Codium fragile which can be traced back to Suringar and Hariot (1889) (Muha et al., 2019). The use of eDNA in the monitoring of rare species is as efficient as the eDNA monitoring programs that were accepted by environmental institutions. For instance, in 2014, Nature England, a non-governmental organization sponsored by the UK Department of

Environment, Food and Rural Affairs, approved the eDNA analysis protocol (Rees et al., 2014) for the detection of the crested newts Triturus cristatus, Laurenti, 1768, which is listed in the international Red Book.

Estimation of organism abundance:

Various studies have begun to explore the quantification of eDNA as a means of estimating organism abundance or biomass. For example, a study carried out in a Japanese lagoon suggests that the eDNA concentration of common carp is related to fish abundance (Takahara et al., 2012).

Population genetics and genomics:

Beyond presence/absence and abundance information, there is more information to be gained from eDNA surveys. eDNA research provides a great opportunity to study population genetics. Genetic analysis of eggshells, hair, faeces, feathers and other samples promotes advances in non-invasive genetic studies.

Functional genetics and genomics:

Remarkably low cost of biotechnology, mostly HTS, has allowed functional genomic analysis of relevant taxa that were previously limited to model systems (Steiner et al., 2013). Application in practice is for finding of adaptive or loci related to fitness, tracing the loci related to stress events and describing the molecular basis of inbreeding depression (Schwartz et al., 2007; Paige, 2010).

Control of the spread of the parasite:

EDNA technology is applicable in the containment of infectious parasitic invaders. Thus, a system has been developed for the detection and monitoring of *Schistosoma mansoni* by extracting eDNA from the water samples (Sengupta et al., 2019).

Detection of plant pathogen:

The application of eDNA method appears to be promising for the identification of plant pathogens in crops. Consequently, the Precision Biomonitoring campaign (Pinakhina et al., 2020) is a service for the detection not only of bacteria but also for fungi that belong to human health and plant threats represented in Cannabis samples.

Healthcare:

eDNA technology is employed in healthcare to detect fungi that can cause allergic reactions when their spores and mycelium fragments get airborne and hence become sources of infection. The deployment of metabarcoding can heat up the taxonomic coverage of fungi dwelling in the air by 10 times more than microscopy (Banchi et al., 2018). Tong et al. (2017) proved such analysis of fungal diversity in air samples in hospitals can be of importance in

providing for preventing potential infections and for the selection of the best decontamination procedures.

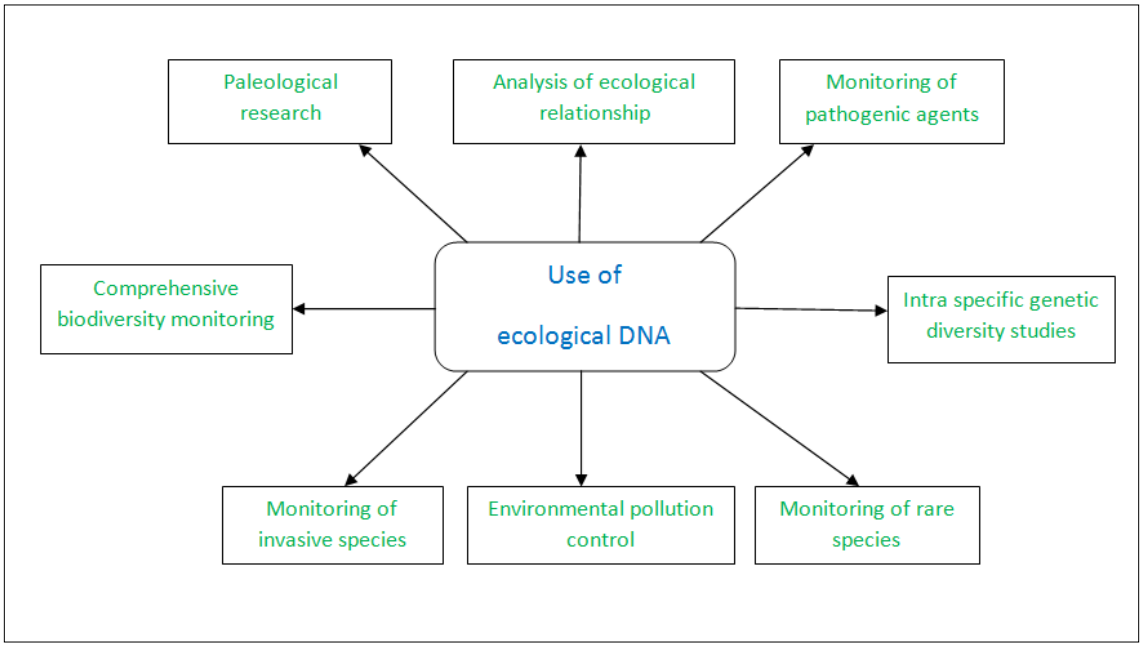

Figure 2. The main areas where eDNA studies are applied for sustainable monitoring.

Challenges:

Nowadays eDNA method is widely used in various aspects of biodiversity conservation. But this technology also faces some challenges viz. degradation of DNA, marker problem, contamination in the sample, inhibition of Taq polymerase by impurities present in eDNA samples etc.

Opportunities:

The evolution of DNA sequencing technologies has broadened eDNA use possibilities, with the advances to be expected in the future. While initial eDNA studies relied on clone-based subsequent Sanger sequencing of PCR products, the impact of new emerging sequencing techniques is obvious (Shokralla et al., 2012) and eDNA would be fully integrated into ecologist tools (Baird and Hajibabaei, 2012; Taberlet et al., 2012; Valentini et Besides, new generations of modern technologies including next generation sequencing techniques as PacBio RS invented by Pacific Bioscience or Nanopore-based sequencing by Oxford Nanopore Technologies, carbon nanotube chips (Mahon et al., 2011) and real-time laser transmission spectroscopy (Egan et al., 2013; Li et al., 2 Traditionally eDNA has been used in the specific context of species or communities to analyse single markers. But going forward, we will explode out into meta-genomic surveys of entire ecosystems with the goal of predicting spatial and temporal biodiversity patterns (Davies et al., 2012; Kelly et al., 2014). This is what we want ultimately - to apply the eDNA through the most holistic method for the sake of the planet and living creatures. Environmental DNA will only be handy in detecting biodiversity,

providing quick and quality assessments of species' present status, their distribution, abundance and the overall size of their population. These aspects suitably render conservation decisions. Thus, it will never involve direct action against the biodiversity crisis which so far is a more challenging problem demanding mostly political will, determination, and activity.

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