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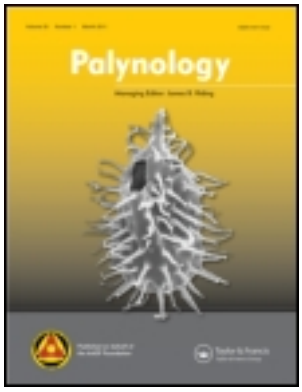

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Pollen morphology of the three subgenera of *Alnus*

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The three subgenera of alder (*Alnus*, *Clethropsis* and *Alnobetula*) are taxonomically distinctive, but how does their pollen match up with their gross morphology? This study links pollen characters with the taxonomic divisions of the genus alder based on nrDNA ITS sequence data. The value of the study is in the breadth of the taxonomic coverage of the three subgenera, as the samples per species only range from 1 to 6. From 29 *Alnus* species, the authors studied pollen from 59 modern reference collections (prepared by acetolysis) and recorded the number and type of apertures (pores), thickness of the arci, pollen size and polar arci. Tallies of the number of pores on the pollen grains in a reference collection typically have a range of $\pm$ 1 or 2. Exceptions are presumed hybrid collections in *Alnus rugosa* with a range of $\pm$ 5, which have malformed pollen cells suggesting infertility. The presumed hybrids show a greater range of both pore number and size of pollen grains. The dominant pore number is a key feature that separates the subgenus *Alnobetula (= Alnaster)* from the other two subgenera by consistently having predominantly 5-, 6- or 7-pored pollen grains, while all other *Alnus* taxa sampled have pollen with predominantly 4- or 5-pored pollen. The dominant pore number is a consistent feature within the subgenus *Alnobetula* (10 species), but is inconsistent in 3 out of 16 species of the subgenus *Alnus* and within the subgenus *Clethropsis* (3 species). Thickenings of the pore lip, aspidate (protruding) and vestibulate pores are important features of *Alnus* pollen on a species level. *Alnus*-like pollen morphology occurs occasionally in certain other betulaceous genera (*Betula* and *Carpinus*). Differences in character or thickness of arci (band-like thickenings on the pollen wall between pores) tend to be somewhat consistent within a species and vary within subgenera. Two *Alnobetula* species in the *Alnus viridis* complex (*Alnus crispa* and *Alnus sinuata*) have arci that are weakly developed or appear as simple folds; walls are fragile and pores are typically less aspidate. Pollen sizes in different species overlap greatly, may be partly influenced by processing methods and do not seem to be generally helpful in characterizing species. Circular doughnut-shaped arci thickenings on the polar area of pollen grains are a common feature (16–40%) in two closely related East Asian taxa of subgenus *Alnobetula* with large pollen ('Clade A': *Alnus firma* and *Alnus sieboldii*). These 'Clade A' features have been reported from Miocene collections of *Alnus* pollen from Alaska, and support megafossil evidence in determining the biogeography of this group along the Pacific Rim in the Neogene. The Miocene appearance of subgenus *Alnobetula* megafossils in both Alaska and Japan can only have occurred via a Bering Land Bridge. This relation is now confirmed by Reinink-Smith's Miocene 'Clade-A-type' pollen data of Alaska. These taxa had Pacific Rim distributions, similar fruiting carposamaras and now correspond to a DNA ITS subgroup within *Alnobetula*. Depending on the geographic location with limited number of local alder species, certain Holocene identifications are possible based on pore structure and arci thickness, e.g. the *Alnus crispa*-type in eastern Canada and the *Alnus sinuata*-type in the Pacific northwest.

Keywords: *Alnus* pollen morphology; DNA systematics; hybrid collections; Bering Land Bridge

1. Introduction

Pollen of flowering plants is generally considered a conservative organ because the morphology of pollen grains tends to be a consistent feature within plant species and within many genera. This study of alder pollen aims to evaluate morphological differences in pollen across species of *Alnus* in order to link pollen morphology with classification of species based on gross plant morphology and/or nrDNA ITS sequence data. Such characteristics may be useful in interpreting the fossil pollen record of *Alnus*.

