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Key words: ant–plant interactions, domatia, fungi, mutualisms, myrmecophytes, symbiosis, tripartite.

Letters

Radiocarbon dates reveal that *Lupinus arcticus* plants were grown from modern not Pleistocene seeds

Introduction

In 1967, Porsild *et al.* published the unprecedented report of Arctic lupine (*Lupinus arcticus* Wats., Fabaceae) plants grown from seeds of presumed Pleistocene age [>10 000 yr before present (BP)]. These seeds were recovered from an ancient rodent burrow in frozen silt considered to have been deposited during the Pleistocene. At the time of publication, these Arctic lupine seeds (Porsild *et al.*, 1967) were considered to be several thousand years older than any other ancient seeds previously reported to have successfully germinated (Godwin, 1968). As such, Porsild *et al.*'s publication was remarkable for the understanding of seed longevity and the potential for the preservation of viable ancient organisms in permafrost (Black, 1967; Kjøller & Ødum, 1971; Fabel *et al.*, 2002). However, Porsild *et al.*'s Arctic lupine germination has been regarded with controversy in the discussion of ancient seed viability (Godwin, 1968; McGraw *et al.*, 1991;

Gugerli *et al.*, 2005). Some cite the Pleistocene Arctic lupine as the greatest example of extreme seed longevity (Taylorson & Hendricks, 1976; Leck, 1980; Basu, 1995; Duarte *et al.*, 1996; Gugerli, 2008), whereas others criticize the validity of this report because of the lack of independent dates (Roos *et al.*, 1996; Baskin & Baskin, 2001; Daws *et al.*, 2007; Sallon *et al.*, 2008a).

To help resolve this issue, we used accelerator mass spectrometry (AMS) analysis to radiocarbon (^{14}C) date two of the remaining Arctic lupine seeds and an associated lemming (*Dicrostonyx torquatus*) skull from the burrow to determine their actual age. We report here the first independent radiocarbon dates that reveal that Porsild *et al.* (1967) did not germinate and grow Pleistocene Arctic lupine seeds as presumed, but instead grew modern seeds that apparently contaminated their ancient sample.

Background

In order to put our new radiocarbon data into context, it is crucial to relate the events that led to the discovery and germination of the presumed ancient Arctic lupine seeds. Harold Schmidt, a mining engineer and placer gold miner, discovered a number of rodent burrows exposed *c.* 3–6 m below the surface within a 'muck' deposit (a local term for frozen organic silt of loessic origin) which overlay gold-bearing gravel at his mining site on Miller Creek, Yukon Territory, Canada (64°00′N, 140°49′W) (C. R. Harington field notes, June 14, 1966). Several of the burrows contained rodent nests, fecal pellets, skulls, skeletons and seeds. Schmidt collected the rodent skull and a number of seeds from one of the burrows. The scientific significance of the burrow contents was not recognized until palaeontologist C. R. Harington visited Schmidt 12 years later. Schmidt gave the skull and *c.* 30 of the seeds to Harington to take them back to the National Museum of Canada in Ottawa for study.

The seeds from Schmidt's rodent burrow were then presented to A. E. Porsild, chief of the Botany Division at the National Museum, for identification. The seeds were readily identified as belonging to Arctic lupine (*Lupinus arcticus*), a common herbaceous plant of the present-day boreal forest across northern Canada. Chromosome counts performed on the seeds showed that they were identical to modern specimens. As Porsild noted that half the seeds were remarkably well preserved, he passed those on to G. A. Mulligan (a research scientist at the Plant Research Institute, Department of Agriculture in Ottawa) for testing. Mulligan placed them on wet filter paper in a Petri dish, where six germinated within 48 h. The young plants were transferred to pots where they grew into normal, healthy plants indistinguishable from *Lupinus arcticus*, with one plant developing a few flowers after 11 months (Hinds, 1967; Porsild *et al.*, 1967).

