Ancient DNA of NW Europe reveals responses to climate change

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Primary objective:
To explore the occurrence of boreal species at northern latitudes by ancient DNA analyses.

Subgoals:

1) Confirm the occurrence of trees and other boreal taxa during the LGM period (22,000-13,000 BP) at Andøya.
2) Determine the occurrence of boreal species on Svalbard during the Holocene warm period.
3) Evaluate the representation of current surrounding vegetation in modern lake DNA.
4) Test if pollen may contribute to DNA recovered in ancient soils.
5) Evaluate implications for inferred species migration rates as well as climate reconstruction.

Summary
Andøya (69 °N) is a key area for data on terrestrial flora and vegetation in Norway during the Late Weichselian (25,000 to 10,000 years (uncalibrated) B.P.). Cores covering most of this time interval has been retrieved from several lakes, confirming the existence of an ice-free area potentially serving as a 'cryptic' refugium at a time when almost all of Scandinavia was covered by ice. The sediments contain small quantities of pollen of tree species (*Betula, Picea, Pinus*); so far interpreted as deriving from long distance dispersal. A recent study of ancient sediment DNA in one of the lakes yielded DNA of pine (*Pinus*) and spruce (*Picea*) dated at c. 22,000 and 17,500 cal years BP, respectively, indicating that these species possibly survived in northern *in situ* refugia. If the source of this DNA is confirmed as locally growing trees, this means that pine and spruce were present in Scandinavia more than 10,000 years earlier than previously assumed. Similarly, recent findings of ancient DNA of thermophilic taxa in the geographically isolated arctic archipelago Svalbard indicate that species had a more northern distribution in the Holocene hypsithermal (4000 – 8000 years BP) than today. We propose to investigate the late glacial flora of Andøya and Svalbard by: 1) using recently developed high throughput sequencing technologies to analyse ancient sediment DNA in four new sediment cores from each of Andøya and Svalbard, 2) analysing modern lake sediments and current vegetation from the two sites to evaluate the correlation between species frequency in the vegetation, and likelihood of representation in the sediment DNA samples, and 3) assess if the methods used for extraction and amplification of ancient sediment DNA are likely to yield DNA from pollen grains within the sediments. The results of this study will increase our understanding of how species responded to climate change in the past, which is essential to forecast effects of current global warming.

1. Relevance relative to the call for proposals

The project is in the forefront of the research field by using and further exploring newly developed methods of ancient DNA analysis to study the response of plants to past climate changes. Strong international collaboration with leading experts within the field will ensure high quality and success of the project.

2. Aspects relating to the research project

2.1. Background and status of knowledge

The fate of northern species during previous periods of climate change has been debated for nearly 150 years (Brochmann *et al*. 2003). The most significant shift in northern biomes is the border between boreal forest and the arctic tundra. Understanding rates of migration and resilience to climate change of arctic as well as boreal species is thus important not only to understand the distribution of single species, but also to understand how ecosystems as a whole respond to climate changes. A few arctic species may have survived the last glacial/interglacial cycle within small unglaciated refugia in NW Europe (Westergaard *et al.* 2010; Westergaard *et al.* 2011) whereas the majority probably immigrated postglacially from late glacial tundra zones south and east of the main ice sheets (Brochmann *et al.* 2003). While the open arctic environment may facilitate dispersal (Alsos *et al.* 2007), there is less knowledge about the dispersal rate of boreal species. Trees are among the plant species that are best adapted to dispersal, and thus could be assumed to be able to track any climatic changes (Hamrick 2004). However, their dispersal rate is highly debated (Welk & Bruelheide 2006; Svenning & Skov 2007; Stewart *et al.* 2010). There is extensive evidence for tree species surviving the glaciation far south, and postglacially colonizing NW Europe (Lascoux *et al.* 2004; Cheddadi *et al.* 2006). Several recent studies using fossil and genetic data indicate that late glacial tundra zones also supported small populations of boreal trees in Alaska (Brubaker *et al.* 2005; Anderson *et al.* 2006), Yukon (Zazula *et al.* 2006), Siberia (Binney *et al.* 2009; Tarasov *et al.* 2009), and Estonia (Heikkilä *et al.* 2009). It remains controversial, however, if tree taxa grew within the limits of the Scandinavian ice sheet (Birks *et al.* 2005; Kullman 2005) as indicated by megafossils of spruce (*Picea*) and pine (*Pinus*) in the Scandinavian mountains dated to c. 11,700 ^{14}C yr BP (Kullman 2002). Even more controversial are the recent findings of pine and spruce DNA in lake sediments from a probable refugium at Andøya (north-western Norway) already at c. 22,000 cal years BP and 17,500 cal. years BP (Jørgensen 2011; Parducci *et al.* In prep) and boreal species in the arctic archipelago Svalbard (EcoChange EU project, Figure 1). These findings, which are based on ancient sediment DNA (*sed*aDNA) analyses, may alter our understanding on plants' responses to past climate change and thus deserve further investigation.