The genus *Alnus* has one of the oldest pollen records of all dicot genera, as its distinctive pollen first appeared in the Late Cretaceous (Srivastava 1967; Rouse 1971; Brown 1993). *Alnus* pollen and leaves have been consistent elements in North American floras of late Paleocene and Eocene age (MacGinitie 1941; Leffingwell 1971; Wolfe 1977; Lavrenko and

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Fot'janova 1993) and from the Oligocene (Barnett 1989). In Paleocene floras along the Pacific Rim, *Alnus* leaves (Wolfe et al. 1966) and pollen (Barnett and Fisk 1980; Barnosky 1984; Leopold and Wright 1985; Leopold and Liu 1994; Liu and Leopold 1994; Reinink-Smith 2010) are ever-present elements. Identification of taxonomically informative patterns in pollen morphology will provide new tools for clarifying the biogeographic and evolutionary history of the genus.

The purpose of this paper is to document major pollen features of 29 species and 'subspecies' (59 collections in this report) as classified by Chen and Li (2004) within the genus *Alnus*. The value of this study is in its coverage of species, but the number of collections per species is limited. The collections include subgenera *Alnus*, *Alnobetula* and *Clethropsis* to determine if any diagnostic pollen features can be helpful in identifying sections of the genus. This study records the frequency of different aperture (pore) numbers, aspidate condition of the pores, conspicuousness of the outer wall (ektexine) arci thickenings and occurrence of circular arci markings at the poles in a wide number of *Alnus* species. The localities of specimens and source material used in this study are listed in Appendix 1.

2. Background

The genus *Alnus* Miller of the Betulaceae includes between 29 and 35 species, mainly occurring in the Northern Hemisphere. Of these, at least 18 *Alnus* species occur in Asia (Hulten 1968; Murai 1968), about five of which are only known from one to three collections (Global Biodiversity Information Facility [GBIF] website 2010). About eight *Alnus* species occur in North America (Johnson 1968; Flora of North America Editorial Committee 1997) including two species in Mexico, one of which extends southwards into the Andes of South America. Four *Alnus* species occur in Europe (Chen and Li 2004, p. 326).

The subgenera *Alnus* and *Alnobetula* (formerly *Alnaster*) differ significantly by their seed morphology and in the position of the fruiting bodies. In subgenus *Alnobetula*, the seeds have short wings and catkins are borne on leafy twigs of the current year. In subgenus *Alnus*, however, the seeds for several species are wingless or wings are smaller than the seed (Navarro et al. 2003) and catkins are borne on (leafless) twigs of the prior year.

The third (and minor) subgenus *Clethropsis* with three species differs from the others by blooming in the fall. *Clethropsis* species cannot be differentiated from subgenus *Alnus* species by nrDNA ITS studies (Navarro et al. 2003), and their pollen is not discernible from the subgenus *Alnus* in the collections reviewed here.

Previous studies of *Alnus* taxonomy by various authors (reviewed by Chen and Li 2004) have recognized subgenera and sections based on gross morphology and geographic distribution. Chen and Li (2004) (Figure 1) and Navarro et al. (2003) each present recent reclassification of *Alnus* species based on nrDNA ITS sequence data. Navarro et al. (2003) and several previous taxonomists recognized subgenus *Alnobetula* (under the old name *Alnaster*) and a single sister subgenus *Gymnothyrsus* (= subgenus *Alnus*). Murai (1968) and Navarro et al. (2003) both concluded that the genus *Alnus* is a monophyletic group, and that the fundamental dichotomy lies between the subgenus *Alnaster* (= *Alnobetula*) and the rest of the genus. The subgenus *Alnaster* (*Alnobetula*) appears to be "basal in the genus and diverges only slightly from the genus *Betula*". They consider Northeast Asia, which has the largest number of *Alnus* species, to be the position of origin of the genus. Chen and Li (2004) concluded that the low sequence divergence within the *Alnus viridis* complex indicates their recent history of diversification in the circumpolar areas.

