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Plant macrofossils from lake sediment as the material to assess ancient genetic diversity: Did deforestation influence Norway spruce (*Picea abies*) in the South Carpathians?

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ABSTRACT

How genetic diversity of populations reacts to neutral or adaptive processes such as population bottlenecks, immigration or local adaptation are central questions of population genetics. They may be directly answered through ancient DNA analysis, however such studies in plants are remarkably scarce, owing to the difficulty of gaining population-scale samples with good DNA preservation. Plant macrofossils are abundant in lake sediments, and here we assessed if they can be valuable material for population genetic studies. We extracted ancient DNA from early to late Holocene seed and needle remains of Norway spruce (*Picea abies* L. Karst) recovered from the sediment of a lake in the Retezat Mts. (South Carpathians). We aimed to reveal whether deforestations starting in the late Holocene and reaching maximum in the 19th and early 20th centuries have caused decreased genetic diversity in the modern Norway spruce population of the Retezat Mts. In a combined approach, we sequenced two plastid regions and generated detailed paleobotanical data to reconstruct the population history of Norway spruce. Our results show that genotyping success of the ancient material was high in samples younger than *c.* 2000-year-old, but was very low in early Holocene samples, and needles allowed genotyping with higher success compared to seeds. We discovered macrofossil samples that contained DNA from more than a single Norway spruce individual. This phenomenon, together with the possible multiple sampling of the same individual needs to be kept in mind when interpreting the genetic data obtained from macrofossils. Our results overall indicate a genetic diversity decrease during the late Holocene. We demonstrate that macrofossils preserved in sediments are invaluable material for population genetic studies.

1. Introduction

The rapid development of ancient DNA (aDNA) research during the past years has opened new perspectives in the field of Quaternary paleoecology. It became possible to gain insights far beyond the local presence of species based on fossil finds, including real-time inference of their genetic diversity, population history and phylogeographic origin. The temporal changes in genetic diversity of animal populations have been in the focus of attention since the early days of aDNA research (e.g. Hardy et al., 1995; Hadly et al., 1998; Leonard et al., 2000). In contrast, so far only a limited number of genetic studies dealing with ancient plant remains aimed to analyse population-scale evolutionary processes over time (cf. Dumolin-Lapègue et al., 1999; Tani et al., 2003; Parducci et al., 2005; Bennett and Parducci, 2006; Suyama et al., 2008; Magyari et al., 2011; Parducci et al., 2012; Schmid et al., 2017). The scarcity of such studies may be caused, on the one hand, by difficulties common in most plant aDNA studies, namely the weak

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preservation of tissues with initially high DNA content, difficulties to authenticate ancient DNA or accumulation of PCR inhibitors (Gugerli et al., 2005; Parducci et al., 2005; Liepelt et al., 2006; Anderson-Carpenter et al., 2011). On the other hand, they require a population-scale sampling, which is difficult to achieve.

Abundant, well-preserved and temporally distributed plant remains can often be found in lake sediments. These plant remains are typically recovered by sediment coring, which makes them an ideal subject to gain population-scale genetic data and carries the advantage that genetic results can be directly linked to population size fluctuations (derived from pollen accumulation rates, PAR) or other paleo-proxies (e.g. vegetation changes) (Magyari et al., 2011; Parducci et al., 2017; Schmid et al., 2017). The two types of subfossil plant remains abundant in lake sediments are microfossils (pollen) and macrofossils (e.g. seeds, buds, leaves). To this day all studies based on ancient pollen encountered the difficulty of genotyping millennial old pollen grains (i.e. Parducci et al., 2005, 2012; Bennett and Parducci, 2006; Magyari et al., 2011). Although new genotyping technologies are proposed in order to increase the success of such studies, no such survey has been completed yet (Parducci et al., 2017; Parducci and Bennett, 2017). Population studies involving subfossilized plant macrofossils evoked even less attention (cf. Parducci et al., 2017). Macrofossils preserved in lake sediments as the source material for DNA analyses were introduced by Anderson-Carpenter et al. (2011). Their two main findings were the strong excess of genotyping success in samples younger than 2000 years old and the existence of exogenous DNA in DNA extracts from macrofossils. Only the very recent study by Schmid et al. (2017) aimed at analyzing a larger number of macrofossils, mid-Holocene subfossil *Abies alba* (Mill.) needles in particular, to compare genetic diversity of a population before and after a demographic bottleneck. Their study proved that conifer needles from lake sediments have a great potential for genetic studies.

