

ON THE NATURE OF THINGS: ESSAYS

New Ideas and Directions in Botany

The real significance of ancient DNA¹

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And why do the shells... remain quite unaltered ... after such migration to milder seas. Be so good as to explain all this in your next letter. — Charles Lyell, letter to Charles Darwin 17 June 1856 (Burkhardt and Smith, 1990, p. 146).

The arctic forms, during their long southern migration and re-migration northward, will have been exposed to nearly the same climate, and ... they will have kept in a body together... (Darwin, 1859, p. 368).

One of the great challenges for science today is to explain what shapes the biodiversity we see around us: some 8.7 (± 1.3) million species and counting (Mora et al., 2011), of which about 400,000 are vascular plants. In a deep sense, an understanding of this biodiversity is rooted in the past. Historically, species were identified in the fossil record by phenotypic characters, so before molecular techniques became available, our understanding of the taxonomy and evolution of past forms has depended exclusively upon approaches akin to the taxonomy of modern organisms. In the last decade, however, a large array of approaches has become available for analyzing the DNA of past organisms (ancient DNA [aDNA]) and to look directly into the genomes of past organisms. These approaches have provided the opportunity to gain clear insights into past genotypes, to understand how they evolved toward the present, and to determine the exact time and region where extinction and divergence of species took place (e.g., Orlando et al., 2013). The importance of aDNA lies in the provision of temporal depth, with geologically dated sequences of genetic change, to the most recent parts of the tree of life.

The field of aDNA has entered its third decade and has undergone a massive methodological transformation since its introduction by Higuchi et al. (1984). Early analyses focused on organellar DNA (mitochondrial in animals and chloroplast in plants) because

these are present in multiple copies in the cells and are covered by extra membranes of protection, making preservation, isolation, and analyses much easier. Within the last decade however, with the advent of more rapid next-generation sequencing (NGS) techniques, it has become possible to analyze the more informative nuclear genome of a larger number of ancient samples. At the same time, technical improvements in aDNA bioinformatic analyses have increased both the geographical range and the types of organisms studied, and understanding of the taphonomy of DNA in sediments is also advancing (Sjögren et al., 2017). Together, these advances mean that we are on the threshold of an explosion of new information on the identification of fossils at the species level (taxonomy) and the precise linking of past organisms, populations, and communities with modern ones, within a tree of life that includes fossil as well as living organisms (evolution) through periods of environmental change.

DNA molecules of sufficient length for sequencing can be found in sediments up to 800,000 yr old (Willerslev et al., 2007), short in the overall context of evolutionary history, but long enough to include the major environmental changes of the ice ages (with a periodicity of ca. 100,000 yr between glacial and interglacial extremes: Bassinot, 2013). These environmental changes forced shifts in the distributions and abundances of organisms, especially at higher latitudes, but also at low latitudes (Hewitt, 2000). Through this period, there have been lineages with populations that may have had different sizes and occupied different regions through time as climate changes. At the same time, there has been a continuity of genotypes along lineages that evolved through neutral mutations and adaptive changes in response to new environmental conditions. Analysis of aDNA is the only tool to look directly into these genotypes, providing the only chance to track these changes through time (Parducci et al., 2005), identify the exact points where lineages split and determine the time and region where this divergence took place (Orlando et al., 2013). With aDNA, we can infer whether any current genotypically distinctive features of a living population appeared before a past distribution shift (possibly enabling it) or afterward. We thus have a better understanding of what happens at the genetic level within populations when environments change. Additionally, identification of aDNA in sediments is proving complementary to classic palaeoecological analyses, extending flora lists (e.g., Pedersen et al., 2016) and, particularly with NGS techniques,

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FIGURE 1 Coring through ice at Rundtjørna Lake (Trøndelag) in central Norway. Photo: Yoshihisa Suyama.

will increase the taxonomic resolution of fossil identification (Bennett and Parducci, 2006). Our basis for understanding speciation and explaining the biodiversity we see today is becoming correspondingly stronger.

By combining data sets on genotypes (modern and aDNA), phenotypes (from morphology of fossils) and environmental conditions (from sediment records) through space and time, we will be able to fully comprehend how these interact and how, collectively, they

(tively) and at different rates after the ice melted. In the 1990s, however, megafossils of spruce dated more than 11,000 yr ago were discovered in Scandinavia, and it was hypothesized that spruce survived glaciations in small ice-free pockets (refugia) (Kullman, 2002). The possible survival of trees through the last glaciation was finally tested with aDNA. If the widely accepted notion that all trees were killed off by the massive ice sheet that covered the region is true, then all the Scandinavian conifer trees should

have the same recent ancestors. Investigation of modern spruce populations of northern Europe identified a rare mitochondrial DNA haplotype unique to Scandinavia with its highest frequency in the west—the area believed to sustain ice-free refugia during most of the last ice age—and this rare haplotype has been found in lake sediments and pollen of Trøndelag (central Norway) (Fig. 1) dating back ~10,300 yr, as well as chloroplast DNA of pine and spruce in lake sediments adjacent to the ice-free Andøya refugium in northwestern Norway (Fig. 2) as early as ~22,000 and 17,700 yr ago, respectively (Parducci et al., 2012). These aDNA findings imply that coniferous trees were present in ice-free refugia of Scandinavia during the last glaciation and, together with similar results elsewhere (e.g., McLachlan et al., 2005), challenge traditional views on survival and spread of trees as a response to climate changes.

The new age of aDNA studies is particularly important for plants (e.g., Brown and Barnes, 2015), as DNA from the plant fossil record has been traditionally more difficult to obtain compared with animal DNA (usually well preserved in the skeletal remains). But what new knowledge do we need to acquire in



FIGURE 2 Endletvatnet Lake in Andøya (northern Norway). Andøya is an area that has been central to recent discussions on glacial survival in Scandinavia because it was ice-free during the coldest period of the last ice age, about 22,000 yr ago. Photo: Tina Jørgensen.

the next decade of studies, and where do we hope to see breakouts in the plant aDNA field? Pollen grains are certainly the best-preserved and most abundant remains in the plant fossil record, and the new possibility now offered by the single-cell sequencing technique to assess single pollen grains likely will lead to great advances in the future (Parducci et al., 2017). However, it will be only through a combination of discoveries from multiple fields as informatics, biochemistry, chronology, genomics, and taphonomy that our basis for making accurate predictions, or even generalizations, about what might happen to plant and animal populations under future climate change scenarios will be more securely based. Such collaborations are therefore critical.

Then there is the really big challenge—with increasing collaborations, combined NGS-based aDNA data sets, development of sophisticated sequencing and bioinformatic tools, denser aDNA sampling around times of environmental change and times of extinction, development of complex phylogenetic, gene clustering, and coalescent models, it ought to be possible to use a mix of genotypic and phenotypic characters in both fossil and modern taxa to understand how patterns of diversification developed and how ancient species and populations diverge, interact and finally evolve separately through periods of environmental change. Even though we may only be able to see this over the last few hundreds of millennia, this time period will encompass the final lineage-splits leading to modern species, enabling generalizations of the process in time and space. We can then better understand the history of lineages through time in relation to changing climates.

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