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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

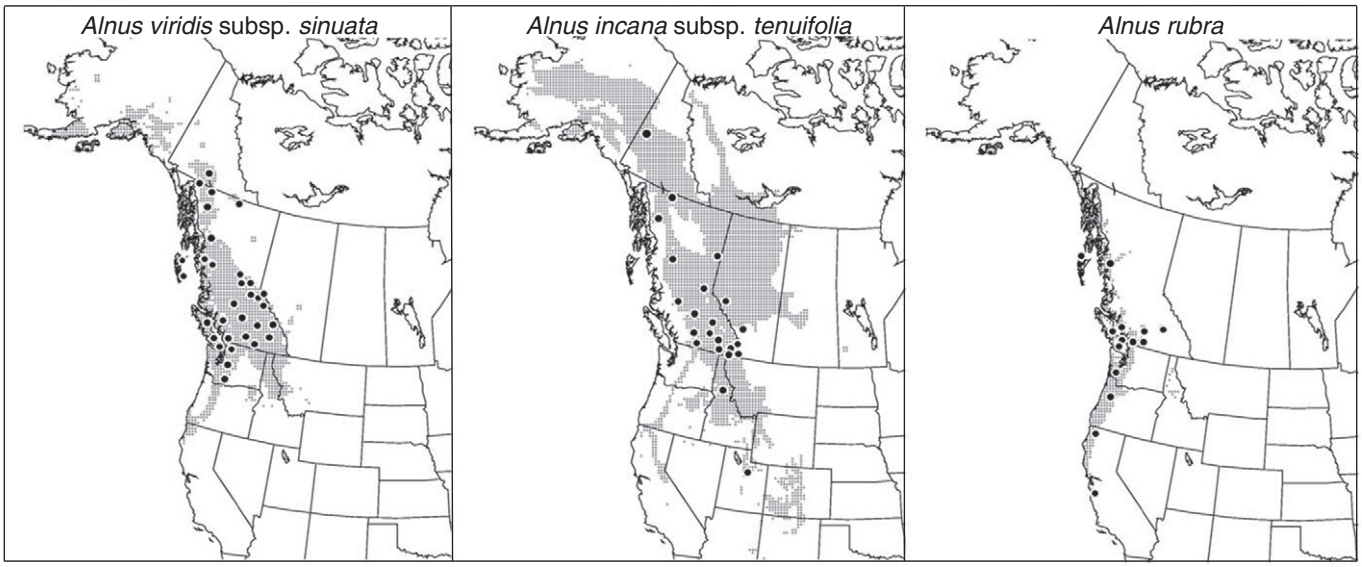

Fig. 1. Species range maps for *Alnus viridis* subsp. *sinuata* (green alder), *Alnus incana* subsp. *tenuifolia* (mountain alder) and *Alnus rubra* (red alder). Circles represent pollen sample locations, though some cannot be seen due to overlap in sample location. Distribution map source: Thompson et al. (1999).

species and between individuals within a species. As more than one catkin per individual plant was used, within catkin variability is not assessed. A Bonferroni correction was applied to each pair-wise nested ANOVA model to adjust the *p*-value for multiple comparisons and decrease the probability of Type I errors. ANOVA model significance was set at $\alpha = 0.05$. The amount of variation in each morphological trait attributable to a given nested factor was also determined as percent variance for each full ANOVA model. To assess the degree of overlap in morphological traits between species, Mann–Whitney U two-way comparisons were performed between each of the three alder species for each quantitative trait. As per Clegg et al. (2005), the resulting U statistic was scaled by the multiplier $(2/n_1n_2)$, where n_1 and n_2 are the number of

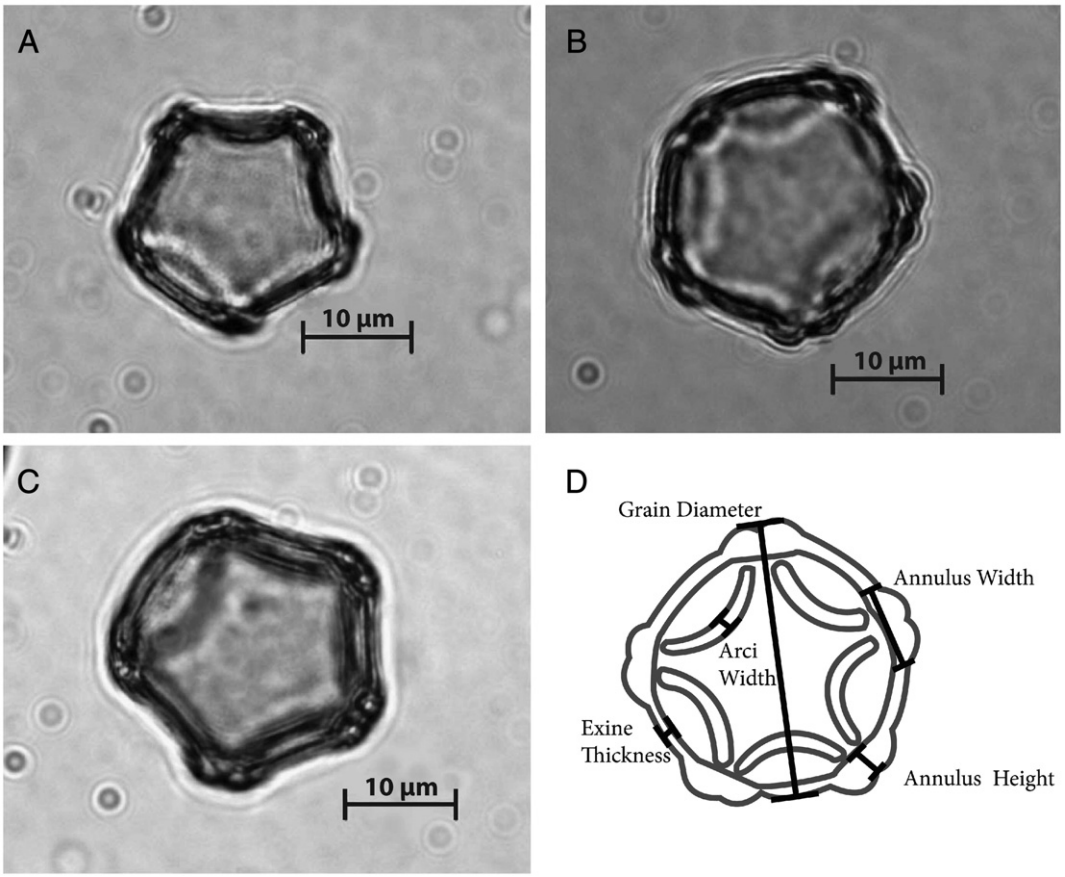

Fig. 2. Isopolar views of (A) *Alnus viridis* subsp. *sinuata*, (B) *Alnus incana* subsp. *tenuifolia*, and (C) *Alnus rubra* pollen at 1000× magnification under oil immersion. Isopolar view (D) of a simplified 5-pored convex alder pollen grain showing the five measured morphological traits.