In the present paper, the taxonomy follows the taxonomic system used by Chen and Li (2004) who recognized three alder subgenera: cf. *Alnobetula*, *Clethropsis* and *Alnus*. They have elevated a wide number of lineages that have been traditionally recognized as subspecies or varieties to the species level (Figure 1). Chen and Li (2004) grouped closely related species into two complexes: the *Alnus incana* complex and the *Alnus viridis* complex. These complexes appear to have modern hybrids between certain member taxa (Johnson 1968; Murai 1968; Furlow 1979).

Chen and Li (2004) show distribution maps for the three *Alnus* subgenera: *Alnus*, *Clethropsis* and *Alnobetula*. Two species in the subgenus *Alnobetula* are northern shrubs (Hulten 1968, p. 586–590). The three species of subgenus *Clethropsis* (mainly trees) are geographically limited to Japan, southeast Asia and southeast USA. The many taxa of subgenus *Alnus*, which are also mainly trees, tend to have distributions in the warm temperate zone.

2.1. *Survey of* Alnus *pollen morphology*

The morphology of *Alnus* pollen is unique within the Betulaceae, even among other angiosperms. As in other genera of Betulaceae, *Alnus* pollen grains are oblate in shape and have protruding aspidate pores along the equatorial plane (stephanoporate: Faegri et al. 1989; zonoporate: Blackmore et al. 2003). Photographs of *Alnus* pollen in Plate 1 show the range