As the rodent burrow and associated seeds were discovered within frozen silt that was well documented to have been deposited during the Pleistocene, Porsild *et al.* (1967) assumed that their plants were grown from seeds that were at least 10 000 yr old. Porsild *et al.* (1967) suggested that these lupine seeds were of similar age to Pleistocene ground squirrel burrows and nests discovered in the mining district near Fairbanks, Alaska that were radiocarbon dated to 14 860 ± 840 yr BP (Péwé *et al.*, 1965). Identification of the skull associated with the Arctic lupine seeds as being that of a collared lemming (*Dicrostonyx torquatus*) (C. R. Harington field notes, June 14, 1966), a rodent of Arctic and alpine tundra that is no longer found in the vicinity of Miller Creek, Yukon, further suggested that these lupine seeds were indeed ancient. As the discovery was made before the development of AMS radiocarbon dating, there was no way of providing an independent age assessment on the skull or the Arctic lupine seeds to support the presumed Pleistocene age.

Yukon fossil rodent burrows

Since the work by Porsild *et al.* (1967), many other rodent burrows and nests have been discovered from Pleistocene permafrost deposits of Eurasia, Alaska and Yukon (Harington, 1977; Guthrie, 1990; Fraser, 1995; Gubin *et al.*, 2003; Zazula *et al.*, 2007). Indeed, Harington (1997) noted several ground squirrel burrows with nests at Sixtymile Loc. 3, a fossil locality *c.* 1.6 km northeast of the site at which the Arctic lupine seeds were found. Research by Zazula documented over 100 such Pleistocene fossil nests from the Klondike mining district of central Yukon (Zazula *et al.*, 2005, 2007; Zazula, 2006). It was determined that most of the burrows and nests were constructed by Arctic ground squirrels (*Spermophilus parryii*), although the recovery of bones from lemming (*Dicrostonyx* and *Lemmus*) and vole (*Microtus*) indicated that microtine rodents also used these subterranean tunnels (Harington, 2003; Zazula *et al.*, 2007). The recovery of these nests in close stratigraphical association with Pleistocene volcanic ash beds of known age, and many radiocarbon dates, indicated that the nests spanned the duration of the Late Pleistocene ~100 000–10 000 yr BP (Zazula *et al.*, 2005, 2007; Zazula, 2006). Palaeobotanical analysis of the nest contents has resulted in an exceptional record of fossil seeds, fruits and leaves from over 60 plant taxa, although Arctic lupine was conspicuously lacking (Zazula, 2006; Zazula *et al.*, 2007).

Considering the controversy surrounding the age of the Arctic lupine seeds studied by Porsild *et al.* (1967), and the fact that seeds of this species were absent from the extensive collection of nests analysed by Zazula (Zazula, 2006; Zazula *et al.*, 2007), we undertook the present study to date the remaining seeds and skull (Fig. 1) using the AMS radiocarbon method.

Radiocarbon dating

Two of the remaining Arctic lupine seeds recovered in 1954 by Harold Schmidt were selected for AMS radiocarbon dating. As they were received in 1966 by Harington, these seeds were curated in the Quaternary Zoology Collection at the National

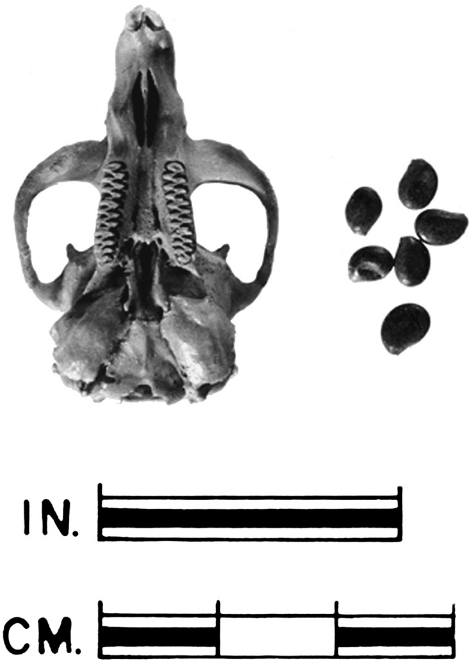

Fig. 1 Photograph of lemming (*Dicrostonyx torquatus*) skull (occlusal view) and Arctic lupine (*Lupinus arcticus*) seeds taken before display in Life Through the Ages Hall, Canadian Museum of Nature, Ottawa, ON, Canada (C. R. Harington).