Table 1
Summary of quantitative morphological traits for the pollen of each alder species.

	Alnus viridis subsp. *sinuata* $n=1050$			*Alnus incana* subsp. *tenuifolia* $n=810$			*Alnus rubra* $n=930$		
Morphological trait	Mean ± SE[a]	Min	Max	Mean ± SE	Min	Max	Mean ± SE	Min	Max
Arci width (μm)	1.57 ± 0.02	0.00	2.69	1.67 ± 0.01	0.00	2.78	1.82 ± 0.01	0.00	2.90
Annulus width (μm)	7.03 ± 0.02	4.73	9.27	7.51 ± 0.03	4.72	10.19	7.86 ± 0.02	5.84	9.97
Annulus height (μm)	2.67 ± 0.01	1.51	4.09	2.86 ± 0.01	1.43	4.02	2.88 ± 0.01	1.88	3.99
Annulus area (μm^2)	18.79 ± 0.12	8.66	37.51	21.67 ± 0.14	9.22	39.45	22.84 ± 0.14	11.80	34.45
Exine thickness (μm)	1.91 ± 0.01	1.17	3.11	2.04 ± 0.01	1.33	3.24	2.11 ± 0.01	1.31	3.37
Diameter (μm)	22.08 ± 0.06	16.22	28.83	22.95 ± 0.06	16.73	28.28	23.99 ± 0.06	18.39	30.05

[a] SE = standard error of the mean.

pollen grains included in the dataset for each species. The resulting U statistic gives a quantitative measure of variable distribution overlap, with 0 indicating no overlap in trait distribution and 100 indicating complete overlap. The statistical significance of interspecific differences in arci strength, annulus protrusion, grain shape and pore number was tested using Wilcoxon rank-sum tests. Statistical analyses were performed using R (R Development Core Team, 2007).

Classification and regression tree (CART) analysis was used to devise multi-trait classification methods for identifying alder pollen to species. CART uses recursive partitioning of independent variables to create a binary decision tree that is conceptually similar to a standard dichotomous identification key (Breiman et al., 1984). Graphical output consists of a tree encompassing internal binary nodes that coincide with specific splitting variables and threshold values, and terminal nodes that unify data into a specific class (i.e., a species). The probability of correct classification for each specific terminal node is quantified via the number of correctly classified cases within that node. Total model classification error is a function of misclassification across the terminal nodes. The tree that results from CART modelling is pruned to minimize cross-validation error and avoid over-fitting via an assessment of model complexity parameters i.e., tree nodes that over-fit data are removed until the decision tree is of an optimal size and misclassification cost is minimized (Breiman et al., 1984; Therneau et al., 2009). Pollen classification begins at the top of the tree. If the internal splitting variables are true for an individual pollen grain, then the right branch is followed. If the criterion is not met, the left branch is followed until end nodes and species classifications are reached.

A classification tree including all three alder species was grown using all quantitative morphological traits as well as arci strength and pore protrusion as model inputs. To determine the accuracy of the resulting decision tree, a randomly selected test set of 30% of the data was held in reserve and used to test model predictions. To assess the possibility of identifying pollen in regions where only two of the three species co-occur, two species classification trees were also built for *A. rubra* and *A. viridis* subsp. *sinuata*, and for *A. viridis* subsp. *sinuata* and *A. incana* subsp. *tenuifolia*. Again, 30% test sets were held in reserve. Two further CART models were derived for the three species and the *A. rubra* and *A. viridis* subsp. *sinuata* datasets, but with qualitative traits excluded. CART analysis was performed using the 'Rpart' package (Therneau et al., 2009) in the R statistical environment (R Development Core Team, 2007). As CART derived decision trees can be unstable i.e., small changes in the data used to create the tree can result in changes in important splitting variables (Sutton, 2005), random forest analysis was used to create an overall ranked list of morphological trait importance and to support CART models for species identification. Random forest models generate large quantities of bootstrapped trees via random sampling and classify data input by combining the results of all generated trees. Ranking of morphological trait importance for classification is a function of each trait's mean Gini decrease. Random forest modelling was performed using the 'randomForest' package (Liaw and Wiener, 2002) in R.

3. Results

3.1. Alnus pollen morphology and variability

Despite different ecologies, life history traits and species ranges, the pollen morphology of the three western North American alders is very similar (Fig. 2). Alder pollen appear psilate, i.e. with no exine ornamentation, when observed under standard light microscopy, but scanning electron microscopy reveals that alder pollen are finely scabrate (Blackmore et al., 2003). Alder pollen range in diameter from 16.2 to 30.1 μm, with almost complete overlap between the three species (Table 1). Alder pollen are stephanoporate with three to six annulate pores (Table 2) arranged equatorially (Fig. 2), with the endexine detached from that of the pores, forming a vestibulum. Pores tend to protrude with each surrounded by exine thickening (an annulus) that is 1.4–4.1 μm in height and 4.7–10.2 μm in width (Table 1). The overall shape is oblate (i.e., two flattened sides opposite each other) and often there are thickened, curved bands (arci) connecting the pores. The strength of the arci varies in all three alder species and grains with no visible arci occur in all three species (Table 2). For all of the quantitative traits, the smallest mean dimensions occur in *Alnus viridis* subsp. *sinuata* and the largest occur in *A. rubra* (Table 1; Fig. 3). Mean values for *A. incana* subsp. *tenuifolia* are intermediate across all quantitative traits. However, there is extensive overlap in morphological traits between all three species (Tables 1 and 2; Fig. 3).