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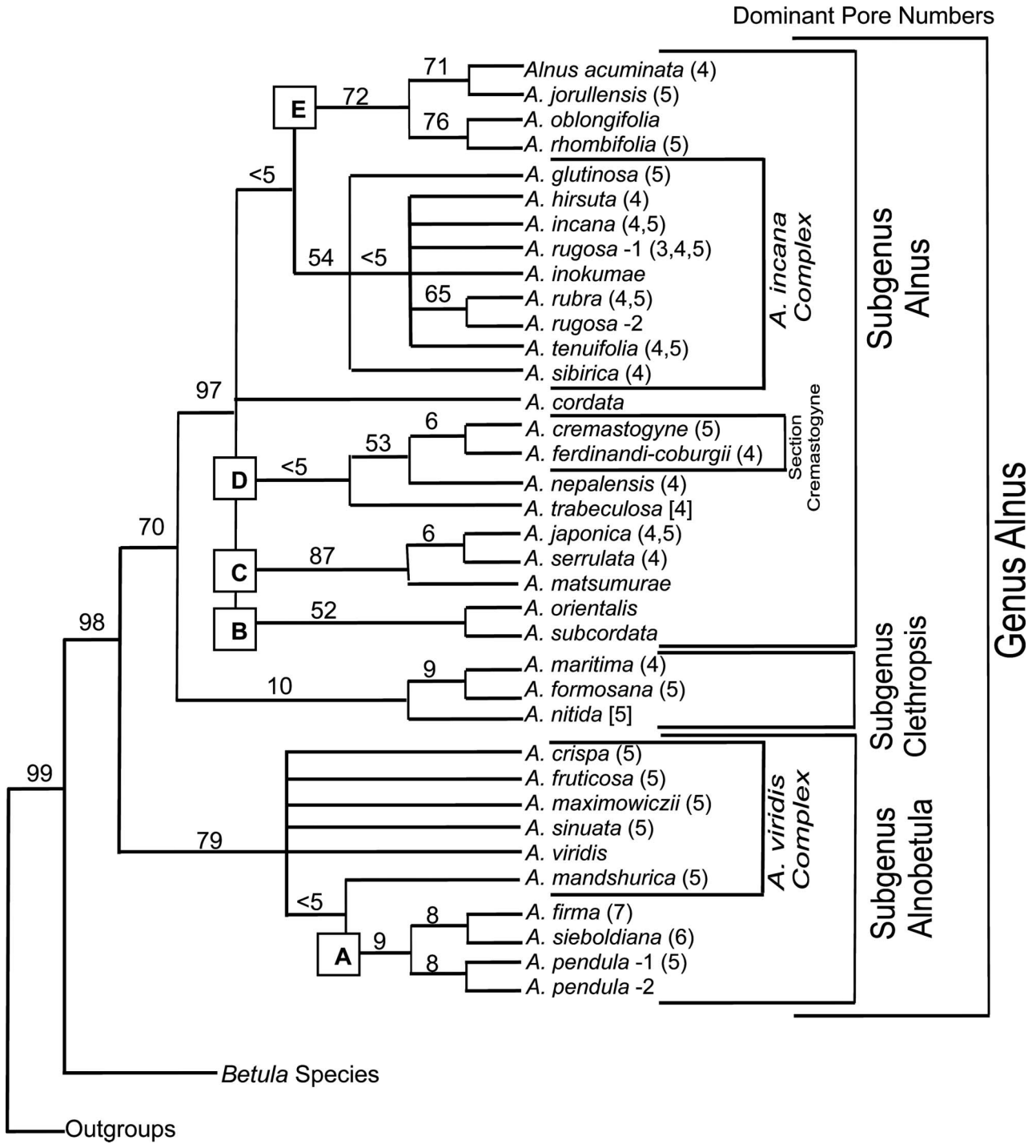
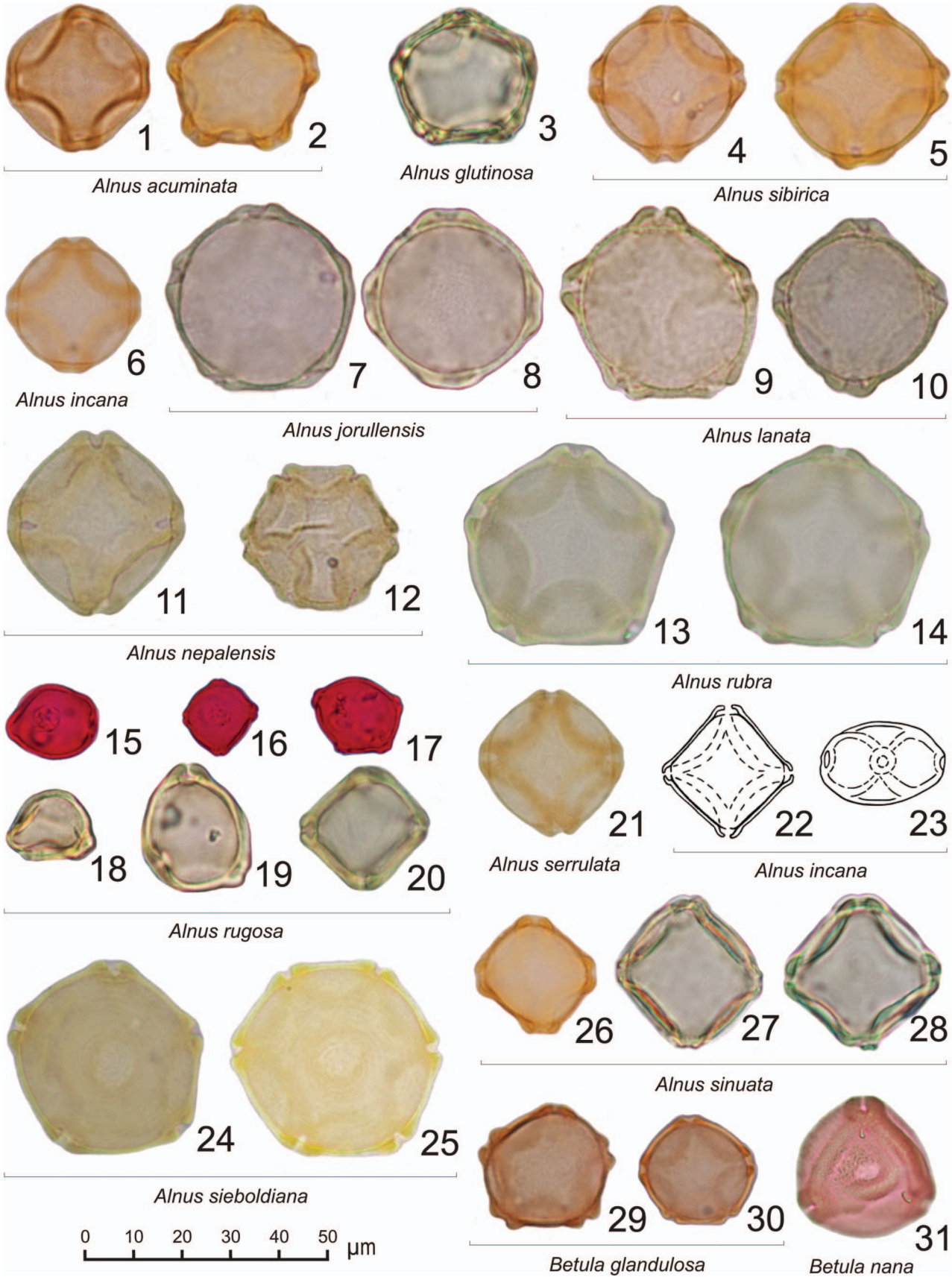