Museums of Canada in Ottawa (now the Canadian Museum of Nature). However, the seeds had been embedded in paraffin wax in a Petri dish for a museum display between the years 1971 and 2004. Because of the potential contamination of residual carbon from the paraffin wax into the Arctic lupine seeds, we needed to use a refined extraction technique before AMS radiocarbon dating. The seeds were decontaminated and dated at the Oxford Radiocarbon Accelerator Unit. As a test to determine the success of the paraffin wax decontamination, we also coated an Indian paintbrush (*Castilleja* sp.) seed capsule with paraffin wax that was subsampled from a Pleistocene fossil nest (GZ.05.44; YG 343.42) that had previously been radiocarbon dated to 25 800 ± 310 yr BP (Beta-210522) (Zazula *et al.*, 2007).

Initially, the seeds were individually subjected to a thorough Soxhlet-type solvent extraction, consisting of sequential extractions with tetrahydrofuran, chloroform, petroleum ether, acetone and methanol for a minimum of 8 h each to remove any paraffin wax (Bruhn *et al.*, 2001). The samples were left to air-dry before undergoing an acid–base–acid (ABA) pretreatment consisting of sequential washes with 1 M hydrochloric acid (80°C, 20 min), 0.2 M sodium hydroxide solution (80°C, 20 min), 1 M hydrochloric acid (80°C, 1 h) and 2.5% (w/v) sodium chlorite solution at pH 3 (80°C, 5 min), with thorough rinsing with ultra-pure water after each wash.

The samples were freeze-dried before being combusted at 1000°C and measured for their elemental and stable isotopic composition using a CF-IRMS system consisting of a PDZ-Europa Robo-Prep biological sample converter (combustion elemental analyser) coupled to a PDZ-Europa 20/20 mass spectrometer (PDZ-Europa, Northwich, Cheshire, UK). Carbon dioxide from each sample was cryogenically distilled and reduced to graphite over an iron catalyst in the presence of excess hydrogen (Dee & Bronk Ramsey, 2000) before AMS radiocarbon measurement.

To test whether the Arctic lupine seeds and the collared lemming skull from Miller Creek were contemporaneous, an ~46-mg portion of the left zygomatic arch from the collared lemming skull (CMN 12062) was removed and submitted to the KECK Carbon Cycle AMS facility for radiocarbon dating.

Results and Discussion

The collared lemming maxilla yielded a radiocarbon age of 23 380 ± 130 yr BP (UCIAMS-48241), confirming its Pleistocene age. However, the two Arctic lupine seeds yielded ages of 1.03492 ± 0.00307 F14C (OxA-18596) and 1.04487 ± 0.00295 F14C (OxA-18999), which calibrate approximately to the years AD 1955–57 using the OxCal 4.05 (Bronk Ramsey, 2001) and the Northern Hemisphere Zone 1 calibration curve dataset (Hua & Barbetti, 2004). The Indian paintbrush seed capsule yielded an age of 23 700 ± 300 yr BP (OxA-18521).

The radiocarbon age on the collared lemming skull confirms that Harold Schmidt did indeed collect Pleistocene fossil remains from the frozen silt at Miller Creek, Yukon. This age is comparable with ages on nests recovered from similar burrows in central Yukon by Zazula *et al.* (2007). However, radiocarbon ages on the seeds indicate that the Arctic lupine seeds were not contemporaneous with the burrow and associated collared lemming skull. The radiocarbon age on the *Castilleja* sp. capsule is comparable with the previous age obtained from the nest sample (GZ.05.44; YG 343.42), thus indicating that the paraffin wax contaminant was effectively removed from this and the Arctic lupine seed samples (Table 1).

These radiocarbon ages indicate that Porsild *et al.* (1967) did not grow Arctic lupine plants from ancient seeds, as presumed, and now effectively negate their Arctic lupine publication from the discussion on ancient seed germination. This revelation is not entirely surprising considering that recent palaeobotanical research on Pleistocene rodent burrows and nests from Yukon failed to recover any Arctic lupine seeds (Zazula, 2006; Zazula *et al.*, 2007). Furthermore, Arctic lupine is a common understorey plant of the present-day boreal forest across northern Canada and would not be expected to have inhabited the cold, arid and, possibly, treeless steppe–tundra ecosystem of Late Pleistocene Beringia (Zazula *et al.*, 2003, 2007).