3.2. Morphological trait comparisons between Alnus species

Qualitative morphological traits and the number of pores per pollen grain vary within each alder species, but most alder pollen are 4- or 5-pored (Table 2). The number of pores differs significantly between *A. incana* subsp. *tenuifolia* and each of the other two species ($p=<0.001$ for both pair-wise comparisons), but not between *A. viridis* subsp. *sinuata* and *A. rubra*. Pore protrusion is also variable, with moderate protrusion (class 2) most common in all three species (Table 2), but no statistically significant differences were found between the three alder species in this trait. However, the pollen of the three alder species differ significantly in arci strength (*A. viridis* subsp. *sinuata* and *A. incana* subsp. *tenuifolia*, $p=0.006$; *A. viridis* subsp. *sinuata* and *A. rubra*, $p=<0.001$; *A. incana* subsp. *tenuifolia* and *A. rubra*, $p=0.002$) with arci less pronounced in *A. viridis* subsp. *sinuata* than in the other two species (Table 2). Statistically significant differences in overall shape also exist (*A. viridis* and *A. incana* subsp. *tenuifolia*, $p=0.002$; *A. viridis* subsp. *sinuata* and *A. rubra*, $p=<0.001$; *A. incana* subsp. *tenuifolia* and *A. rubra*, $p=0.001$). Overall shape varies in all three species with most *A. rubra* having a convex shape (Table 2). However, considerable intraspecific variability in these qualitative traits indicates that none of the traits on their own are sufficient for distinguishing the pollen of these three alder species.

Table 2
Percentage of *Alnus* pollen grains classified as each qualitative morphological trait.

Morphological trait	*Alnus viridis* subsp. *sinuata* $n=1050$	*Alnus incana* subsp. *tenuifolia* $n=810$	*Alnus rubra* $n=930$
Number of pores[a]			
3	0.3	0.9	0.5
4	22.5	47.8	23.4
5	70.3	49.4	72.4
6	6.9	1.8	3.6
Pore protrusion			
1	15.4	12.0	14.7
2	51.1	47.9	52.4
3	33.4	40.1	32.9
Arci strength			
0	6.0	4.3	1.3
1	10.3	4.1	1.7
2	26.5	17.5	9.5
3	27.6	27.4	19.1
4	22.8	29.8	36.9
5	6.9	16.9	31.5
Pollen shape			
Concave	26.9	12.2	5.7
Mixed	46.2	44.4	31.2
Convex	26.9	43.3	63.1

[a] For the number of pores, $n=8050$ for *A. viridis* subsp. *sinuata*, $n=6210$ for *A. incana* subsp. *tenuifolia*, and $n=7130$ for *A. rubra*.

Nested ANOVA models comparing morphological traits between species indicate that there are statistically significant differences in pollen morphology both between and within species (Table 3; Fig. 3). This general trend of statistically significant differences is consistent across all quantitative morphological traits with the exception of annulus height, which does not differ significantly between *A. rubra* and *A. incana* subsp. *tenuifolia*. Between species variation accounts for 74.4–91.4% of total model variance for all quantitative traits, with variation within species and variation within individual plants accounting for the remaining 8.6–25.6% of variation (Supplementary Table 2). The low amount of variation within individual plants, relative to the variation between species, suggests that individual alder plants produce pollen that is morphologically similar. This contrasts with the morphometric results of Clegg et al. (2005) who found that morphological variation within individual *Betula* plants accounted for between 30.1 and 49.1% of the total variation.

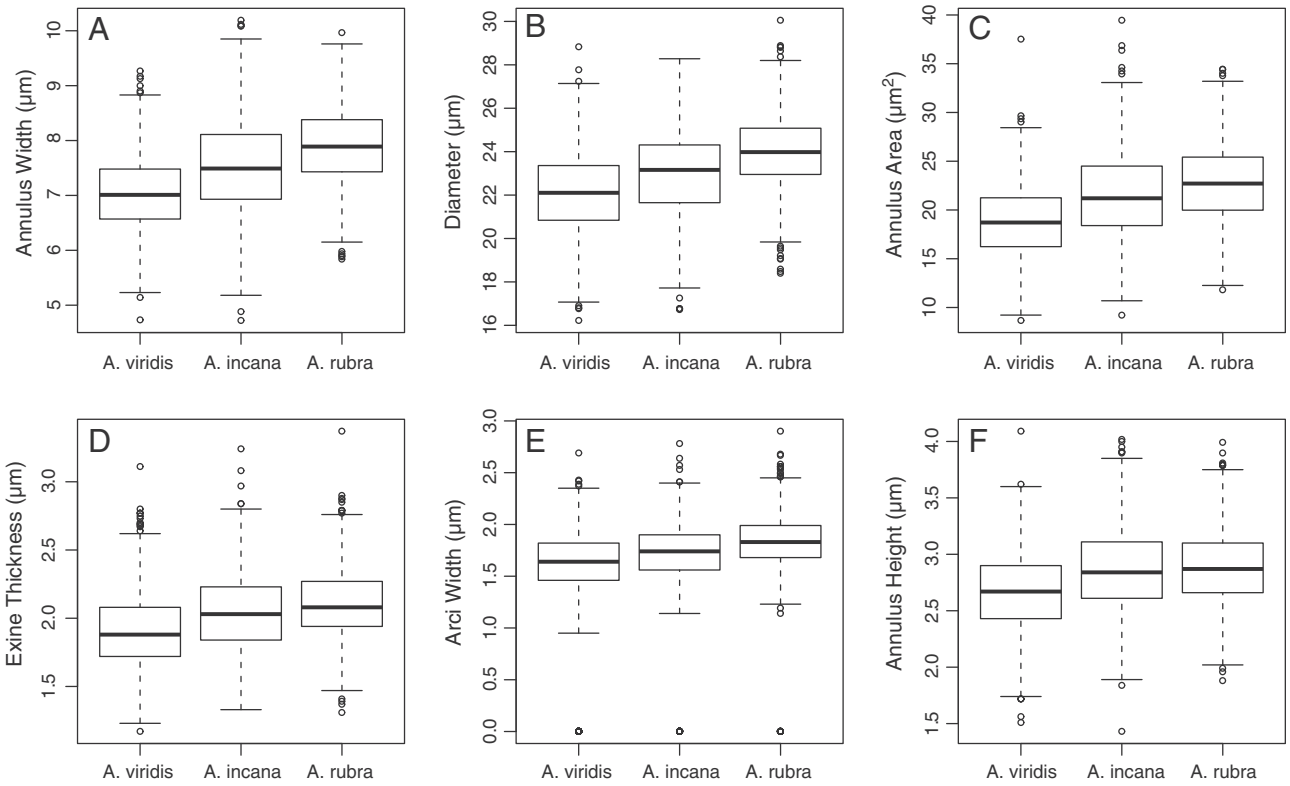

Fig. 3. Box plots showing within-species variability and between-species overlap for each quantitative morphological trait in *Alnus* pollen: (A) annulus width, (B) diameter, (C) annulus area, (D) exine thickness, (E) arci width, and (F) annulus height. Solid lines bisecting each box plot represent the trait median for that species across all samples. Box edges mark the first and third quartiles. Whiskers extend to the smallest and largest non-extreme data points. Species are *A. viridis* = *Alnus viridis* subsp. *sinuata*; *A. incana* = *Alnus incana* subsp. *tenuifolia*; and *A. rubra* = *Alnus rubra*.