Part of the controversy may be due to the limitation and uncertainties of the methods used. While macrofossils give a firm proof of local occurrence of a species, their resolution is limited by being relatively rare in the sediments (Birks & Birks 2000). Pollen, on the other hand, is generally better preserved, but may derive from long-distance dispersal, and trace amounts are in particular difficult to interpret (Birks & Birks 2000; Hicks 2006). Also, the taxonomic resolution of pollen is often limited to genera or family level, e.g. major taxa groups of northern biomes such as e.g. sedges (*Carex*), willows (*Salix*), and grasses (Poaceae). It has previously been demonstrated that ancient DNA extracted from sediments may detect presence of taxa not detectable and/or not distinguishable by pollen or macrofossils (Willerslev *et al.* 2003; Haile *et al.* 2007; Jørgensen 2011). This technique has been further improved in the ongoing project EcoChange (http://www.ecochange-project.eu/), in which all of the current applicants participate and/or collaborate. One of the biggest advantages of analysing *sed*aDNA

compared to the traditional methods of pollen and macrofossils, is the use of deep massive parallel sequencing with high throughput sequencing techniques. Here, in theory, one molecule should be enough to detect a species. Compared to analyses of macrofossils of seeds and pollen, which are associated with sexual reproduction, *sed*aDNA occurs as extracellular DNA in the sediments, and originates from all tissues. This allows the detection of plants not reproducing sexually, a common scenario for plants at their range limits.

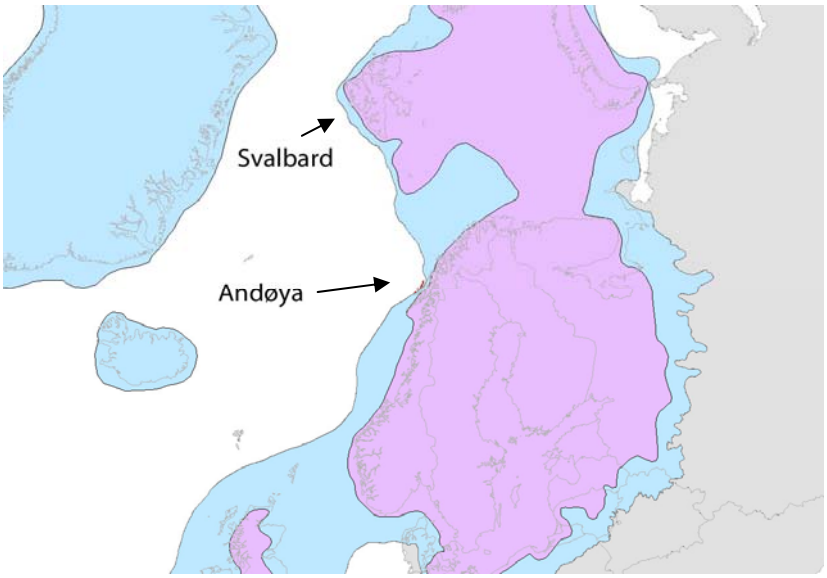

Figure 1. Extent of the glaciations at the Last Glacial Maximum (LGM, blue) and at 17,000 cal BP (purple). The planned research sites Andøya, where pine and spruce dated to c. 22,000 cal years BP and 17,500 cal. years BP were found, as well as the remote arctic archipelago Svalbard, were several subarctic species have been found, are indicated. Ice limits after (Dyke *et al.* 2003; Gyllencreutz *et al.* 2007)

