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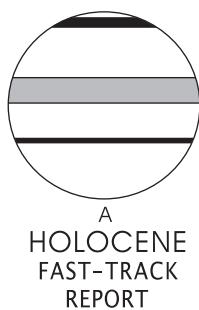
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DNA from pollen: principles and potential

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Abstract: This paper describes our recent extraction of ancient DNA (aDNA) from Holocene pollen and discusses the potential of the technique for elucidating timescales of evolutionary change. We show that plastid DNA is recoverable and usable from pollen grains of Scots pine *Pinus sylvestris* from 10 ka and 100 years ago. Comparison of the ancient sequences with modern sequences, obtained from an extant population, establish a first genetic link between modern and fossil samples of Scots pine, providing a genetic continuity through time. One common haplotype is present in each of the three periods investigated, suggesting that it persisted near the lake throughout the postglacial. The retrieval of aDNA from pollen has major implications for palaeoecology by allowing (i) investigation of population-level dynamics in time and space, and (ii) tracing ancestry of populations and developing phylogenetic trees that include extinct as well as extant taxa. The method should work over the last glacial oscillation, thus giving access to ancestry of populations over a crucial period of time for the understanding of the relationship between speciation and climate change.

Key words: Ancient DNA, pollen, plant population history, fossil, DNA sequence, Holocene.

Introduction

There is a well-developed pollen-based record of plant dispersal on the northern continents at the end of the last glaciation (Davis, 1976; Huntley and Birks, 1983). Ferris *et al.* (1995) and Petit *et al.* (1997) have related these fossil-based distributions to modern patterns of DNA variation, providing important and significant linkage between the fossil temporal patterns and modern spatial patterns. However, although fossil patterns display past distributions simply and effectively, they cannot display ancestries of populations. Hypotheses about the ancestry of populations and how they are affected by Quaternary-scale climate change (eg, Cwynar and MacDonald, 1987; Bennett *et al.*, 1991) remain untested.

Modern taxonomy relies heavily on the DNA similarities and differences between individuals, populations and species. In the last 10 years, ancient DNA (aDNA) has been successfully extracted from fossil organisms and results from specimens of late Quaternary remains (up to 100 ka) have provided insights into many evolutionary processes, particularly in animal species (Krings *et al.*, 1997; Poinar *et al.*, 1998; Brown, 1999; Leonard *et al.*, 2000; Cooper *et al.*, 2001; Lambert *et al.*, 2002; Endicott *et al.*, 2003; Shapiro *et al.*, 2004).

Initial aDNA reports suggested that the time period open to investigation was vast (Golenberg *et al.*, 1990; Soltis *et al.*, 1992). Later, theoretical and empirical considerations showed that aDNA is highly affected by hydrolytic and oxidative damage (Pääbo and Wilson, 1991; Lindahl, 1993). As a consequence, the retrieval of DNA sequences older than about

100 ka is expected to be difficult to achieve, and the criteria that need to be met in order to eliminate the possibility of contamination are rigorous (Willerslev and Cooper, 2005; Hebsgaard *et al.*, 2005). Methodological difficulties, problems with contamination and the rarity of suitable fossilized samples have often prevented a broader utility for aDNA studies at the population level. This is particularly so with plants, where well-preserved fossilized hard tissues, such as wood, can be difficult to obtain in sufficient number, over large enough geographical areas. Nevertheless, in the last two decades, aDNA has also been successfully extracted from plant material too (for a review see Gugler *et al.*, 2005), demonstrating that it is there to be found, but so far with little direct impact on ancestry reconstruction.

Pollen contains haploid DNA and is the means of its dispersal to the haploid DNA of the ovules. In flowering plants, it consists of two or three haploid cells (large vegetative cells enclosing one single or two generative cells) that comprise the pollen grain cytoplasm, including several plastids and mitochondria. Organellar DNA (ie, from plastids and mitochondria) is therefore present in multiple copies in the pollen of flowering plants. In addition, during pollen maturation, because of the different mode of inheritance of the organelles (paternal or maternal) there is a selective increase or decrease of the amount of organellar DNA in each pollen grain (Nagata *et al.*, 1999). As a result, pollen from species with paternal inheritance of plastid DNA, such as the majority of conifers, should be rich in plastid DNA at maturation.

A number of recent investigations have extracted DNA from bulk sediments of permafrost (Willerslev *et al.*, 2003; Lydolph *et al.*, 2005) or lakes (Coolen *et al.*, 2004). Since the organism

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that produced the DNA is not visible, the technique is most useful in identifying and listing taxa that may have been present. The alternative approach, extracting DNA from identified fossils, has been the most useful in reconstructing past lineages (eg, Leonard *et al.*, 2000). The difference is perhaps not dissimilar to that between choosing bulk sediment or individual fossils for radiocarbon dating.