Table 3
Summary results of nested ANOVA analyses for each quantitative morphological trait.

Pair-wise comparisons[a]	Nested comparison (between species)		Nested comparison (within species)		U Statistic (U)($2/n_1n_2$)
	F-ratio	*p*-value	F-ratio	*p*-value	Overlap (%)
Arci width					
V–I	38.64	<**0.001**	5.90	<**0.001**	79.2
V–R	232.21	<**0.001**	6.01	<**0.001**	57.1
I–R	67.43	<**0.001**	7.54	<**0.001**	78.9
Annulus width					
V–I	277.02	<**0.001**	15.72	<**0.001**	67.1
V–R	969.77	<**0.001**	9.75	<**0.001**	37.2
I–R	157.51	<**0.001**	15.93	<**0.001**	73.5
Annulus height					
V–I	182.38	<**0.001**	10.39	<**0.001**	72.3
V–R	257.98	<**0.001**	9.84	<**0.001**	64.6
I–R	1.56	0.213	9.92	<**0.001**	98.0
Annulus area					
V–I	340.39	<**0.001**	16.62	<**0.001**	65.1
V–R	780.12	<**0.001**	13.10	<**0.001**	44.5
I–R	48.38	<**0.001**	16.22	<**0.001**	84.6
Exine thickness					
V–I	153.74	<**0.001**	15.93	<**0.001**	74.5
V–R	394.92	<**0.001**	13.43	<**0.001**	56.7
I–R	44.37	<**0.001**	18.24	<**0.001**	86.5
Grain diameter					
V–I	161.98	<**0.001**	23.63	<**0.001**	74.8
V–R	924.46	<**0.001**	19.00	<**0.001**	42.5
I–R	230.48	<**0.001**	20.31	<**0.001**	71.1

[a] V = *Alnus viridis* subsp. *sinuata*, I = *Alnus incana* subsp. *tenuifolia*, R = *Alnus rubra*. Significant *p*-values are in bold.

Scaled U statistics indicate extensive overlap in morphological traits between all three alder species. The greatest amount of morphological overlap occurs between *A. rubra* and *A. incana* subsp. *tenuifolia* (71.1–98.0%) and the least amount of overlap between *A. rubra* and *A. viridis* subsp. *sinuata* (37.2–64.6%), again reflecting the intermediate morphological position of *A. incana* subsp. *tenuifolia* relative to the other two species (Table 3). This trend is consistent across all quantitative morphological traits as well as arci strength and overall pollen shape. While there are statistically significant interspecific differences in mean values for most traits, the large amount of intraspecific morphological variability as well as the interspecific overlap in morphology precludes the use of mean values for pollen identification to species. As with the qualitative morphological traits, none of the quantitative traits can be used on their own for identifying alder pollen to species.

3.3. Multi-trait CART models for identifying fossil alder pollen to species

CART analysis provides a multi-trait method for identifying alder pollen. The full CART model derived for all three alder species classifies pollen grains based on annulus width, arci strength and exine thickness (Fig. 4A). Model accuracy is 89.1% and 61.0% for *A. viridis* subsp. *sinuata* pollen and *A. rubra* pollen, respectively; however, of the *A. incana* subsp. *tenuifolia* pollen used to create the decision tree, only 5.5% are classified accurately (Table 4). Total model classification error is 44.6%, which is explained in part by the misclassification of *A. incana* subsp. *tenuifolia* pollen as both *A. rubra* and *A. viridis* subsp. *sinuata*. When model classification accuracy is tested using the 30% test set (Table 4), 89.8%, 3.3% and 58.8% of *A. viridis* subsp. *sinuata*, *A. incana* subsp. *tenuifolia* and *A. rubra* pollen are classified to species correctly. The intermediate morphology of *A. incana* subsp. *tenuifolia* pollen prevents the model from accurately classifying pollen from this species, which in turn inflates overall model classification error. Misclassification and high model error are even more pronounced if the CART model is built using only