Within the EcoChange project, we have developed a DNA database for 842 arctic vascular plant species, representing all widespread and/or ecologically important taxa of the species-poor arctic flora (Sønstebø et al. 2010). This has recently been extended with 1000 boreal vascular plant species (Brochmann et al., unpublished). The databases are for the chloroplast *trn*L barcode (Taberlet *et al.* 2007), which has several advantages when working on rather short and degraded ancient DNA. Chloroplasts are much more abundant in plant cells compared to the single copy of nuclear DNA; the *trn*L primers binds to a conserved region in most plants; and the insert is short (between 10-120 bp) and varies among most taxa. This allows for identification of all families, >75 % of genera, and one-third of species (Sønstebø *et al.* 2010). To increase taxonomic resolution in some taxa, ITS primers, as well as a ITS database for more than 200 taxa of Poaceae, Asteraceae and Cyperaceae, have been developed (EcoChange unpublished, Taberlet et al. unpublished). In addition, primers have been developed for mammals, birds, bryophytes, and some invertebrate groups (Brochmann et al. unpublished), which can be used to further get insight into past biota.

Calibration of the *sed*aDNA method using modern soil samples and vegetation analyses show that the majority of common species as well as some rare species in the vegetation are represented in the soil DNA at a subarctic site in N Norway (Yoccoz *et al.* Submitted) and Svalbard (EcoChange, in prep). No similar study has so far been conducted on lake sediments. When analysing ancient material, degradation processes may affect which species are found. A study on permafrost sediments from Siberia indicate that the three methods (pollen, macrofossil and *sed*aDNA analyses) are complementary rather than overlapping, and that the overlap between pollen and DNA is poor (Jørgensen *et al.* In review). This suggests that the amplified DNA does not come from pollen, or that the method and/or barcode used does not amplify pollen DNA. When using the chloroplast *trn*L barcode, the method would only amplify DNA from pollen if the pollen contains chloroplast. In contrast to gymnosperms, in which the chloroplast is mainly paternally inherited, about 80 % of the angiosperms show

maternal inheritance of chloroplast (Zhang *et al.* 2003). However, even in species with mainly maternal inheritance of chloroplast, biparental inheritance may occur (Ellis *et al.* 2008). Thus, especially species critical for interpretation of past climate and/or dispersal rates should be investigated in detail. If any *sed*aDNA is amplified from pollen, the method is still promising due to the high taxonomic resolution and its ability to detect taxa otherwise not recorded, but the same precautions as for pollen data should be taken when interpreting the results. More systematic studies on what *sed*aDNA represents in terms of vegetation and pollen are therefore needed.

Andøya has the oldest late glacial sediments known in NW Europe. During Last Glacial Maximum (LGM), a small lowland and nunatak just outside the Late Weichselian ice sheet remained ice-free (Figure 1). Extensive pollen and macrofossil analyses suggest high arctic vegetation from ca. 22,000 cal. yr BP followed by a more herb rich tundra vegetation from ca. 15,000 cal. yr BP (Alm & Birks 1991; Alm 1993). Theoretically, the pine (*Pinus*) and spruce (*Picea*) dated to c. 22,000 and 17,500 cal. years BP discovered by ancient sediment DNA analyses of a core from this site (Jørgensen 2011; Parducci *et al.* In prep) could have four possible sources of origin: drift wood, reworked material from an earlier warm interglacial, wind-blown pollen, or local vegetation. In the sediment core studied, marine macro-algae occur at ca 20,500 cal. yr BP, but not in the layers where pine or spruce were detected, thus making drift wood a less likely source. However, to rule out the possibility, sediments from a lake basin which never experienced a marine incursion should be investigated. Reworked sediments is not a likely explanation for the observed pine and spruce DNA, as there is no evidence for reworked organic material for this period at Andøya (Vorren 1978; Vorren *et al.* 1988; Alm 1993). Windblown pollen is an unlikely source as the success rate of *sed*aDNA analyses of pollen is very low (<2 %, Parducci *et al.* 2005). Also, we did not find any pollen in the same soil samples as we extracted *sed*aDNA from (pollen prepared according to Faegri & Iversen 1989). The dates of the tree *sed*aDNA coincide with dates at which tree pollen have been found in other cores taken on Andøya (Figure 2). These pollen findings have previously been interpreted as long-distant dispersed (Alm & Birks 1991; Alm 1993). However, if *sed*aDNA is from local growing trees, the pollen may rather be of local origin as well, and thus supporting the interpretation that trees existed at Andøya during the late glacial period. Both the pollen