The study of Magyari et al. (2011) assessed the early Holocene genetic diversity of Norway spruce (*Picea abies*) in the sediment of Lake Brazi (Retezat Mountains, South Carpathians), and compared it with the genetic diversity of modern samples. Magyari et al. (2011) found higher genetic diversity in the early Holocene compared to the modern population of the Retezat Mts, which was explained by genetic depauperation through cycles of population bottlenecks. It has remained unknown, however, when such loss of genetic diversity took place. This study was primarily based on fossil pollen, but also involved an early Holocene subfossil cone and its six seeds. Numerous Norway spruce seeds and needle remains were found along the entire length of the sediment core with macrofossils present from the late glacial (Magyari et al., 2011, 2012). Hence, the sediment of Lake Brazi may be suitable to further evaluate the utility of macrofossils for genetic studies. We are particularly interested in how genotyping success depends on the type and age of macrofossils, the length of genotyped DNA fragments, whether we encounter exogenous DNA and if yes, how this influences the outcome of population genetic studies.

We extracted DNA from early to late Holocene Norway spruce seeds and needles and sequenced short plastid regions following Magyari et al. (2011). We aimed to assess some general aspects of using macrofossils from lake sediments; those that were feasible to address by our applied traditional PCR-based sequencing technology. We expected to find age dependence of the genotyping success, but an overall considerably higher genotyping success than what was found in the case of pollen by Magyari et al. (2011). As seeds have a seed cover composed of hard tissues and are apparently much better preserved than needles, we expected to find higher genotyping success in seed samples compared to needles.

By combining our genetic data with those of Magyari et al. (2011) and reconstructing the population history of Norway spruce around Lake Brazi using paleobotanical data, we aim to further investigate the Holocene genetic diversity changes of Norway spruce in the Retezat Mts. Specifically, we aim to assess whether massive deforestations – starting in the late Holocene and reaching their peak in the 19th and early 20th century – may have caused genetic diversity loss of Norway spruce.

2. Regional setting and forest history

The Retezat Mountains are located on the western side of the South Carpathian ranges in Romania (Fig. 1). These mountains have a relatively warm climate (mean July temperature 11.9 °C at 1740 m a.s.l.) and high precipitation (1010 mm at 1740 m a.s.l.; Magyari et al., 2013) in comparison with the more northerly situated ranges of the Carpathians (calculated using CARPATCLIM data for the period between 1961 and 2010, Spinoni et al., 2015), and probably that is why Norway spruce found refugial habitats here during the last glaciation. This was recently attested by both population genetic and paleobotanical studies (Bucci and Vendramin, 2000; Ravazzi, 2002; Tollefsrud et al., 2008; Magyari et al., 2012).

Lake Brazi (Tăul dintre Brazi, 45°23′44″ N, 22°54′06″ E) lies on the northern slope of the main east-west mountain ridge in a small plateau of the Valea Galesu glacial valley at 1740 m a.s.l. (Fig. 1). This small and shallow lake has a surface of 0.4 ha and maximum depth around 1 m, and is primarily fed by meltwater and rainwater. The lake is surrounded by mixed Norway spruce–Swiss stone pine (*Pinus cembra* L.) forests, while dwarf pine (*Pinus mugo* Turra) grows abundantly on a floating moss carpet of its northern shore.

Today Norway spruce is present in the Retezat in the mixed deciduous-coniferous forests between 1200 and 1400 m a.s.l., and as a dominant tree species at higher elevations up to 1850 m on the northern, and 1900 m on the southern slope (Nyárády, 1958). Stunted individuals (50 cm tall shrubs), however, grow higher up on both slopes, up to ~2000–2050 m a.s.l. No other spruce species is native to the Retezat, and no remains of other spruce species were found in the Late Glacial and Holocene sediments in the mountain region (Vincze et al., 2017, Orbán et al., this issue).

Forest exploitation on the northern slopes of the Retezat Mts began centuries ago (Maderspach, 1868). In the early 19th century, immense deforestation of the conifer forests started, and by the 1860s only 2% of the spruce forests were unaffected by cuttings. The wood was primarily used to produce roof shingle, and local people were allowed to cut trees without limitation after paying 10% of their harvest as tax. In other areas, where forests were not cut for shingle, sawmills were constructed. On the southern slopes massive deforestation started slightly later, at the end of the 19th century, after Italian workers were settled in the area. The cuttings reached maximum intensity during World War I, and by the 1930s the entire spruce forest was logged on the southern mountain side (Stelian, 2004). Clear cuts were not followed by afforestation, neither on the northern nor on the southern slopes. The core area of the Retezat Mts, including the area surrounding Lake Brazi, falls in the area of the Retezat National Park established in 1935 (Ioras, 2003). Since then, forest exploitation has ceased in the vicinity of Lake Brazi and conifer forests have regenerated by natural secondary succession (Grodzińska et al., 2004).

3. Materials and methods

3.1. Sediment drilling and sample preparation

Two sediment cores were obtained from Lake Brazi. The first in the summer of 2007 (TDB-1), while the second in the summer of 2011 (TDB-2011). In both cases an 8 cm diameter modified Livingstone piston corer was applied, operated from a platform. The drill