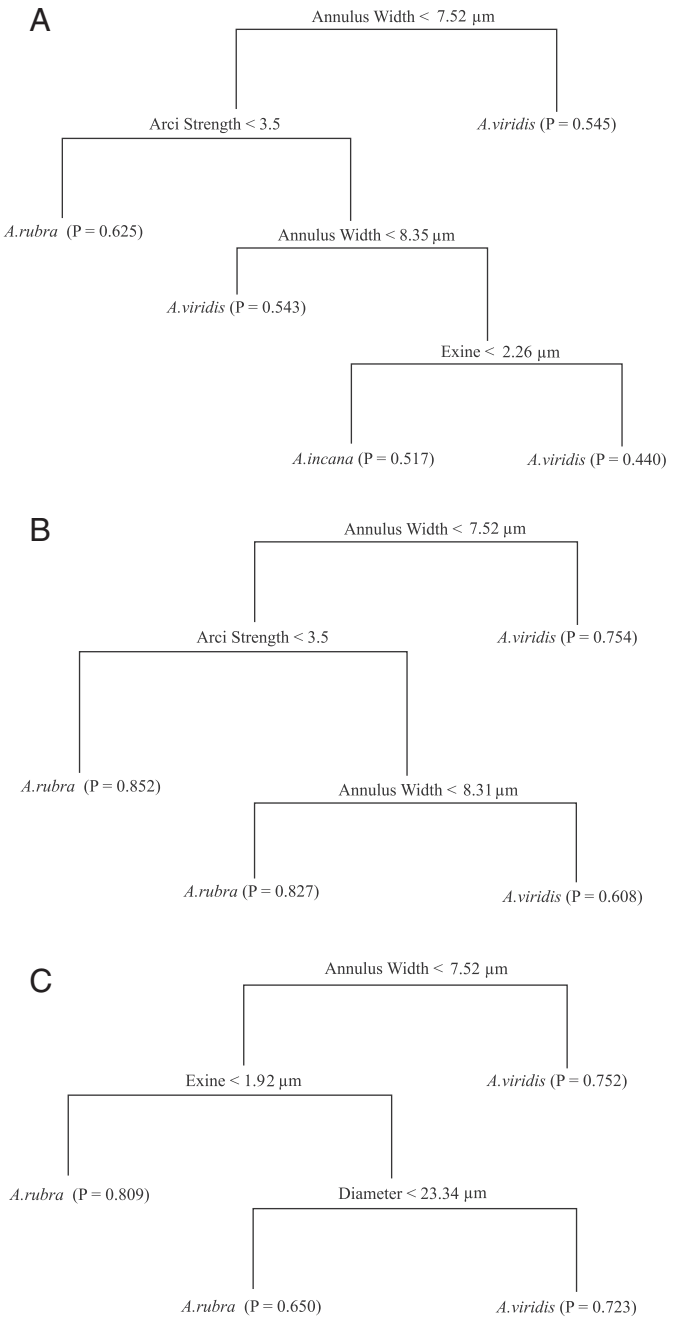

Fig. 4. CART derived decision tree for the simultaneous classification of (A) *Alnus viridis* subsp. *sinuata*, *Alnus incana* subsp. *tenuifolia* and *Alnus rubra* pollen, and (B) *Alnus viridis* subsp. *sinuata* and *Alnus rubra* pollen with all quantitative traits and arci strength and pore protrusion are model input. Shown in (C) is the CART model for separating *Alnus virdis* subsp. *sinuata* and *Alnus rubra* built using quantitative traits only. Morphological splitting variables and threshold values occur at each internal node. Terminal nodes indicate species classification and the within-model probability of correct classification.

quantitative traits. This decision tree classifies pollen solely on the basis of annulus width and classifies pollen as either *A. rubra* or *A. viridis* subsp. *sinuata* i.e., classification accuracy for *A. incana* subsp. *tenuifolia* is 0%.

The two species CART model for *A. viridis* subsp. *sinuata* and *A. incana* subsp. *tenuifolia* failed to separate these two species: model accuracy for *A. incana* subsp. *tenuifolia* is only 46% and 55% of test set pollen grains of this species are misclassified as *A. viridis* subsp. *sinuata*. However, the two species CART model for separating *A. rubra* and *A. viridis* subsp. *sinuata* is reasonably successful as differentiating these two species. This CART model uses annulus width and

Table 4
Three species and two species CART classification accuracies for model and test set pollen.

A. Three species CART model (Fig. 4A)

		Alnus viridis subsp. *sinuata* ($N=700$)		*Alnus incana* subsp. *tenuifolia* ($N=540$)		*Alnus rubra* ($N=620$)	
Identified as	Data	*n*	%	*n*	%	*n*	%
A. viridis subsp. *sinuata*	Model	624	89.1	336	62.2	224	36.2
	Test set	314	89.8	174	64.6	119	38.5
A. incana subsp. *tenuifolia*	Model	10	1.5	30	5.5	18	2.8
	Test set	9	2.6	9	3.3	8	2.7
A. rubra	Model	66	9.4	174	32.3	378	61.0
	Test set	27	7.6	87	32.1	183	58.8

B. Two species CART model (Fig. 4B)

		Alnus viridis subsp. *sinuata* ($N=700$)		*Alnus rubra* ($N=620$)	
Identified as	Data	*n*	%	*n*	%
A. viridis subsp. *sinuata*	Model	631	90.2	237	38.2
	Test set	321	91.7	128	41.2
A. rubra	Model	69	9.8	383	61.8
	Test set	29	8.3	182	58.8

C. Two species CART model (quantitative traits only) (Fig. 4C)

		Alnus viridis subsp. *sinuata* ($N=700$)		*Alnus rubra* ($N=620$)	
Identified as	Data	*n*	%	*n*	%
A. viridis subsp. *sinuata*	Model	584	83.5	200	32.3
	Test set	289	82.6	91	29.4
A. rubra	Model	116	16.5	420	67.7
	Test set	61	17.4	219	70.6

arci strength to differentiate *A. rubra* and *A. viridis* subsp. *sinuata* pollen (Fig. 4B). Model and test set accuracy for *A. viridis* subsp. *sinuata* and *A. rubra* classification are improved compared to the three species CART model (Table 4). The model classifies *A. viridis* subsp. *sinuata* pollen most accurately, with 90.2% of pollen grains used in creating the model correctly identified and 91.7% of test set grains classified accurately. Model classification for *A. rubra* pollen is less accurate at 61.8%, with 58.8% of *Alnus rubra* test set grains classified accurately. Total model error is reduced to 23.1%, compared to 44.6% in the three species CART model.

To determine the model that best differentiates *A. viridis* subsp. *sinuata* and *A. rubra* pollen, CART analysis was also performed with all qualitative traits excluded. This model classifies 83.5% of *A. viridis* subsp. *sinuata* and 67.7% of *A. rubra* pollen correctly (Table 4), on the basis of annulus width, exine thickness and diameter (Fig. 4C). Test set pollen classification is comparable to model prediction, with 82.6% and 70.6% of *A. viridis* subsp. *sinuata* and *A. rubra* test grains classified correctly. In general, the CART identification methods for distinguishing *A. viridis* subsp. *sinuata* and *A. rubra* pollen are supported by random forest analysis, with annulus width and overall diameter ranked as the two most important traits for distinguishing the pollen of these species (Supplementary Table 3).

4. Discussion

4.1. Alder pollen identification

What is most clear from the morphometric analyses on alder pollen is that no morphological trait on its own is sufficient for species-level pollen identification. There are statistically significant differences in the pollen morphology of all three species, but given the substantial degree of overlap in morphological variability between species, these differences in mean trait values are not particularly useful for identifying fossil pollen. Instead, the pollen morphologies of the three alder species form a morphological continuum, where *A. viridis* subsp. *sinuata* pollen are the smallest across traits, *A. rubra* are the largest, and *A. incana* subsp. *tenuifolia* are intermediate (Table 1; Fig. 3). A comparison of mean trait values and/or reliance on one or a few morphological traits is insufficient when attempting to differentiate the pollen of these three alder species.

CART analysis produces a multi-trait method for pollen identification by defining important morphological traits and threshold values for these traits. In doing so, CART analysis provides a better tool for classification of alder pollen than is possible by examining individual morphological traits in isolation. However, the classification error for *A. incana* subsp. *tenuifolia* was over 90% in the three species CART model (Fig. 4A, Table 4) and 100% in a model that excluded qualitative morphological traits. As the morphology of *A. incana* subsp. *tenuifolia* pollen is intermediate between the other two species, morphological overlap is extensive enough to prevent CART models from defining appropriate trait thresholds by which to classify *A. incana* subsp. *tenuifolia* pollen. *Alnus incana* subsp. *tenuifolia* pollen cannot be reliably distinguished from that of the other two species.