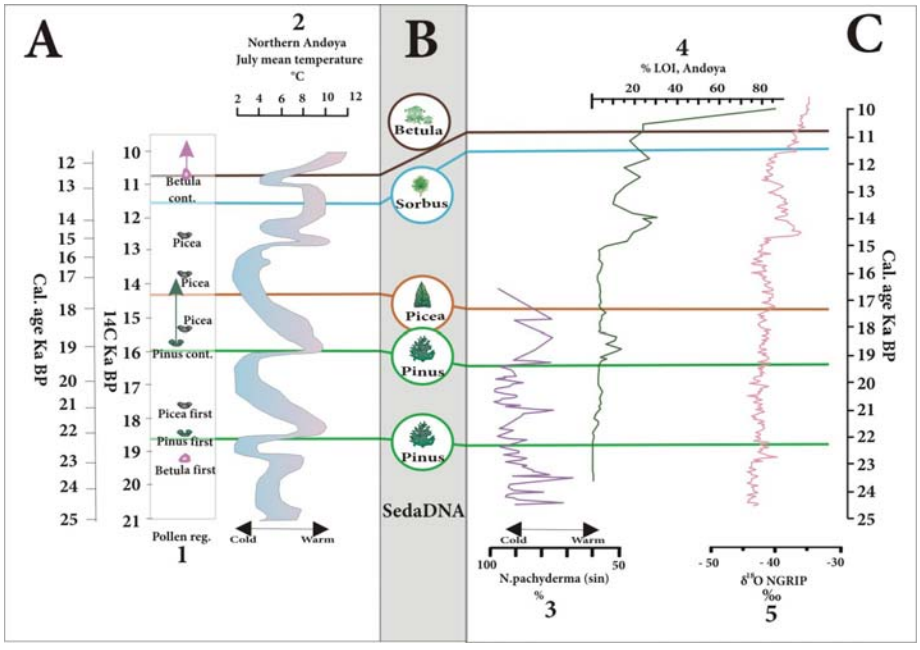

Figure 2. Temporal occurrence of boreal trees at Andøya based on fossil and *sed*aDNA data. Panel A: Tree pollen records and July mean temperature redrawn from earlier investigations. Panel B: *sed*aDNA tree findings. Panel C: Loss on ignition (LOI%), $\delta^{18}O$ values, and occurrences of *Neogloboquadrina pachyderma*, as a proxy for detection of temperature fluctuations. From (Parducci *et al.* In prep).

and *sed*aDNA of trees are found in periods with July mean temperatures around 10°C (Figure 2), corresponding to the temperature at the tree line. A genetic identification of a macrofossil to *Urtica dioica,* dated to ca. 20,000 Cal. yr BP (Jørgensen 2011; Parducci *et al.* In prep), further strengthens the view that the locality may have supported more thermophilic species than previously inferred. Using and further developing the *sed*aDNA techniques to explore the occurrence of tree species in northern refugia is of high importance as this may revolution our view on tree species abilities to resist and/or disperse in response to climate change.

