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Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity

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ABSTRACT

The continuous decline in Earth's biodiversity represents a major crisis and challenge for the 21st century, and there is international political agreement to slow down or halt this decline. The challenge is in large part impeded by the lack of knowledge on the state and distribution of biodiversity – especially since the majority of species on Earth are un-described by science. All conservation efforts to save biodiversity essentially depend on the monitoring of species and populations to obtain reliable distribution patterns and population size estimates. Such monitoring has traditionally relied on physical identification of species by visual surveys and counting of individuals. However, traditional monitoring techniques remain problematic due to difficulties associated with correct identification of cryptic species or juvenile life stages, a continuous decline in taxonomic expertise, non-standardized sampling, and the invasive nature of some survey techniques. Hence, there is urgent need for alternative and efficient techniques for large-scale biodiversity monitoring. Environmental DNA (eDNA) – defined here as: *genetic material obtained directly from environmental samples (soil, sediment, water, etc.) without any obvious signs of biological source material* – is an efficient, non-invasive and easy-to-standardize sampling approach. Coupled with sensitive, cost-efficient and ever-advancing DNA sequencing technology, it may be an appropriate candidate for the challenge of biodiversity monitoring. Environmental DNA has been obtained from ancient as well as modern samples and encompasses single species detection to analyses of ecosystems. The research on eDNA initiated in microbiology, recognizing that culture-based methods grossly misrepresent the microbial diversity in nature. Subsequently, as a method to assess the diversity of macro-organismal communities, eDNA was first analyzed in sediments, revealing DNA from extinct and extant animals and plants, but has since been obtained from various terrestrial and aquatic environmental samples. Results from eDNA approaches have provided valuable insights to the study of ancient environments and proven useful for monitoring contemporary biodiversity in terrestrial and aquatic ecosystems. In the future, we expect the eDNA-based approaches to move from single-marker analyses of species or communities to meta-genomic surveys of entire ecosystems to predict spatial and temporal biodiversity patterns. Such advances have applications for a range of biological, geological and environmental sciences. Here we review the achievements gained through analyses of eDNA from macro-organisms in a conservation context, and discuss its potential advantages and limitations for biodiversity monitoring.

Contents

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1. Introduction

The continuous decline in Earth's biodiversity remains one of the most critical challenges in the 21st century (Butchart et al., 2010). Worldwide, populations of wild flora and fauna are being depleted due to anthropogenic disturbances (Barnosky et al., 2011; Dirzo et al., 2014) and species extinctions rates exceed those of pre-human periods (Pimm et al., 1995; Barnosky et al., 2011), which greatly impacts human health and sustainability of our planet (Diaz et al., 2006). Although knowledge on biodiversity is incomplete or even un-described for numerous taxa and geographical regions (Vié et al., 2009), there is international political agreement to halt the current loss in biodiversity (UNEP, 2011). All such conservation efforts to save biodiversity essentially depend on biological monitoring for obtaining precise data on species distributions and population sizes on a relevant ecological and political time scale. Species monitoring has traditionally relied on physical identification of species by, for example, visual surveys and counting of individuals in the field using distinct morphological characters. However, in some cases these techniques fall short of actually performing efficient and standardized surveys, due to, for example, phenotypic plasticity and closely related species with very similar appearance in juvenile stages. Thus, there are examples of species databases flawed with errors (Daan, 2001). Additionally, traditional monitoring techniques have sometimes proven to be invasive on the species or ecosystem under study, such as marine surveys that has relied on highly destructive techniques (Baldwin et al., 1996; Jones, 1992), although see Robertson and Smith-Vaniz (2008). Furthermore, morphological identification is heavily dependent on taxonomic expertise, which is often lacking or in rapid decline (Hopkins and Freckleton, 2002; Wheeler et al., 2004). All such limitations of traditional biodiversity monitoring have created demand for alternative approaches.

Obtaining information of species, populations and communities by retrieving DNA from environmental samples (*environmental DNA – eDNA*) holds the potential of combating many of these challenges associated with biodiversity monitoring (Baird and Hajibabaei, 2012; Kelly et al., 2014b). The fact that DNA from higher organisms persists in the environment, where it can be sampled, extracted and analyzed, has been a major technological and scientific breakthrough within the last decade (Fig. 1). As species interact with the environment, they will continuously expel DNA to their surroundings. For higher organisms, this DNA may come from excreted cells or tissue such as urine (e.g. Valiere and Taberlet, 2000), faeces (e.g. Poinar et al., 1998), hairs and skin (e.g. Bunce et al., 2005; Lydolph et al., 2005), and obviously from dead individuals leaking genetic material. The macrobial eDNA may in some systems exists predominantly inside mitochondria or small cells (Turner et al., 2014), but owing to eventual membrane degradation, extracellular DNA will also be present in the environment (Nielsen et al., 2007). Once DNA is left in the environment, its preservation, and thus availability, varies with several orders of magnitude from weeks in temperate water (Dejean et al., 2011; Thomsen et al., 2012b) to hundreds of thousands of years in cold, dry permafrost (e.g. Willerslev et al., 2003). Accordingly, eDNA has been used to address applied and fundamental research questions within areas ranging from molecular biology, ecology, palaeontology and environmental sciences.

Within a single standardized sample, DNA from entire communities across taxonomic groups can potentially be analyzed simultaneously. The content of an eDNA sample is typically analyzed by amplification using *polymerase chain reaction* (PCR) and subsequent DNA sequencing. The amplification is done either by a single-species approach using specific primers or by multiple-species (multiple-taxon) approach using generic primers for a given focal group of organisms. Especially the fast advancing *next-generation sequencing* (NGS) technologies has made comprehensive biodiversity surveys possible for limited effort and costs (Shokralla et al., 2012). It has thus made the multiple-species eDNA approach especially powerful by *DNA metabarcoding* – mass DNA sequencing for the simultaneous molecular identification of multiple taxa in a complex sample (Taberlet et al., 2012a). Although similar in principle to classical *DNA barcoding* of simple DNA extracts (Hebert et al., 2003), the practical approach and target sequence is very different. While both rely on the fact that short standardized DNA regions – typically mitochondrial, chloroplast or ribosomal RNA (rRNA) genes – can be amplified by PCR, sequenced and subsequently used as *barcodes* to identify and discriminate taxons, DNA metabarcoding cannot efficiently utilize protein coding genes such as cytochrome oxidase I (COI), since interspecific genetic variation impedes the use of universal primers (Deagle et al., 2014). Also, proposed standardized DNA barcodes are usually >500 bp and seem to reach consensus on the mitochondrial COI for animals (Hebert et al., 2003), the plastid ribulose 1,5-bisphosphate carboxylase gene (rbcL) and the maturase K gene (matK) for plants (Hollingsworth et al., 2009) and the internal transcribed spacer (ITS) for fungi (Nilsson et al., 2009; Bellemain et al., 2010; Schoch et al., 2012). These target genes have basically been chosen due to their high resolution at the species level, but high copy number per cell of mitochondria, chloroplasts and rRNA genes also make them useful in eDNA studies, since they are more likely to be picked up than single-copy nuclear DNA. However, as

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