The intermediate pollen morphology of *A. incana* subsp. *tenuifolia* does not reflect the genetic relatedness of these three species of *Alnus*. The *A. incana* species complex and *A. rubra* both fall within the *Alnus* subgenus in the broader genus *Alnus*, whereas *A. viridis* subsp. *sinuata* is part of the phylogenetically distinct *Alnobetula* subgenus (Chen and Li, 2004). These phylogenetic patterns are not reflected in pollen morphology. Changes in pollen morphology are likely driven by within-range selection pressures related to plant–pollinator interactions, climate, and/or other factors (e.g., Ejsmond et al., 2011).

Alnus incana subsp. *tenuifolia* does not currently occur along the Pacific coast of North America (Fig. 1), so a method for distinguishing pollen of the other two *Alnus* species is useful for increasing the taxonomic resolution of Quaternary pollen records from this region. With *A. incana* subsp. *tenuifolia* excluded, CART analyses provide robust solutions for the separation of pollen from *A. rubra* and *A. viridis* subsp. *sinuata*, both of which are common along the north Pacific coast (Fig. 4). CART classification accuracies for the two species models range between 61.8 and 91.7% (Table 4), results that are comparable or better than CART classification accuracies for species of *Picea* (Lindbladh et al., 2002) and *Pinus* (Barton et al., 2011). The two species CART models are appropriate for differentiating alder pollen only when it can be safely assumed that *A. incana* subsp. *tenuifolia* has not occurred in the pollen source area at any time over the period of record. Small, light seeds are characteristic of *Alnus*, as is the ability to disperse rapidly, particularly along waterways. These life history characteristics combined with substantial changes in climate (Wright et al., 1993; Williams et al., 2004) suggest that large shifts in the late Quaternary ranges of alder species occurred along the Pacific coast of North America. However, while *A. incana* subsp. *tenuifolia* macrofossils (e.g., seeds) have been found in northern locations such as northern Yukon (Matthews, 1975), no macrofossils of this species have been found in late Quaternary sediments along the Pacific coast, where *A. rubra* and *A. viridis* subsp. *sinuata* macrofossils are common (e.g., Cwynar, 1987; Wainman and Mathewes, 1987; Lacourse et al., 2007). Therefore, it is unlikely that *A. incana* subsp. *tenuifolia* occurred along the Pacific coast during the late Quaternary in conjunction with the other two species of alder. It is possible that alder species that do not currently occur in the region were present in the past. For instance, *Alnus rhombifolia*, currently native to southern Oregon and California (Thompson et al., 1999), may previously have had a more northern distribution (Furlow, 1979; Reinink-Smith, 2010), in which case its pollen would confound species identification. Furthermore, studies of modern pollen

distributions show that alder pollen are routinely blown far outside the boundaries of current species ranges, travelling distances in the hundreds of kilometres (MacDonald and Ritchie, 1986; Fægri and Iversen, 1989; Mayle et al., 1993; Whitmore et al., 2005). For these reasons, the absence of *A. incana* subsp. *tenuifolia* and reliable discrimination of *A. viridis* subsp. *sinuata* and *A. rubra* pollen must be assumed with caution, especially in areas close to modern range limits.

If the absence of *A. incana* subsp. *tenuifolia* pollen can be safely assumed, then separation of *A. viridis* subsp. *sinuata* and *A. rubra* pollen can be achieved via CART modelling (Fig. 4B, C). Even with *A. incana* subsp. *tenuifolia* excluded, CART models are not able to differentiate *A. viridis* subsp. *sinuata* and *A. rubra* pollen in all cases (Table 4). We recommend that the two species CART models be used for separating *A. viridis* subsp. *sinuata* and *A. rubra* pollen into two morphotypes that are analogous to species separation and representative of their shrub vs. tree growth forms. In general, these results are similar to those of Clegg et al. (2005), who found that pollen of congeneric *Betula* species, the closest relatives to *Alnus* (Navarro et al., 2003), could not be identified to species, but could be separated into 'tree birch' and 'shrub birch' pollen based on differences in pore depth and pore-diameter ratio.

The two species CART model built using all morphological traits isolates *A. viridis* subsp. *sinuata* and *A. rubra* pollen based on annulus width and arci strength (Fig. 4B), whereas the two species CART model built using only quantitative traits separates the two species based on annulus width, exine thickness and diameter (Fig. 4C). Accordingly, we propose criteria for morphotype separation that combines the most important quantitative and qualitative traits: individual alder pollen should be classified as *A. viridis*-type if they have an annulus width <7.5 μm, arci that are weak to moderate in strength (1–3), an overall diameter of <23 μm, and exine thickness <1.9 μm, otherwise pollen are classified as *A. rubra*-type. Overall shape may be useful for assigning fossil pollen grains to *A. rubra*-type, as *A. rubra* has a much higher proportion of convex pollen than *A. viridis* subsp. *sinuata* (Table 2). If opposing group qualifiers are met for an individual pollen grain, then it should be classified as '*Alnus* undifferentiated'. The traits identified as important for the separation of alder pollen into an *A. viridis*-type and an *A. rubra*-type support the morphological differences recognized previously by palynologists separating alder pollen into two morphotypes in western North America (e.g. Lacourse, 2005) as well as eastern North America (e.g. Mayle et al., 1993). Using the number of pores per alder pollen grain to differentiate species, which has been suggested as a possible identification method by Reinink-Smith (2010), is not supported by our large dataset ($n = 21{,}390$) and was similarly rejected as a method for separating alder species in fossil pollen records by Hansen and Easterbrook (1974).

4.2. Implications of alder pollen identification for paleoecological studies

Identifying fossil alder pollen as *A. viridis*-type or *A. rubra*-type is valuable for paleoecological reconstructions because it allows for the distinction between tree (*A. rubra*-type) and shrub alder (*A. viridis*-type) in areas where *A. incana* subsp. *tenuifolia* is not present such as along the north Pacific coast of North America. This distinction, while primarily associated with growth form, also reflects important ecological and functional life history differences: in addition to obtaining a larger adult height, *A. rubra* has a longer lifespan, larger seed mass, lower cold tolerance, and higher shade and drought tolerance than *A. viridis* subsp. *sinuata* (Lacourse, 2009). Separating the pollen of these two taxa is therefore important for enhancing our understanding of Holocene plant community dynamics in western North America.