While molecular studies show that arctic species have a high dispersal rate, and have frequently colonized even the most remote of all arctic archipelagos, Svalbard, during the postglacial period (Alsos et al. 2007), it is not known if more southern taxa also have such high dispersal rates. In a recent analysis of soil samples from several locations in Svalbard, *sed*aDNA of boreal species such as *Viola biflora, Rumex* sp., and *Anthoxantum* were found (EcoChange, unpublished). These species do not occur in Svalbard today, but could have thrived there during the Holocene hypsithermal, when pollen and marine mollusc data indicate that the climate was 1-2 ºC warmer than at present (Birks et al. 1994). A few other records of macrofossils and pollen of species not occurring in Svalbard today are known (van der Knaap 1985; van der Knaap 1988; Wohlfart et al. 1995). Thus, the Svalbard flora during the Holocene warm period may have been much richer than the current one. Further analyses using *sed*aDNA may provide more knowledge of the Holocene warm flora and thus dispersal abilities of subarctic/boreal species.

By obtaining more and better samples from Andøya and Svalbard, as well as exploring further what *sed*aDNA is likely to represents in terms of modern vegetation and pollen, we expect to obtain firmer knowledge on plant responses to past climatic changes, especially on tree species dynamics and dispersal abilities.

2.2. Approaches, hypotheses and choice of method

In this study, we will further explore the discovery of exotic species at a key glacial refugium in NW Europe, Andøya, and on the isolated arctic archipelago, Svalbard, by applying *sed*aDNA analyses to new sediment cores. As the method is newly developed, we will also explore if *sed*aDNA is likely to detect pollen DNA, and thus could represent non-local species, as well as explore to which degree DNA from modern lake sediments represents species in the surrounding vegetation.

Our hypotheses are that:

1) Exotic species existed at northern latitudes, but have been overlooked due to poor taxonomic resolution of traditional paleobiological methods used in previous studies, and/or due to misinterpretation of pollen as long-distance dispersed.
2) Trees existed at the glacial *in situ* refugium Andøya during the late glacial maximum (LGM), and/or the late glacial period.
3) Boreal species have as high dispersal ability at northern latitudes as arctic ones.
4) Chloroplast *sed*aDNA mainly represents DNA from plant material other than pollen.
5) The probability of discovering species in modern lake DNA is related to their abundance in the surrounding vegetation and their distance from the sampling site.

The results of this study will first of all be important for applying *sed*aDNA methods in a wide array of studies, such as reconstructing past vegetation assemblage and niche stability over time and space, evaluating past responses to climate changes, estimating past dispersal rates, study the recent and contemporary dynamics of soil and lacustrine ecosystems, etc. Second, any confirmation of the discovery of trees at high latitudes during LGM and/or the late glacial period will imply that our current understanding of tree-species dynamics and responses to

climate change is wrong, and that trees are either substantially more resistant to climate change, or have a considerably better dispersal ability than currently believed. Third, by increasing our knowledge of past dispersal also to subarctic/boreal species, we may better be able to forecast the effect of climate change on this large group of cold tolerant species, which are expected to lose considerable parts of their ranges due to global warming (ACIA 2006; Parmesan 2006).

Sampling of new cores on Andøya
Three closely spaced lakes at Andøya have been thoroughly investigated using traditional paleoecological methods. Of these, Nedre Æråsvatn (35 m a.s.l.) and Endletvatn (36 m a.sl.) are situated below or at the Late Weichselian relative maximum sea level. This opens a theoretical possibility for influx of drift-wood; a problem in terms of interpreting the origin of ancient DNA of tree species, as recorded in Endletvatn. This may be avoided by extracting new cores from Øvre Æråsvatn (44 m a.s.l.), which contains a long and purely lacustrine sediment sequence dating back to 22,000 B.P. (ca 26,000 cal. BP) (Alm 1993). It includes an extensive (ca. 2.5 m thick) gyttja layer deposited at 18,000 to 15,000 B.P. Despite being highly organogenic, it is sparse in pollen and macrofossils and has probably been formed mainly by droppings from birds. Ancient DNA is a possible tool for unlocking the secret of the origin of this organic material. Additional core samples will also be collected closer to the shore to increase the chances of finding plant remains.