Here we describe our recent attempt to extract DNA from Holocene pollen and discuss the potential of the method for elucidating larger evolutionary questions, including the firm linkage of the observed, dated, fossil record with patterns of ancestry inferred from modern populations.

Methods and results

Full details of the methods and authentication procedures used in the extraction of aDNA from fossil pollen are available in Parducci *et al.* (2005). A brief summary is presented below and in Figure 1.

Pollen of Scots pine (*Pinus sylvestris* L.) was isolated from a core of Holocene lake sediments (0–11 ka) retrieved from Holtjärnen, central Sweden at 60°39'N, 15°56'E (Giesecke, 2005). This species is abundant in the region, and its pollen is readily recognizable, and also available from contemporary trees. An age-depth model was constructed based on six radiocarbon determinations from the same core. Pollen grains 100 and 10 000 years old were removed from the core and analysed independently in Sweden (Uppsala University) and in Japan (Tohoku University). By sequencing clones from two short plastid DNA sequences and by using multiple controls, it was shown that the ancient sequences were endogenous to the fossil grains (Parducci *et al.*, 2005). Comparison of ancient sequences and those obtained from an extant population establishes the first genetic link between extant and fossil samples in this species, providing genetic continuity through time. We found one common haplotype present in each of the three periods analysed (modern, 100 years and 10 000 years),

which suggests that it could have persisted near the lake throughout the postglacial period (Parducci *et al.*, 2005: figure 3).

Discussion and conclusions

The results presented in Parducci *et al.* (2005) are not in themselves adequate to demonstrate how Scots pine populations have moved (or otherwise) in space and time. The data are consistent with genetic change through time locally, but other scenarios are also possible. Extension of this work to additional sites in Scandinavia and elsewhere in the past and present distribution of Scots pine should enable a fuller picture of population-level spatial dynamics over time, which should itself make it possible to understand in greater detail more precisely what happens genetically when species spread over vast areas within relatively short periods of time, as happened with many taxa in Europe (Huntley and Birks, 1983) and eastern North America (Davis, 1976) at the transition between the last glacial and the Holocene. In particular, a test of the hypotheses of Bennett *et al.* (1991) about spread, northern extinction and southern survival should be possible.

Among the implications of this method in palaeoecology, the study of (i) dynamics in space and time, and (ii) the study of ancestry of populations are the most important here. These considerations lead to a number of potential uses, of which we present a selection below.

(1) It is clear that different parts of the genome evolve at different rates, and while some may evolve in a more or less regular fashion ('molecular clocks'), others may be more irregular. Analyses of different components of the genome from dated fossils should make it possible to establish much more precisely than hitherto exactly what these rates are, and which parts of the genome are more constant in rate than others. This contribution alone has enormous implications for understanding many phylogenetic problems.