The ability to distinguish the pollen of different species in fossil records is especially important when species have differential responses to environmental conditions and differing ecologies (Finkelstein et al., 2006), as is the case for *A. viridis* subsp. *sinuata* and *A. rubra* along the north Pacific coast. While most paleoecological studies from this region have not identified alder pollen past the generic level, studies that have separated alder pollen into morphotypes show that *A. viridis* and *A. rubra* have different postglacial histories (e.g. Cwynar, 1990; Lacourse, 2005) and forest associations (Gavin et al., 2005). Lacourse (2005, 2009) demonstrated that individual alder species had strong temporal associations with different conifers on northern Vancouver Island following the last glaciation: *A. rubra*-type pollen increased in abundance during the expansion of *Picea sitchensis* and then decreased and increased along with this conifer through the Younger Dryas, wheras *A. viridis*-type pollen was closely associated with the arrival and dynamics of other conifers i.e., *Pinus contorta* and *Tsuga mertensiana*. A similar association occurs between *A. rubra*-type and *Pseudotsuga menziesii* pollen in Holocene lake sediment records from coastal Oregon (e.g., Long et al., 2007). These patterns suggest that *A. rubra* and *A. viridis* subsp. *sinuata* played different roles in facilitating the establishment of conifers following the last glaciation that are consistent with modern successional trends (e.g., Chapin et al., 1994). However, the distinct postglacial histories for the two species of alder as well as their interactions with conifer populations are lost when *Alnus* pollen are not differentiated. More paleoeclogical studies that differentiate *A. viridis*-type and *A. rubra*-type pollen are needed so that these types of species interactions can be investigated on long ecological timescales. In some instances, increased taxonomic precision in fossil pollen analysis may also permit separation of exogenous drivers of Holocene ecosystem change such as climate from endogenous processes such as succession and competition (Flenley, 2003).

5. Conclusions

This research demonstrates clearly that no morphological trait can be used on its own to distinguish the pollen of the three common alder species in western North America. Despite statistically significant differences in mean values, the pollen morphology of each species is highly variable across traits, with a large degree of morphological overlap between species. The intermediate morphology of *Alnus incana* subsp. *tenuifolia* prevents the pollen of this species from being distinguished from that of the other two species, and pollen of the two shrub alders, *A. viridis* subsp. *sinuata* and *A. incana* subsp. *tenuifolia*, also cannot be differentiated. However, the method derived from our large dataset confirms that individual alder pollen can be assigned to one of two morphotypes (i.e., *A. rubra*-type and *A. viridis*-type), analogous to species separation, where and when *A. incana* subsp. *tenuifolia* is absent. Multi-trait CART modelling provides trait thresholds for differentiating *A. viridis* subsp. *sinuata* and *A. rubra* pollen into these ecologically relevant morphotypes. The principal morphological traits that distinguish these two pollen morphotypes are annulus width, arci strength, exine thickness, and diameter. Given the intermediate morphology of *A. incana* subsp. *tenuifolia* pollen, these morphotype identifications should only be used in regions where it can be safely assumed that *A. incana* subsp. *tenuifolia* has been absent from the pollen source area for the entire period of record e.g., in coastal British Columbia. Differentiating fossil *Alnus* pollen using this morphotype method will greatly enhance the taxonomic resolution of Quaternary pollen records from the north Pacific coast and the paleoecological and paleoenvironmental inferences that can be drawn from them.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.revpalbo.2012.04.007.

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Supplementary Table 1: Herbarium specimens of *Alnus* spp. used for pollen morphometric measurements in this study.

Species	Herbarium Accession#	Sample Location (as noted on herbarium sheets)
Alnus incana	43248[1]	Sumass Mt., 1 km west of Quading farm, British Columbia
subsp. *tenuifolia*	V105976[2]	Juab near Nephi, Utah
	V107227[2]	Similkameen River Valley, British Columbia
	V109975[2]	Jasper National Park, Athabasca River banks, near Pocahontas, Alberta
	V111096[2]	6.4 km north of Trail, Columbia River Valley, British Columbia
	V165973[2]	Bulkley-Nechako Regional District, Maclure Lake near Telkwa, British Columbia
	V170499[2]	10 km north of Clearwater Village (De Kelver's place), British Columbia
	V177016[2]	Stikine River basin, north end of Eddontenajon Lake, British Columbia
	V205824[2]	Fraser-Fort George Regional District, Miworth, Prince George, British Columbia
	V221486[2]	Peace River Regional District, Carbon Creek, Ten Mile Creek, British Columbia
	V45684[2]	Big Lake, Caribou, British Columbia
	V61455[2]	above Nelson, British Columbia
	V6417[2]	Dawson, Yukon
	V6421[2]	Armstrong, Okanagan, British Columbia
	V65180[2]	Sheep Creek at Turner Valley, Alberta
	V72230[2]	Jaffray, British Columbia
	V73200[2]	Cedar Mt, Latah County, Idaho
	V134341[3]	Trail, British Columbia
	V29468[3]	Nelson, British Columbia
	V061521[3]	Trail, Topping Creek, British Columbia
	V082070[3]	near Lower Post, British Columbia
	V160213[3]	Anarchist Mt, British Columbia
	V149481[3]	White Lake, Salmon Arm Forest District, British Columbia
	V151891[3]	McLure Ferry, Kamloops, British Columbia
	V151907[3]	Osoyoos Oxbows, north of Lake, British Columbia
	RM25[4]	Buck Hills Ecological Reserve, near Lumby, British Columbia
	RM26[4]	near Radium, British Columbia
Alnus rubra	003837[1]	Allouette Lake area, British Columbia
	003841[1]	Cowichan Station, southwest Duncan (Koksilah River Drainage), British Columbia
	003843[1]	Beaver Lake, British Columbia