Figure 1. The strict consensus tree of Chen and Li (2004) for 335 sites which have yielded the genus *Alnus* based on nrDNA ITS sequence data. Numbers above the branches are bootstrap percentages. The letters A–E mark their clades referred to in the text. Traditional classifications and subgenera are shown on the right. Dominant pore numbers on pollen collections from our material are shown in parentheses and pore numbers from previously published studies are shown in brackets.

of pollen morphology in the genus. Figure 2 shows a generalized outline of *Alnobetula* pollen in polar view (P6 with polar arci). The pores are typically vestibulate, showing a spatial separation between the endexine and ektexine at the pore rim. The reported size of *Alnus* pollen ranges from 15 to 40 μm



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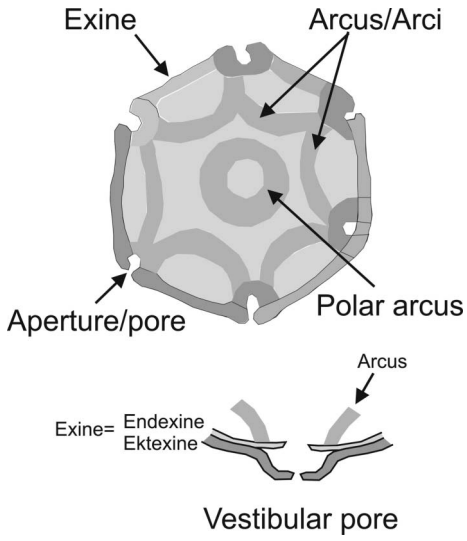

Figure 2. Generalized outline and structure of *Alnus* pollen, subgenus *Alnobetula*, illustrating the pattern of 6-pored pollen with a polar arcus as seen in *Alnus sieboldiana* (after Reinink-Smith 2010).

depending on the species and method of preparation. The dominant pore number in *Alnus* pollen is typically 4 (P4) or 5 (P5), while some species have dominantly 6-pored (P6) or 7-pored (P7) pollen grains. The sister genus *Betula* usually has 3-pored (P3) pollen.

The most distinctive feature of *Alnus* pollen is the presence of thickenings of the ektexine surrounding the pores (Wodehouse 1959; Blackmore et al. 2003). The thickenings extend out from them in arcs swinging from pore to pore (hence the term arci; see Figure 2 and Plate 1, figures 22–23). *Betula* pollen typically lacks arcoidal bands of thickening except in certain genetic variants. Erdtman (1963, p. 372) showed that in some species (e.g. *Alnus glutinosa*) polar arci or doughnut-shaped circles of arcus material (''detached arci'') occur at the poles of some pollen grains (Figure 2; Plate 1, figures 24–25). Among most *Alnus* taxa, however, polar arci are rare (Table 1).

The thickness of the *Alnus* pollen wall is about 1–1.5 μm, although it is somewhat thicker at the arcus. The outer wall (ektexine) is considerably thicker than the inner wall (endexine). Erdtman (1966) described the pollen wall as tegillate, with the ektexine having a thick outer layer and a thinner middle layer with bacculae that are often difficult to trace.