Sampling of new cores in Svalbard
In the EcoChange project, modern soil samples from Svalbard were analysed to evaluate how modern vegetation is represented in the soil samples (EcoChange, in prep.), but only one core of older material dated more than 5000 BP was sampled. Here we want to analyse new cores taken at a location that reveals a long chronology (8110 yr) and the occurrence of several thermophilic species, the lake Skardtjønna (Birks 1991). Additionally, we may analyse a core dated to 14,060-15,000 cal BP, so far the oldest one available from Svalbard (in collaboration with Anne Hormes, University Centre in Svalbard).

Age/depth chronology
^{14}C-dating of sediments will show if any exotic species established immediately after deglaciation in Svalbard, and after climate became suitable at Andøya (Alm 1993; Parducci *et al.* In prep), or if there was a time lag before species arrived, as observed in NW Russia (Väliranta *et al.* 2011). The datings will be based on macrofossils if possible, otherwise bulk sediments.

sed*aDNA analyses*
Small (ca. 10 g) samples of soil will be taken from the cores in the sterile laboratory at Tromsø Museum. Thereafter, DNA extraction and analyses will be done in the newly establish ancient DNA laboratory at National History Museum, University of Oslo, and in the internationally leading ancient DNA laboratory at Centre for GeoGenetics, University of Copenhagen. For samples were exotic species are found, the consistency of the results will be checked by analysing samples in both laboratories. For each sediment sample, the short chloroplast DNA sequence *trn*L, as well as ITS, will be amplified. We may also amplify other species groups such as birds and mammals. To get as much information as possible on past biodiversity, the amplified DNA products from the cores will be deep-sequenced using second generation sequencing (either 454 (Roche) or Solexa (Illumina)). The sequences will be matched with both the arctic and boreal databases mentioned above.

Calibration of sed*aDNA with pollen*
To examine how much, if any, DNA is gained from pollen compared to leaf material, we will do multiple tests. Presence of plastid DNA in pollen will be determined by epifluorescence microscopy (Zhang et al. 2003). We will attempt to amplify DNA from pollen using the chloroplast *trn*L primers (and ITS primers when relevant) for: 1) three different concentrations of modern pollen of 30 species, 2) three different concentrations of DNA from leaf material of 30 species, 3) a mixture of modern pollen from up to 30 species and 4) a mixture of leaf DNA from the same up to 30 species, 5) a mixture of modern pollen DNA for 5 species with leaf DNA from 5 different species, and 6) fossil pollen of different concentrations. The tests will be done on the five northernmost tree species in the area, five species of grass, five sedges, five dwarf shrubs, five terrestrial, and five aquatic herbs. These tests will be repeated with different extraction methods (with and without crushing the samples). All tests will be made in the laboratory at Tromsø Museum, University of Tromsø. For this, we need a technical assistant.

Calibration of modern lake sediment DNA with vegetation
The vegetation in the total catchment area of the lakes will be investigated by noting the frequencies of species (rare, scattered, common, dominant). This will be done in consecutive concentric circles around the lake (every 10^{th} m), and in eight directions from the lake. Twenty samples of the uppermost layer of the lake sediments will be samples, and taxon assemblages from DNA amplifications will be compared, both quantitatively and qualitatively, with the estimated vegetation composition at different radii from the lake.

2.3. The project plan, project management, organisation, and cooperation
Project plan
Before the start of the project, Alm and Alsos will revisit the location at Andøya during the summer of 2011 to explore the best places to obtain new cores. A post doc will be hired at project start. A likely candidate for this position is Tina Jørgensen, who has experience from previous analyses of *sed*aDNA from Andøya, as well as analyses of pollen, macrofossils, and *sed*aDNA from Taymyr (Jørgensen 2011).

Depending on whether the best sites on Andøya are found in open water or ancient lakes now overgrown by peat, drilling will then either take place during the first summer (when peat is thawed), or during the second winter (when lakes are frozen). Vegetation analyses (Andøya and Svalbard), as well as collection of pollen for the calibration studies, will be done by Alm, Alsos and the post doc during the first summer. After the first field season, a lab technician will be hired to quantify pollen in samples and do DNA analyses of modern pollen samples. This is estimated to take about one year, and will be done in parallel to the *sed*aDNA analyses of the first cores (either obtained from Andøya first summer or the one already collected in Svalbard).

