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Research paper

Morphological differentiation of *Alnus* (alder) pollen from western North America

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ABSTRACT

Increasing the taxonomic resolution of fossil pollen identification is critical for advancing Quaternary paleoecology to a point where species-specific ecologies can be addressed in the fossil record. Here, we determine the critical morphological features that permit species-level differentiation of *Alnus* pollen, an abundant pollen type in Quaternary records from western North America. We examined over 21,000 pollen grains from the region's three common alder species: *Alnus viridis* subsp. *sinuata* Regel, *Alnus incana* subsp. *tenuifolia* Nuttall and *Alnus rubra* Bongard. Modern pollen samples were collected from 27 to 35 individual plants from across the range of each species. Nine morphological traits were measured on 30 pollen grains from each plant, and the number of pores was determined for an additional 200 pollen grains from each individual. Nested ANOVA analyses suggest that for individual *Alnus* plants, pollen morphology appears relatively stable, compared to variation between species. Statistically significant differences exist between the pollen of all three alder species in most morphological traits, but there is a high degree of within-species variability and between-species overlap in pollen morphology. Since no morphological trait on its own was sufficient for pollen identification to species, classification and regression tree (CART) analysis was used to derive multi-trait classification models. CART analyses show that *A. rubra* and *A. viridis* subsp. *sinuata* pollen can be differentiated into two distinct morphotypes, analogous to species separation, based on annulus width, arci strength, exine thickness and overall diameter. The intermediate pollen morphology of *Alnus incana* subsp. *tenuifolia* prevents identification of *Alnus* pollen to species when all three species are present in the pollen source area. This research lends support to paleoecological studies in western North America that have differentiated *Alnus* pollen into two morphotypes and revealed distinct postglacial histories that are masked when *Alnus* pollen are not differentiated.

1. Introduction

Pollen analysis is one of the most widely used tools in paleoenvironmental science. It is the primary technique for determining vegetation dynamics on long time scales, but also provides paleoenvironmental records of changes in climate, hydrology, edaphic conditions, and the impact of anthropogenic activity. The broad utility of fossil pollen analysis relies on accurate and precise pollen identification, and these identifications assume spatial and temporal stability in pollen morphology. However, fossil pollen records often suffer from low taxonomic resolution due to the difficulty in identifying many pollen types beyond the family or generic level (Birks, 1993; Seppä and Bennett, 2003). Given the large ecological differences between species within genera and between genera within plant families, low taxonomic resolution constrains the paleoecological and paleoenvironmental inferences that can be drawn from fossil pollen analysis. The prevalence of important autoecological differences between species means that grouping pollen types by genus masks species changes in reconstructions of paleovegetation dynamics, as well as differential responses of congeneric species to changes in climatic and environmental conditions (Finkelstein et al., 2006). Low taxonomic resolution also hinders the field from answering questions about species specific ecologies and interspecific interactions through time (Flenley, 2003; Payne et al., 2011) and inhibits correlations between paleovegetation reconstructions and modern plant survey data (Finkelstein et al., 2006).

In particular, improving the taxonomic resolution of *Alnus* pollen identification in paleoecological records is important for a number of reasons. Alder are important early seral species on landscapes undergoing plant community succession (Connell and Slatyer, 1977; Bormann and Sidle, 1990; Chapin et al., 1994; Titus, 2009) and are important indicator species for forest fire and ecosystem disturbance regimes (Lantz et al., 2010). It is likely that alder played a similarly important role in plant succession and ecosystem dynamics throughout the late Quaternary period (Hu et al., 2001; Lacourse, 2009). Due to their ability to fix atmospheric nitrogen, alder species facilitate the establishment of conifers (Chapin et al., 1994), but interactions between specific alder and conifer species through time cannot be documented in fossil pollen records if alder pollen are only identified to the generic level. In western North America, alder species are often

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integral components of plant communities and are ecologically disparate such that grouping them into a single taxonomic unit results in a substantial loss of paleoecological information. For instance, the coastal tree species *Alnus rubra* Bongard (red alder) is the largest alder in North America and often forms extensive stands on open coasts, wet slopes, and along lakeshores and riverbanks to a maximum elevation of 300 m (Douglas et al., 1998). *Alnus incana* subsp. *tenuifolia* Nuttall (mountain alder) and *A. viridis* subsp. *sinuata* Regel (green alder) have shrubby growth forms, but *A. incana* subsp. *tenuifolia* is able to persist at higher elevations (to 3000 m) than *A. viridis* subsp. *sinuata* or *A. rubra* (Douglas et al., 1998). These three alder species also have substantial differences in life history and tolerance to shade, drought, fire, waterlogging, and soil pH and texture (Niinemets and Valladares, 2006; NRCS, USDA, 2011), as well as different forest associations (e.g., Gavin et al., 2005). Since *Alnus* can account for up to 80% of fossil pollen assemblages in Quaternary sediments from western North America (e.g., MacDonald and Ritchie, 1986; Hansen and Engstrom, 1996; Brown and Hebda, 2003; Lacourse, 2005; Lacourse et al., 2005), differentiating the pollen of these three species could greatly increase the taxonomic resolution of pollen records from this region and the paleoenvironmental inferences that can be drawn from them. In eastern North America, Mayle et al. (1993) showed that *Alnus viridis* subsp. *crispa* (formally *A. crispa*) is an important indicator of Younger Dryas cooling in fossil pollen records from Altantic Canada and highland areas of New England. Additional studies that differentiate the pollen of alder species are needed to maximize paleoecological and paleoclimate information in Holocene reconstructions.

