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# Using carbon economics of tree height to estimate evolutionary timing of cold tolerance in conifers

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ABSTRACT

Modern conifer species express many cold tolerant traits that allow for their persistence and dominance in ecosystems at high latitudes. The evolution of these traits is believed to have occurred at some point after the Cretaceous, but there exist no studies that support this hypothesis. Understanding when frost tolerance evolved within the conifer lineage is imperative as the presence of this flora is often used as a palaeoclimatic indicator under the nearest living relative (NLR) method. A trade-off relationship between tree height and cold stress tolerance is indicated for modern-day conifer species, providing clues as to what would have occurred physiologically and structurally to conifers upon the evolution of these traits. To approximate the evolutionary timing of frost tolerance, conifer basal stem diameters were obtained, from the Triassic to present day, and used to predict tree height measures using allometric equations. The plotting of tree height through time and statistical analyses suggest a significant decline of 11.31 m between the Cenozoic and Mesozoic, and height similarities within the Cenozoic and Mesozoic. This decline suggests carbon resource reallocation from tree height to frost tolerance which is further supported using genetic data, during the Cenozoic Mesozoic boundary. However, since there is only minimal research on the phylogeny of frost tolerance in conifers, we are unable to refine our estimate any further. Our overall goal was to incite collaborative research to address this critical evolutionary step in modern-day conifers.


## 1. Introduction

Modern conifer species express many cold tolerant traits that allow for their persistence and dominance in ecosystems at high latitudes. Conifer species have needle leaf traits such as increased lignification that help prevent needle deformation in response to freezing temperatures (Loehle, 1998). Other freezing adaptations such as dormancy, maintenance of high cold hardiness throughout winter, deep undercooling, and resistance to photodamage have also evolved in a variety of conifer species. Deep undercooling is a process by which cellular water is lost to extracellular ice allowing plants to survive subfreezing temperatures (George et al., 1982). Resistance to photodamage is necessary to protect chloroplasts of temperate conifers from the effects of drought and low winter temperatures when exposed to high light levels (Gillies and Vidaver, 1990).

Conifers, when they first evolved in the Permian, were warm-adapted and likely did not exhibit any of the cold tolerance mechanisms observed in conifers today. Information on the physiological development of frost tolerance and cold hardiness of coniferous trees over geologic time is surprisingly lacking within the scientific literature. Similarly, studies of cold tolerance and genetic variation of phenological traits responsible for cold hardiness focus on the identification of traits and genes, completely overlooking discussions of their evolutionary origin (e.g., Aitken and Hannerz, 2001; Bannister and Neuner, 2001; Sperry and Robson, 2001; Sutinen et al., 2001). The evolution of these traits is believed to have occurred at some point after the Cretaceous, but there exist no studies that support this hypothesis. As a result, one of the most obvious unresolved questions concerning the life history of coniferous trees is the geological period in which cold tolerance first evolved. Descriptive and/or quantitative research on frost tolerant traits is essential for expanding our knowledge about conifer physiology and for forestry applications such as developing management strategies for tree breeding (e.g., Bigras et al., 2001; Clapham et al., 2001; Colombo et al., 2001). Furthermore, this gap in our knowledge severely limits our ability to use the presence of conifers to infer cool/cold palaeoclimates.

Fossil species are typically used as potential indicators of palaeoclimates under the nearest living relative (NLR) method (Mosbrugger, 1994). This method assumes that fossil specimens have undergone little morphological evolution and experienced little change in its ecology. The concern with the use of this method, particularly for coniferous taxa, is that it assumes that cold tolerance was always present. One study conducted using the NLR method by LePage (2003) proposes that due to the physiological responses and requirements seen in extant species of *Thuja*, together with the presence of *Thuja* and other conifers in the Cretaceous, dark polar winters were cold to freezing. Osborne and Beerling (2002) develop a $CO_2$ model for the predication of

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tree height and wood growth at global scales in the late-Jurassic and mid-Cretaceous using modern-day conifer parameters. Other studies which infer cooler climates based on the presence of coniferous trees include those of Chung and Koh (2005), Liu et al. (2007), Francis and Poole (2002), Francois et al. (2011) Reguero et al. (2002) and Larsson et al. (2011).

The overall objective of our research is to begin estimating a specific geological time for the evolution of frost tolerance in coniferous trees. To meet this objective, we will use tree height data obtained from fossil specimens and research about the genes that enable cold hardiness. A trade-off relationship between tree height and cold stress tolerance is indicated for modern-day conifer species (Darychuk et al., 2012), providing us clues as to what we might look for in the palaeo-literature in the absence of phylogenetic data. From purely a carbon economic perspective (Bloom et al., 1985), adaptations that require the building of extensive enzyme/hormone-signalling response systems or other morphological structures to maintain adaptations, will require a large source of carbon. The addition of a stress tolerant mechanism means that carbon from another plant function must be reduced (often tree height) to fulfill the carbon requirements that will maintain survival of the species during the stress (i.e. freezing temperatures).

To further refine our estimates of the time range for the evolution of cold hardiness in conifers, sequenced conifer genes with putative roles in frost tolerance are discussed. Although we will not be able to provide definitive support to when cold tolerance evolved in conifers, we have conducted this research as a way to strongly reinforce the lack of research on the topic and to incite researchers from multiple fields to collaborate in addressing this topic of high importance.