National and international research expertise, facilities, and network-building
The project will lead to strong national networking among partners which are leading scientist in different disciplines (Alsos, Alm, Brochmann, Yoccoz). Further, the project will utilize national facilities in terms of molecular laboratory, as the molecular work will take place both at Tromsø University Museum, and in the newly established ancient DNA laboratory at National History Museum, UiO. Necessary infrastructure for drilling cores will be obtained from the University of Tromsø.

The network further builds on cooperation with the ongoing EcoChange project, in which Alsos, Brochmann, and Yoccoz, as well as the international partners Edwards, Willerslev, Cheddaddi, and Gielly, participate. This will ensure that the current project takes advantage of

any further progress in the EcoChange project, as well as having access to the competence of the partners and their respective research groups.

PI **Alsos** has 19 years of experience in arctic and subarctic flora, with special competence on dispersal and persistence of the more thermophilic elements in the amphi-Atlantic region, applying molecular as well as ecological methods. **Alm** has thorough knowledge of northern Scandianvian flora (>400 publications) as well as paleorecords of Andøya. **Yoccoz** has extensive expertise in developing and using advanced statistical tools to analyse variability in climate and ecosystems parameters. **Brochmann** has published >100 papers mainly on molecular taxonomy, phylogeography and the glacial survival problem of northern plants, and he has high activity in the ancient DNA laboratory at UiO. **Edwards** has an international reputation as a northern-regions palaeoecologist, and is leading the palaeoecological activity team in EcoChange. **Willerslev** has pioneered the work on ancient DNA, and is the leader of the renowned Ancient DNA and Evolution Group at the University of Copenhagen. **Gielly** is among the most experienced molecular laboratory research engineers, and has been central in the development of the *sed*aDNA and other molecular techniques in the laboratory of Pierre Taberlet. **Cheddadi** has a wide experience in reconstructing past environments based on pollen, macrofossils, genetic analyses, and species distribution models, and he has especially studied past distribution of tree species. Together, the group provides the necessary competence in flora, paleoecology, molecular methods, and statistics. The successful applicant for the post doc position will be enrolled in the Norwegian Research School for Biosystematics (ForBio, http://www.nhm.uio.no/english/research/forbio/), which arrange courses, workshops, and conferences relevant for the project. The post doc will also spend up to 8 months abroad in Willerslev's laboratory, as well as up to 8 months in Brochmann's laboratory in Oslo, in total 1 year.

2.4. Budget

We are applying for money to cover a 3 year post doc (including 1 year abroad/in Oslo), 1 year laboratory assistant, field work, DNA analyses, radiocarbon dating, and project meetings. We are aware that the Norwegian Research Council usually does not finance research stays at other institutes within Norway. However, we wish to strengthen the bonds between UiT and UiO, as well as utilize the newly established ancient DNA laboratory at UiO. The rates we have used for covering the stay of the post doc in Oslo are the Norwegian Research Council rate for studies abroad.

3. Key perspectives and compliance with strategic documents

3.1. Compliance with strategic documents

Biosystematic focusing on molecular techniques is of highest priority at Tromsø Museum, which recently has hired Alsos (2010) to strengthen this research field. It is also a national priority, as reflected in the allocation of funds by the Norwegian Research Council, Norwegian Biodiversity Information Centre, and the four Norwegian University Museum for establishing a research School in Biosystematics, with Brochmann as a project leader, and Alsos as a representative for Tromsø Museum.

3.2. Relevance and benefit to society

Society needs to adjust to climate change in terms of agriculture, livestock keeping, settlements, and management including conservation. With increased knowledge of past responses of plants to climate change, we may improve models for estimating future plant distribution, and thereby adjust accordingly (Araújo *et al.* 2011; Thuiller *et al.* 2011).

3.3. Environmental impact

The project will significantly help protect species diversity as knowledge on past species responses to climate change may help adjust conservation plans. There are no negative impacts of the project.

3.4. Ethical perspectives and 3.5. Gender issues

The project raises no specific ethical questions. The project will promote the Research Council's general objectives to increase recruitment of women and improve gender balance in projects by encouraging women and applicants with minority background to apply for the post doc position.

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