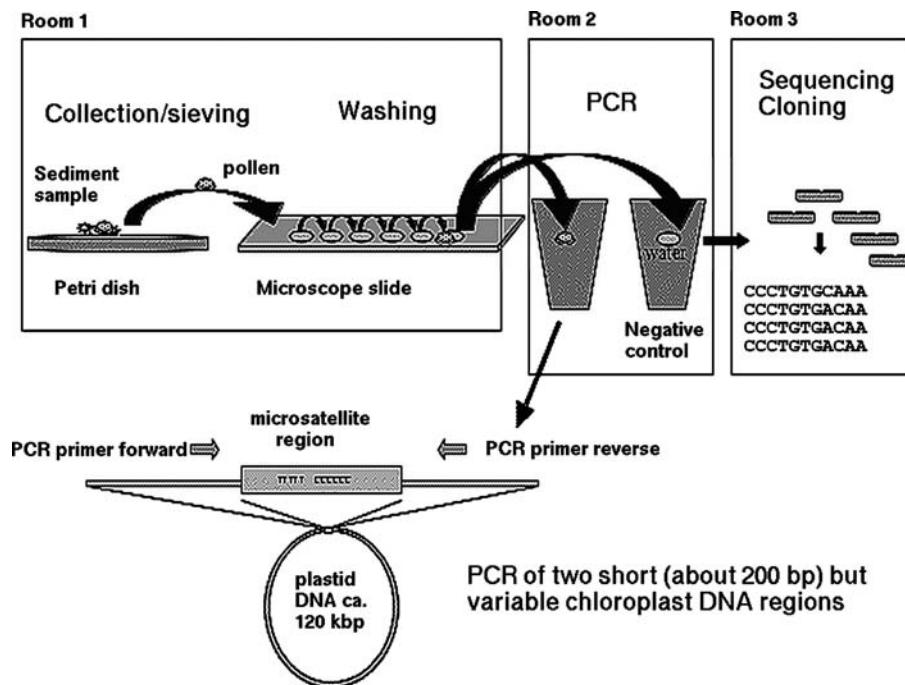


Figure 1 Direct DNA amplification from single pollen grains. Individual grains were transferred by mouth pipetting to microscope slides with specially made glass micropipettes (Room 1). They were washed repeatedly with sterile water before being transferred to the PCR tubes. A negative control contained all PCR reagents and a drop of water used to wash the pollen grain. The PCR primers (Room 2) select a predetermined region, known to be variable, and this is then cloned and sequenced (Room 3).

(2) There is an excellent record of the distribution and abundance of terrestrial plants through the identification of fossil pollen in sequences of peat and lake sediments (Berglund *et al.*, 1996), and there is also an excellent record of modern genetic diversity in equivalent taxa (eg, Comps *et al.*, 2001; Petit *et al.*, 2002; Lascoux *et al.*, 2004). There may be no group of fossils with a potentially more detailed or more accessible aDNA record than pollen, and it should therefore be possible for aDNA from pollen to take the lead in questions of rates of genomic change, and understanding of the tempo of evolution on timescales of tens of thousands of years (at least).

(3) Locally, there may also be abundant macrofossil finds (for example, pine remains in peatlands of northern Europe). Here, there may be good supplementary material that can complement aDNA from pollen. It should be possible to use the aDNA from macrofossils to define genotypes that are present locally at a certain time, and then use aDNA in fossil pollen to distinguish the proportion of pollen that was produced locally from that with different genotypes that should have come from further away.

(4) The choice of appropriate plastid DNA regions should now make it possible to relate DNA from ancient and modern species in order to distinguish taxa for which the morphological identification is not possible. In principle, it should be possible to separate different species within genera (or families), where the pollen identification to generic (or familial) level is routine, but it is difficult to get further. Candidate taxa in the Northern Hemisphere include Poaceae, Cyperaceae, *Alnus*, *Betula*, *Quercus* and *Pinus*. Extraction of aDNA from grains of these taxa and making species-level identifications has the potential to replace much repetitive work previously done with measurements, or by supplementary macrofossil analyses, or simply not done at all. The possibility of developing 'DNA barcodes' for all species is now being seriously discussed (Hebert *et al.*, 2004; Lambert *et al.*, 2005). Such a project would clearly form a strong basis for species identification of fossil materials, and should even open up the possibility of pointing to fossil taxa that should not be considered as identical to modern taxa, even if morphologies are basically similar.

(5) Mapping the distribution of genotypes in modern populations is now routine, and such maps are used to infer past population movements. There are some good examples for several tree taxa (Petit *et al.*, 2002; Lascoux *et al.*, 2004). Analysis of aDNA from fossil pollen provides a technique that can test these inferences directly. A question of particular direct interest, and which cannot be resolved solely from modern populations, is whether populations with modern genotypic composition have shifted their distributions across continents, or whether the present genotypic composition is the product of *in situ* change.

(6) The method we have described works with pollen types that contain (sufficient) plastid DNA for aDNA studies, but has so far been tested only with conifers, which have paternal inheritance of this genome and tend to have more organellar DNA at maturation. It should be tested also with species showing maternal inheritance of plastid DNA (Parducci *et al.*, 2005), which would include many angiosperm trees for which there is an abundant pollen record, such as *Alnus* and *Betula*, or *Nothofagus* and *Eucalyptus*.

(7) Ancient DNA from extinct taxa would allow these to be placed in phylogenetic trees, and thus allow these 'trees' to show variation in diversity towards the present, instead of simply connecting up extant populations backwards to a single common ancestor. The possible time range is not great (because of the degradation of aDNA), but it should be

possible to cover all taxa (extinct and extant) in lineages back to the last interglacial, and thus allow investigation of the degree to which evolutionary changes are influenced by climatic changes of a glacial–interglacial oscillation (Bennett, 2004).

Our understanding of evolution is based upon observations of modern organisms, including their genotypes and variations of these in space, together with observations based on fossils that may be well dated but have hitherto offered only morphology as a means of connection with the present. A particular limitation is the absence of molecular data from extinct species and populations.

Data from aDNA provides that link, by offering the potential to deal with fossil populations and species on the same molecular basis as modern populations and species. The wider vision that this achieves may be the most significant development in the understanding of the tempo of evolution since the development of radiometric dating.

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