## 2. Methodology

### 2.1. Creation of a meta-database

A meta-database was constructed compiling fossil conifer basal stem diameters from 28 previously published studies and several secondary resource texts. Taxonomic designations were collected at as fine a scale as possible, most frequently reflecting family and genera, with a few instances of species-level identification. The meta-database consists of 214 conifer individuals spanning the early Triassic to the Pliocene and reflecting 9 species and 19 genera. In some cases, studies provided only predicted heights, omitting trunk diameter measures. In this situation, only studies which provided height predictions using the method described by Niklas (1994) were incorporated in the meta-database.

### 2.2. Estimating fossil tree height

Due to the influence of factors affecting the preservation of fossil specimens, complete fossil trees are rarely available. As a result tree height data must often be predicted, and can be done so through the use of allometric equations. For this study, tree height for each conifer individual was reconstructed using the allometric equation described by Niklas (1994) for woody species:

$$\mathrm{Log}_{10}H = 1.59 + 0.39(\log_{10}D)^2 0.18(\log_{10}D)^2 \quad (1)$$

where $H$ is the tree height (m) and $D$ is the tree diameter (m). This allometric model that was developed by direct and published measurements from 75 species proved to be rather accurate, explaining 94.9% of the variation reflected in Niklas' (1994) study. Although this method reflects several limitations (related to the incomplete nature of fossil specimens) it is widely used and agreed upon within the scientific literature (e.g. Enquist et al., 1998; Ash and Creber, 2000; Davies-Vollum et al., 2011; Hinz et al., 2010; Brea et al., 2011).

### 2.3. Comparisons against modern-day tree heights

To assess whether a significant transformation has occurred with conifer tree height over time, fossil data was compared to average heights of living boreal coniferous species. Modern conifer height was obtained from published studies, yielding data for 25 boreal species, reflecting 9 genera. Modern conifer height data was limited to boreal species because it ensures the presence of cold hardiness, as opposed to incorporating tropical conifer species which may be lacking cold tolerant genes. As a result, the Cupressaceae family has been largely omitted from this study being a tropical coniferous species, apart from three genera, all other tree height data belonged to the Pinaceae family. These modern conifer tree heights were assigned to our current epoch, the Holocene.

### 2.4. Collection of genetic data on frost tolerance

Due to the lack of extensive phylogenetic research concerning the evolution of frost tolerant genes in conifers, a basic review of these genes, their functions and appearance is discussed. Genetic data was collected from various publications on the genetic control on frost tolerance for modern-day conifers, only one study by Schneider-Poetsch et al. (1998) referenced gene emergence at geologic timescales. Conifer genes with putative roles in the development of frost tolerance used in this study include: (1) red/far-red light receptors; phytochromes *PHYO* and *PHYP*, (2) antifreeze proteins; af70, endochitinase and glucanase, (3) dehydrins; genes coding for 9 kDa proteins, and (4) a dormancy marker; cdc2. These genes have been found in several coniferous genera including; *Picea*, *Pinus*, *Abies*, *Tsuga*, *Thuja* and *Larix* (Table 1).

## 3. Results and discussion

### 3.1. Morphological and biogeographic indications of cold tolerance

In a review of the origin of the boreal forest zone, Taggart and Cross (2009) highlight the importance of the family Pinaceae in dominating boreal zones today and indicate that they likely arose in the Palaeogene evergreen montane confer forests of the western North American Cordillera. Genera of montane conifers began to increase in presence in the fossil record during the mid-Eocene (47–45 Ma) (Axelrod, 1990). Axelrod (1990) suggests that increased volcanism in the Cordillera increased niches characterized by colder upland (montane) climate.

**Table 1**
Presence and absence of freezing tolerance genes and proteins in various conifer species. Antifreeze proteins include af70, ENDO (endochitinase) and GLUCA (glucacanase). Dormancy is represented by presence of cdc2 gene (REF). VSPs refer to vegetative storage proteins. If there is an "X" marked in a column, it means that these genes/proteins were investigated but not found. Dashed lines (–) indicate that cold tolerant genes/proteins may or may not exist as no studies have yet investigated their presence/absence.

| Species | Phytochrome | Antifreeze | Dehydrins | Dormancy | VSPs |
|---|---|---|---|---|---|
| Pinus spp. | – | GLUCA | – | – | – |
| *Pinus abies* | – | – | – | – | – |
| *Pinus sylvestris* | PHYP | – | – | – | yes |
| *Pinus edulis* | – | – | yes | – | – |
| *Pinus strobus* | – | – | – | – | yes |
| Picea spp. | – | – | – | – | yes |
| *Picea abies* | PHYO | af70 | – | cdc2 | X |
| *Picea glauca* | – | ENDO | yes | – | yes |
| *Picea mariana* | – | X | yes | – | – |
| *Abies balsamea* | – | X | – | – | X |
| *Tsuga canadensis* | – | X | – | – | – |
| Thuja spp. | – | X | – | – | X |
| *Larix decidua* | – | – | – | – | yes |