The only other comparable report of Pleistocene seed germination was made by Yashina *et al.* (2002), in which they germinated seeds of campion (*Silene stenophylla* Ledeb.) and persicaria (*Polygonum* spp.) discovered within fossil rodent nests recovered from Siberian permafrost that date between 32 800 ± 400 yr BP (IEMEZH-1178) and 31 800 ± 300 yr BP

Table 1 Comparison of estimated ages with radiocarbon ages from Miller Creek, Yukon and other specimens included in this study

Specimen	Specimen no.	Previous age estimate or age (yr BP)	New radiocarbon age
Lupinus arcticus seed (paraffin wax-coated)		>10 000	1.03492 ± 0.00307 F14C (OxA-18596) (paraffin removed); calibrated to AD 1955–1957
Lupinus arcticus seed (paraffin wax-coated)		>10 000	1.04487 ± 0.00295 F14C (OxA-18999) (paraffin removed); calibrated to AD 1955–1957
Dicrostonyx torquatus left zygomatic arch	CMN 12062	>10 000	23 380 ± 130 yr BP (UCIAMS-48241)
Castilleja sp. seed capsule (paraffin wax-coated)	GZ.05.44; YG 343.42	25 800 ± 310 (Beta-210522)	23 700 ± 300 yr BP (OxA-18521) (paraffin removed)

(Beta-157195). Yashina *et al.* (2002) placed the sterilized ancient seeds in an *in vitro* agar nutrient medium containing salts, sucrose and various phytohormones to compensate for the deficiency of endosperm and reserve nutrients within the seed embryo itself. Their experiment resulted in the campion seeds germinating to the initial stage of embryo root growth and the persicaria growing to the stage of cotyledon leaf formation, but neither developing into full plants. If Yashina *et al.*'s (2002) Siberian seed germination report is authentic, it suggests that permafrost preservation may enable plant cells to remain physiologically viable and able to resume division and growth, even after ~33 000 yr. The oldest independently dated ancient seeds known to have germinated and grown into full plants include an ~2000-yr-old date palm (*Phoenix dactylifera* L.) seed excavated from near the Dead Sea (Sallon *et al.*, 2008b) and an ~1300-yr-old lotus (*Nelumbo nucifera* Gaertn.) seed from China (Shen-Miller *et al.*, 1995, 2002). Our new data affirm that no plants have ever been grown to maturity from Pleistocene seeds or from those independently dated to older than ~2000 yr. Our research highlights the importance of independent radiocarbon dating for any germination experiments involving ancient seeds.

Although our data indicate that Porsild *et al.*'s (1967) claim that Arctic lupine plants were grown from presumably ancient seeds was erroneous, much research has yet to be done on the viability of ancient seeds in permafrost and other archaeological or palaeontological contexts. Most of the research on ancient seed longevity has been conducted in temperate climatic regions, including the Middle East (Sallon *et al.*, 2008b), Asia (Shen-Miller *et al.*, 1995, 2002; Priestly & Posthumus, 1982) and Africa (Daws *et al.*, 2007). We suggest that the permafrost regions of the Holarctic may be an invaluable, but largely unexplored region for similar pursuits. Permafrost deposits in Siberia, Alaska and Yukon are well-known archives of Pleistocene plant and animal genetic material (Willerslev *et al.*, 2003, 2004; Debruyne *et al.*, 2008). Although Yashina *et al.*'s (2002) report of the successful germination of Pleistocene *Silene stenophyllus* and *Polygonum* spp. seeds should be independently replicated to become widely accepted, their experiments represent only the beginning of much needed work on seed longevity in permafrost. Pleistocene rodent nests and burrows from permafrost deposits of northern North America and Eurasia may be the best source of ancient seeds with which to conduct more systematic experiments on ancient seed germination. The burrows and nests are found within well-drained silts that have been permanently frozen for thousands of years. In fact, Arctic ground squirrels are known to collect seeds and store them in winter subterranean hibernation chambers at dry sites, a pattern that extended far back into the Pleistocene (Zazula *et al.*, 2007). Thus, these nests contain a plethora of permafrost-preserved ancient seeds that may be ideal for further germination experiments. With climatically induced permafrost melting and the release of potentially viable Pleistocene seed banks from the cryosphere into the biosphere, there is opportunity for the development of unique plant communities composed of formerly ancient and contemporary organisms.

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Key words: ancient seed longevity, Arctic lupine, permafrost, Pleistocene, radiocarbon dating, Yukon.

Cyanogenic myrmecophytes, redundant defence mechanisms and complementary defence syndromes: revisiting the neotropical ant-acacias

Janzen (1966) first hypothesized that myrmecophytic plants hosting ants that provide an effective biotic defence mechanism will have reduced chemical defences to minimize