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Response to Comment on “Glacial Survival of Boreal Trees in Northern Scandinavia”

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Birks *et al.* question our proposition that trees survived the Last Glacial Maximum (LGM) in Northern Scandinavia. We dispute their interpretation of our modern genetic data but agree that more work is required. Our field and laboratory procedures were robust; contamination is an unlikely explanation of our results. Their description of Endletvatn as ice-covered and inundated during the LGM is inconsistent with recent geological literature.

We thank Birks *et al.* (1) for their comments on our paper (2) and continuing engagement in the long-standing debate on tree survival (or otherwise) in glacial-age Scandinavia (3). We know as well as any that the new field of environmental ancient DNA requires further work; however, we find their specific criticisms to be unfounded. For our results to be merely a set of errors, as might be construed from their Comment, would require contamination by independent spruce and pine genetic markers to occur in two independently obtained sediment samples, including pollen isolates, and/or in three independent ancient DNA laboratories. Additionally, for our interpretation of the genetic patterns to be erroneous, a highly complex alternative explanation of modern spruce genetic data in northern Europe must be invoked.

(i) How reliable is ancient DNA (aDNA) from lake sediments? It is a misinterpretation of Gilbert *et al.* (4) that a specific set of authentication criteria is needed. They recommend adding criteria of relevance to a given study rather than following a predefined list. We (i) took material from two locations with different equipment on different expeditions (minimizing cross contamination); (ii) used two independent DNA markers—chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA)—for each locality (minimizing

the chance that positive findings of conifer DNA result from contamination of amplicons obtained from the other core); and (iii) undertook replication of findings in three ancient DNA laboratories (two in Uppsala and one in Copenhagen).

No piston corer is contamination-free, including that of (5). We followed all conventional protocols for avoiding contamination: careful core storage, cleaning of cores before sampling, and sampling from the undisturbed interior of a core in clean laboratories and with clean instruments (5). The lacustrine Endletvatn sediments were retrieved from a mire, so the corer was never in a lake water column; dissolved DNA from lake water is not a contaminant. Were contamination present, taxa currently abundant in the catchment (e.g., *Calluna vulgaris* and *Vaccinium* spp.) should have been found, but they were not; rather, most taxa were similar to pollen taxa.

We do not report the plant fragment retrieved from lake sediment as contamination. It was bleached to remove modern contamination (standard procedure before ancient macrofossil genotyping) and identified by DNA barcoding as *Urtica*.

(ii) Was glacial survival on Andøya possible? Our record is physically feasible. Current understanding of Last Glacial Maximum (LGM) glacial limits places northern Andøya outside those limits (6). The ice cover of Scandinavia was highly dynamic in space and time, even in inland areas (7), intermittently exposing ice-free areas. Endletvatn remained a lake above sea level during the LGM (8). Areas for plant growth would not have been confined to mountain peaks but would have included lower elevations and a range of topoclimates. Pollen and macrofossil records from previous Andøya studies indicate a mosaic of vegetation in space and/or time (3, 8–11). A local temperature reconstruction (Fig. 1) shows marked fluctuations through the LGM and Late Glacial, including episodes when the mean temperature of the warmest month exceeded 10°C, compatible with the establishment and survival of tree taxa.

(iii) Where were *Pinus* and *Picea* growing? Our data, and other studies [e.g., 12], underpin a growing case for tree survival in locations within otherwise glaciated Scandinavia, but we neither demonstrate nor claim that pine (*Pinus*) and spruce (*Picea*) survived the whole glacial period on Andøya. Indeed, we entertain alternative explanations for our findings, including redeposition, driftwood, and long-distance pollen [see supporting online material (SOM) for (2)] but show them to be unlikely, concluding that survival of conifers through the LGM in northern Scandinavia is the most parsimonious explanation of our results.

We do not expect aDNA results to duplicate pollen results. We do expect that, like macrofossil evidence, results will be complementary to pollen data. Understanding cases where aDNA is present but there is no corresponding pollen data (and vice versa) is an area of continuing research by our group. However, absence of pollen data is not evidence of absence.

Therefore, where did the cpDNA come from? Birks *et al.* cite (13) to suggest that there was long-distance dispersal of environmental DNA. Subsequent papers by the same group (e.g., 14–16) find no evidence for long-distance dispersal of either animal or plant sedimentary DNA.

(iv) Does haplotype A (HapA) reflect *Picea* spreading directions? We report haplotype A as a 21–base pair deletion clearly identifiable by sequencing. The finding of this deletion at ~10,300 calibrated years before the present (BP) in more than 30% of the samples from central Norway [see SOM table S5 for (2)] indicates that HapA individuals were present by the early Holocene. This early abundance at high latitudes indicates that HapA populations arose at an earlier time and then expanded. Deep divergence between northern and southern European spruce populations is demonstrated by the mtDNA marker *nad1*, which likely predates several glacial-interglacial cycles (17); migration from the southern range is therefore less likely.