Ornamentation is similar in *Betula* and *Alnus* pollen: in surface view the sculpture is psilate, scabrate when seen with a scanning electron microscope (SEM). Wittborn et al. (1996) described the sculpture of *Alnus* pollen as seen in SEM as follows:

> Scabrae are short and conical, on short, irregularly arranged micro-rugulate ridges. The ridges may be more or less distinctly defined. Columellae are circular, often in irregular, short rows which correspond with the ridges.

Columellae rest on a foot layer. Blackmore et al. (2003) provide a similar description. Distinct arcus bands are consistently present in *Alnus* pollen, with some taxa having greater thickening of arcus bands than in others and some having only simple folds (Mayle et al. 1993). The morphology of the *Alnus* grain is presented by many authors (Erdtman et al. 1962; Erdtman 1966; Blackmore et al. 2003) and recently by Reinink-Smith (2010).

At the pore margin, the separation of the ektexine from the endexine creates a vestibule (Faegri et al. 1989) which is well developed in certain species (e.g. *Alnus lanata* and *Alnus incana*; Plate 1, figures 9–10,

Plate 1. Photographs of reference collections of *Alnus* pollen (polar views, 920 ×). Pollen slides are in the collections of the University of Washington. Species in Figures 1–23 are of the subgenus *Alnus*. Those of Figures 24–28 are of the subgenus *Alnobetula*. Selected unusual reference collections of *Betula* pollen are included. Figure 1. *Alnus acuminata* Kunth, Slide P1239, polar view, high focus. Figure 2. As in 1, medial focus. Figure 3. *Alnus glutinosa*, Slide JG 8-2-67; polar view. Figure 4. *Alnus sibirica* (Spach) Turcz. ex Kom., Slide P2140; strong arci. Figure 5. As in 4, medial focus. Figure 6. *Alnus incana* (L.) Moench, Slide P2052. Figure 7. *Alnus jorullensis*, Slide JG 3-17-56. UC350582. Figure 8. As in 7, showing vestibulate pore. Figure 9. *Alnus lanata*, Slide P2138; aspidate pore lip thickened. Figure 10. As in 9. Figure 11. *Alnus nepalensis* D. Don, Slide P2135; high focus. Figure 12. As in 11, medial focus. Figure 13. *Alnus rubra* Bong, Slide JG 3-10-56, high focus on arci. Figure 14. As in 13, a medial focus on apertures. Figure 15. *Alnus rugosa* (Du Roi) Spreng. Slide EL 7-23-53, Yale Herbarium; small malformed grains (probable hybrid collection). Figure 16. As in 15, 4-pored. Figure 17. As in 15, 4-pored; pores are not symmetrically placed. Figure 18. *Alnus rugosa* var. *americana*, Slide JG 8-8-67, U. of O., Rock Point, VT; malformed grain, 2-pored. Figure 19. As in 18, 3-pored. Figure 20. *Alnus rugosa*, Slide JG 1-19-67; 4-pored grain. Figure 21. *Alnus serrulata* (Ait.) Wild., Slide P3729; arci moderately developed. Figure 22. Drawing of *Alnus incana* pollen in polar view (after Erdtman 1963, pl. 11, fig. 8). Figure 23. Drawing of *Alnus incana* pollen in equatorial view showing arci thickenings that extend between the pores (after Erdtman 1963, pl. II, fig. 9). Figure 24. *Alnus sieboldiana* Matsum, Slide P2132; Subgenus *Alnobetula*. P5. Figure 25. As in 24, *Alnus sieboldiana* Matsum, Slide P2132; note polar arcus, P6. Figure 26. *Alnus sinuata* (Regel) Rydb., Slide P770, medial focus. Figure 27. *Alnus sinuata* (Regel) Rydb., Slide JG 2-14-68; high focus; arci appear to be simple folds. Figure 28. *Alnus sinuata* (Regel) Rydb., Slide JG 8-01-67, U. of O. High focus. Arci seem to be simple folds. Figure 29. *Betula glandulosa* Michaux, Slide EL 158; collection seems to be a genetic variant as arci seem to be present and pores are 4–5 (instead of 3). Figure 30. As in 29; most of the pollen of this collection is similar to *Alnus*; arci are present. Figure 31. *Betula nana* L., Livingstone Slide 537, Scotland; this anomalous grain shows folds (like arci) and a polar arcus.