	30974[1]	University of Victoria campus, Victoria, British Columbia
	30995[1]	University of Victoria campus, Victoria, British Columbia
	24722[1]	Cordova Bay, Victoria, British Columbia
	V112076[2]	Lighthouse Park, west Vancouver, trail 1, grid-3d, British Columbia
	V125583[2]	west edge of Santa Cruz Mts., 2 miles south of Davenport, California
	V137600[2]	Powell River, British Columbia
	V138413[2]	Spanish Banks, University of British Columbia area, Vancouver, British Columbia
	V196177[2]	1.6 km west of Falls City, Black Rock rd, sec. 17, Polk County, Oregon
	V213587[2]	Vancouver Island, Port Alberni, Somass River delta, British Columbia
	V28165[2]	Spruce Cove, Trinidad, Humboldt County, California
	V6337[2]	Vancouver, West Point Grey, British Columbia
	V6338[2]	Masset, British Columbia
	V6345[2]	Greater Vancouver Regional District, Burquitlam, British Columbia
	V6681[2]	Dryas Island in Fraser River at Hope, British Columbia
	V71258[2]	Oak Ranch Creek, 1.6 km east of route 47, Columbia County, Oregon
	V78952[2]	Vancouver, Point Grey, British Columbia
	V88028[2]	Cheam Lake, British Columbia
	V78631[2]	Oak Ranch Creek, 1.6 km east of route 47, Columbia County, Oregon
	V1391[3]	Esquimalt district, Goldstream, British Columbia
	V34428[3]	Fernwood, Saltspring Island, British Columbia
	V101645[3]	Killarney Rd, Victoria, British Columbia
	V47631[3]	Kitimat, British Columbia
	V148972[3]	Ganges, Salt Spring Island, British Columbia
	V115524[3]	Port Neville Inlet, British Columbia
	V98039[3]	Mayne Island, British Columbia
	V126473[3]	Somass Delta, Port Alberni, British Columbia
	V187605[3]	Kal Lake Provincial Park, Vernon, Cosens Creek, British Columbia
	RM28[4]	University of British Columbia campus, Vancouver, British Columbia
Alnus viridis	23960[1]	Lower Bertha Falls, British Columbia
subsp. *sinuata*	31308[1]	4.8 km south of Nadina River crossing, west end of Francois Lake, British Columbia
	014952[1]	Timothy Mtn, Timothy Lake, Lac la Hache, British Columbia
	003865[1]	Matheson Lake, Vancouver Island, British Columbia
	029190[1]	Haley Lake, Marmot District, Vancouver Island, British Columbia
	003864[1]	Mt. Arrowsmith, Vancouver Island, British Columbia

V125584[2]	King County, Deception Creek, 12.8 km west of Stevens Pass, Washington
V137595[2]	Powell Lake, Rainbow Lodge, British Columbia
V3409[2]	Bennett, British Columbia
V60917[2]	Gray Creek, British Columbia
V45951[2]	Snohomish County, Cascade Mountains, Perry Creek trail, Washington
V87864[3]	Alsek River, ca. 2.5 km north of the British Columbia Boundary, Alaska
V1389[3]	Mt Mclean, British Columbia
V6363[3]	Skidegate, Queen Charlotte Islands (Haida Gwaii), British Columbia
V29472[3]	bank of Kicking Horse Gorge, Yoho National Park, Alberta
V22839[3]	Langara Island, Queen Charlotte Islands (Haida Gwaii), British Columbia
V45469[3]	Allouette Lake area, British Columbia
V47627[3]	Kitimat, British Columbia
V47628[3]	Kitselas Canyon Rd., northeast of Terrace, British Columbia
V49593[3]	South shore of Charlotte Lake, west Chilcotin, British Columbia
V54399[3]	9.6 km north of Prince George, British Columbia
V062037[3]	Copper Island, Atlin, British Columbia
V060843[3]	Silver Creek, Hope, British Columbia
V114447[3]	Rossland, Record Ridge, British Columbia
V069231[3]	Murray Ridge, northeast of Fort St. James, British Columbia
V075162[3]	Red Pass, Park Headquarters, British Columbia
V115522[3]	Port Neville Inlet, British Columbia
V144564[3]	Stewart-Cassiar Hwy, British Columbia
V87436[3]	Alsek River, 5km south of the Yukon border, British Columbia
V112152[3]	4 km southeast of Mackenzie Ranger Station, British Columbia
V182763[3]	McBride Peak, 7 km from highway up Mount View Rd, British Columbia
V165960[3]	Sewell Inlet, south of Sewell Inlet, Moresby Island, British Columbia
V38329[3]	Lake Whatcom, Washington
V89293[3]	Apex Mt. Rd, 1.6 km from intersection with Old Penticton Rd, British Columbia
V139552[3]	Chase Shuswap Territory, near Pillar Lake, British Columbia

[1] University of Victoria Herbarium, Department of Biology, Victoria, British Columbia
[2] University of British Columbia Herbarium, Beaty Biodiversity Museum, Vancouver, British Columbia
[3] Royal British Columbia Museum Herbarium, Victoria, British Columbia
[4] The pollen reference collection of Dr. Rolf Mathewes, Simon Fraser University, Burnaby, British Columbia

Supplementary Table 2: Nested ANOVA results and variance component analyses comparing the six quantitative morphological traits between all three alder species (*Alnus viridis* subsp. *sinuata, Alnus incana* subsp. *tenuifolia*, and *Alnus rubra*). See Table 3 of the main paper for F-ratios and P-values for species pair-wise comparisons.

Quantitative Trait and Source of Variance	df	Sum of Squares	Mean Squares	Variance Component[a]	% Variance
Arci width (µm)					
Between species	2	31.90	15.95	0.4850	74.4
Within species	90	80.93	0.90	0.0250	3.8
Within individual plants	2696	382.85	0.14	0.1420	21.8
Annulus width (µm)					
Between species	2	371.18	185.59	5.8158	91.4
Within species	90	477.42	5.30	0.1637	2.6
Within individual plants	2696	1043.40	0.39	0.3870	6.1
Annulus height (µm)					
Between species	2	30.02	15.01	0.4516	77.6
Within species	90	90.16	1.01	0.0303	5.2
Within individual plants	2696	268.49	0.10	0.1000	17.2
Annulus area (μm^2)					
Between species	2	8635.40	4317.70	133.7310	89.0
Within species	90	15483.00	172.04	5.3603	3.6
Within individual plants	2696	30268.00	11.23	11.2270	7.5
Exine thickness (µm)					
Between species	2	20.99	10.50	0.3132	80.5
Within species	90	71.49	0.79	0.0247	6.3
Within individual plants	2696	136.27	0.05	0.0510	13.1
Grain diameter (µm)					
Between species	2	1781.80	890.87	27.3703	89.1
Within species	90	3815.50	42.39	1.3457	4.4
Within individual plants	2696	5435.40	2.02	2.0160	6.6

[a] Variance explained by each nested factor

Supplementary Table 3: Random Forest derived mean Gini decrease and morphological trait importance for each model. Models shown are those based on *Alnus viridis* subsp. *sinuata* and *Alnus rubra*; *Alnus incana* subsp. *tenuifolia* is excluded.

	Quantitative & Qualitative Traits (OOB[a] =21.3%)		**Quantitative Traits Only** (OOB=24.6%)	
Trait	Gini	Rank	Gini	Rank
Annulus width	139.73	1	242.01	1
Grain diameter	122.76	2	207.93	2
Annulus area	99.62	3	138.31	5
Arci width	84.26	4	138.58	4
Arci strength	82.13	5	-	-
Exine thickness	80.04	6	163.07	3
Annulus height	60.53	7	95.66	6
Pore protrusion	19.28	8	-	-

[a] Out of bag error rates