More genetic markers and improved population resolution are indeed needed to reveal the full history of spruce in Scandinavia. Genetic patterns revealed by other mtDNA and nuclear markers have already revealed a complex history (17, 18). Elevated genetic distances in central Scandinavia and relatively high genetic diversity at nuclear loci in southern Scandinavia argue in favor of a western refugium (18).

(v) Why are *Pinus* and *Picea* absent on Andøya after 17,700 years BP? Interestingly, macrofossils of *Betula pubescens* at ~20,000 years BP (3) provide “conventional” evidence of LGM tree presence on Andøya. However, assuming “once there, always there” is a rather static vision of Quaternary biogeography. Individual populations appear locally and become extinct locally. There is no a priori reason why all populations recorded in the palaeoecological record of the LGM, particularly those in suboptimal habitats, should survive intact until today.

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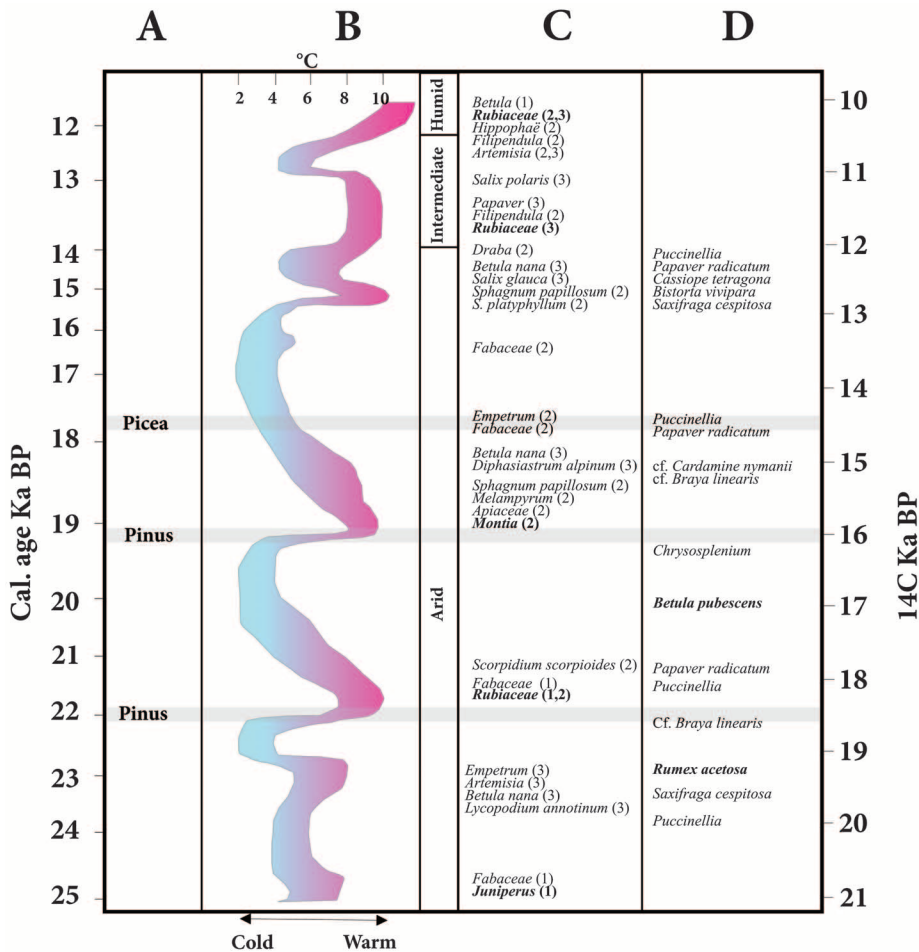


Fig. 1. Temporal occurrence of plant taxa and climate reconstruction at Andøya. (A) Our sedimentary aDNA (sedaDNA) findings in the Endletvatn core. (B) Northern Andøya mean July temperature climate curve and aridity/humidity estimates, redrawn from (9). (C) Climatic bio indicators as identified by (9) (no. 1), (10) (no. 2), and (8) (no. 3). (D) Botanical macrofossils from (11) and a *Betula pubescens* macrofossil from (3). Taxa with their current northern limit in shrub tundra (mean July temperature 10 to 12°C) are shown in bold. Radiocarbon dates have been converted into calendar years using Oxcal 4.1 with IntCal09.

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