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Table 1. Table of *Alnus* pollen features. Species are listed in alphabetical order by subgenus. Based on the list of taxa in Appendix 1 and Vishnu-Mittre and Sharma (1963). For hybrid *Alnus rugosa* collections see Figure 3 and Table 4. Dominant pore percentages are shown in **bold** type. The members of a species complex are underlined, but also hyphens make the assignment more obvious.

				Pollen pore numbers as %							
GENUS *ALNUS*	Sample Number	Arci Strength	Polar arci	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Equatorial Diam. (μm)
SUBGENUS *ALNUS*											
Alnus acuminata	P 1239	Mod	–	–	0.5	**53.9**	45.5	0.1	–	–	23.4–34.2
Alnus cremastogyne	P 2145	Mod	–	–	–	25.0	**74.7**	0.3	–	–	30.6–37.8
Alnus ferdinandi-coburgii	P 2143	Strong	–	–	5.6	**75.3**	19.2	–	–	–	28.2–36.0
Alnus japonica	JG 12-27-61	Weak	–	–	–	**68.3**	31.7	–	–	–	27.0–36.0
	JG 11-14-55	Weak				40.0	**59.0**	1.0	–	–	30.6–34.2
Alnus jorullensis	JG 3-17-56	Mod	–	–	0.3	31.6	**67.1**	0.7	0.3	–	34.2–41.4
Alnus lanata	P 2138	Strong-mod	–	0.3	7.6	**59.3**	32.1	0.7	–	–	28.8–36.0
Alnus nepalensis	P 2135	Strong	–	–	0.3	**67.7**	32.0	–	–	–	28.8–34.2
Alnus rhombifolia	S 272	Strong-mod	–	–	–	11.3	**82.3**	6.3	–	–	25.5–33.9
	JG 3-19-56	Strong	–	–	–	8.9	**60.6**	30.5	–	–	30.6–39.6
	JG12-17-53	Strong	–	–	–	18.3	**74.8**	7.6	–	–	34.2–39.6
	JG 7-13-67	Strong	–	–	–	12.3	**80.8**	7.0	–	–	22.6–31.1
	JG 3-12-56	Strong		–	0.7	42.7	**55.0**	1.3	0.3	–	32.4–39.6
Alnus serrulata var. *vulgaris*	JG 3-13-56	Strong	–	–	5.0	**71.7**	23.3	–	–	–	27.0–32.4
Alnus serrulata	EL 106; P3729	Strong	–	0.6	9.6	**84.0**	5.8	–	–	–	25.5–31.1
***Alnus incana* Complex**											
-*Alnus glutinosa*	JG 1-5-68	Strong	–	–	0.7	23.9	**72.1**	13.3	–	–	23.4–28.8
	JG 8-2-67	Strong	–	–	0.7	18.0	**81.3**	–	–	–	25.2–28.8
-*Alnus hirsuta*	JG 3-12-56	Strong	rare	–	–	**71.8**	28.2	–	–	–	30.6–32.6
-*Alnus incana*	P 2050	Strong-mod	–	–	0.7	**66.9**	32.5	–	–	–	27.0–30.6
	P 2052	Strong-mod	rare	–	0.7	**61.8**	37.5	–	–	–	27.0–30.6
	JG 2-4-68	Moderate	–	–	6.8	**73.5**	19.0	0.6	–	–	16.9–22.6
	JG 7-27-67	Moderate	–	–	1.3	**69.6**	29.0	–	–	–	22.6–31.1
	JG 10-56	Strong-mod	–	–	4.3	**91.1**	4.6	–	–	–	28.8–30.6
	JG 3-16-56	Strong	–	–	1.6	24.8	**71.9**	1.6	–	–	32.4–36.0
-*Alnus rubra*	P 772	Mod-weak	–	–	–	11.9	**85.8**	2.3	–	–	32.4–37.8
	S 19	Moderate	–	–	0.3	9.3	**88.3**	2.0	–	–	27.0–32.4
	JG 5-29-67	Strong-mod	–	–	–	13.9	**81.8**	4.3	–	–	23.4–25.6
	JG 2-28-68	Moderate	–	0.7	1.0	27.7	**69.3**	1.3	–	–	30.6–34.2
	JG 3-10-56	Moderate	–	–	–	23.3	**76.3**	0.4	–	–	36.0–39.6
	JG 7-18-67	Weak	–	–	–	22.7	**65.5**	11.8	–	–	25.5–33.9
-*Alnus rugosa* (see Text- Figure 3)	JG 11-15-55	Strong-mod	rare	–	1.3	**68.3**	30.3	–	–	–	21.6–28.8