To date, no definitive method for species-level identification has been devised for fossil alder pollen from western North America. The pollen morphology of alder from North America and Europe has been described to a limited extent (Heusser, 1969; Richard, 1970; Furlow, 1979; Mayle et al., 1993; Wittborn et al., 1996; Blackmore et al., 2003), but the vast majority of paleoecological studies simply group all pollen from alder species into their genus *Alnus* (e.g., Bryant and Holloway, 1985; Williams et al., 2004; Whitmore et al., 2005; Minckley et al., 2008). Some Holocene paleoecological studies from the Pacific coast of North America (e.g. Cwynar, 1990; Sugita, 1990; Gavin et al., 2001; Lacourse, 2005; Lacourse et al., 2007) have separated alder pollen into two morphotypes, an *Alnus rubra*-type and an *Alnus viridis*-type, and in one instance an *Alnus incana*-type (Arsenault et al., 2007). These morphotype distinctions are based on modern reference collections and morphological descriptions of *Alnus* pollen from eastern North American and European studies, where *Alnus* is also sometimes differentiated into two morphotypes (e.g. Mayle et al., 1993), or on limited examination of overall pollen size and interporal concavity in western North America (Heusser, 1969).

Previous studies used multi-trait classification methods such as discriminant function analysis to identify a suite of morphological traits with which to identify fossil pollen to species (e.g., Birks and Peglar, 1980; Hansen and Engstrom, 1985). Recent studies have used multi-trait classification and regression trees (CART) to differentiate the pollen of *Picea* (Lindbladh et al., 2002) and *Pinus* (Barton et al., 2011) species. CART analysis can provide a more powerful statistical approach than discriminant function analysis when comparing morphological traits that overlap between species (Breiman et al., 1984; Lindbladh et al., 2002). CART models can also incorporate rank and ordinal data, which is not the case with discriminant function analysis, as this technique assumes that multivariate data are from a normal distribution with common covariance (Breiman et al., 1984). Here, we use over 21,000 modern pollen grains from the three common alder species in western North America (i.e., *Alnus rubra*, *A. viridis* subsp. *sinuata,* and *A. incana* subsp. *tenuifolia*) to determine if it is possible to identify *Alnus* pollen to species in Quaternary sediments. We did not include *A. rhombifolia* Nuttall in this study because its range is more or less limited to southern Oregon and California. We measured 10 pollen morphological traits for each of the three alder species. Nested ANOVA and other statistical analyses were used to identify statistically significant differences between species. Classification and regression trees (CART) were then used to create a multi-trait identification method for differentiating *Alnus* pollen.

2. Materials and methods

2.1. Pollen sample collection and preparation

Modern pollen samples from across the range of all three alder species (Fig. 1) were collected from herbaria (Supplementary Table 1). A total of 93 individual alder plants were sampled: 35 pollen samples were collected for *Alnus viridis* subsp. *sinuata* (Fig. 2A), 27 for *A. incana* subsp. *tenuifolia* (Fig. 2B) and 31 for *A. rubra* (Fig. 2C). Botanical nomenclature follows the Flora of North America Editorial Committee (1993+). Male catkins were prepared for light microscopy using standard techniques (i.e., acetolysis) and unstained pollen were mounted in 2000 cs silicone oil (Fægri and Iversen, 1989; Bennett and Willis, 2001). Silicone oil was used because it remains fluid after mounting and other media such as glycerine can cause changes in pollen size and shape (Andersen, 1960; Whitehead, 1961; Fægri and Iversen, 1989; Mäkelä, 1996).

2.2. Morphological measurements

The morphological traits assessed for each pollen grain were chosen based on published identification keys and morphological descriptions of alder pollen in eastern North America and Europe (Richard, 1970; Furlow, 1979; Mayle et al., 1993; Kapp et al., 2000; Blackmore et al., 2003) and on informal criteria used by palynologists when separating fossil alder pollen into two morphotypes, an *A. rubra*-type and an *A. viridis*-type. Five quantitative morphological traits were measured on each pollen grain: diameter, arci width, exine thickness, and annulus width and height (Fig. 2D). Annulus area was derived for each pollen grain based on annulus height and width. For traits where multiple measurements were possible on one grain (e.g., there are up to six arci on any given pollen grain), multiple measurements were taken and then averaged across an individual pollen grain. Three qualitative morphological traits were also assessed on each pollen grain. Arci strength was assigned a relative rank from 0 (arci not visible) to 5 (very prominent, robust arci). The overall protrusion of the annulus was scored on a scale from 1 (annulus flush with the exine) to 3 (annulus protruding substantially from the exine). Overall grain shape when a pollen grain is lying on its isolpolar axis was assessed as concave, convex or mixed based on the inward curvature of the exine between the pores on each pollen grain. These six quantitative and three qualitative traits were determined on 30 pollen grains from each individual alder plant. The number of pores was also counted on an additional 200 pollen grains per individual alder plant. In total, 21,390 pollen grains are included in this dataset. All measurements were made under oil immersion at 1000× magnification using Zeiss AxioVision 4.7.1 (Carl Zeiss MicroImaging, 2008), which includes a measurement interface that allows individual morphological traits to be measured to two decimal places (0.00 ± 0.02 μm). All measurements were made on pollen grains that were lying flat on their isopolar axis.

2.3. Statistical analyses

Due to the hierarchical sampling design (i.e., pollen samples are from only one of the three alder species and pollen grains are from individual alder plants), nested ANOVA analyses were performed for each quantitative trait to test the null hypothesis that means do not differ between the three alder species. The nested model allows for partitioning of the total variability in each morphological trait into components explained by each of the nested factors i.e., between