(*continued*)

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Table 1. (*Continued*).

GENUS *ALNUS*	Sample Number	Arci Strength	Polar arci	Pollen pore numbers as % 2	3	4	5	6	7	8	Equatorial Diam. (μm)
-*Alnus rugosa* var. *americana*	JG 1-19-68	Mod	rare	–	5.7	**82.9**	11.4	–	–	–	17.0–25.5
-*Alnus rugosa* var. *americana*	EL 157; P3726	–	–	–	1.2	**77.2**	21.3	0.3	–	–	15.6–25.5
-*Alnus sibirica*	P 2140	Strong	–	–	8.6	**84.0**	7.3	–	–	–	27.0–32.4
-*Alnus tenuifolia*	P 1388	Mod-weak	–	–	7.7	**75.7**	16.7	–	–	–	27.0–34.2
	S 274	Strong	–	–	5.4	**82.9**	11.7	–	–	–	22.0–25.0
	JG 7-25-67	Moderate	–	–	–	9.3	**85.7**	5.0	–	–	22.6–33.9
	JG 11-14-55	Weak	–	–	–	32.7	**66.7**	0.7	–	–	27.0–34.2
	P1373	Moderate	–	–	5.0	**76.3**	18.3	0.3	–	–	23.4–27.0
SUBGENUS *CLETHROPSIS*											
Alnus formosana	JG 7-6-67	Strong	–	–	–	24.2	**75.5**	0.3	–	–	16.2–21.6
Alnus maritima	JG 11-15-55	Strong	–	–	4.0	**91.7**	4.3	–	–	–	21.6–27.0
	JG 1-22-68	Strong-mod	–	–	24.5	**72.2**	3.3	–	–	–	21.6–25.2
Alnus nitida	Grana v. 4:2; 1963	NA	–		18.8	37.6	**43.5**	–	–	–	NA
SUBGENUS *ALNOBETULA (ALNASTER)*											
Alnus firma	P2142	Moderate	~40%	–	–	–	0.4	36.1	**60.1**	3.6	30.0–41.0
Alnus pendula	P 2133	Moderate	–	–	–	13.6	**82.5**	4.9	–	–	27.0–30.6
	P 2055	Moderate	–	–	–	7.3	**74.1**	18.6	–	–	27.0–32.4
Alnus sieboldiana	P 2132	Mod-weak	~16%	–	–	–	36.0	**66.0**	–	–	36.0–45.0
***Alnus viridis* Complex**											
-*Alnus crispa*	JG 3-17-56b	Weak	–	–	–	25.0	**74.7**	0.3	–	–	27.0–30.6
	JG 3-17-56a	Weak	–		0.3	47.5	**52.2**	1.0		–	25.2–28.8
-*Alnus fruticosa* Rupr.	JG 8-7-67	Strong	rare	–	0.3	21.0	**75.0**	3.7	–	–	19.8–28.3
	JG 2-22-68	Strong	rare	–	–	9.3	**85.7**	5.0	–	–	27.0–32.4
-*Alnus mandshurica*	P 2139	Moderate-weak	–	–	3.0	39.8	**55.3**	1.9	–	–	12.6–16.2
-*Alnus maximowiczii*	P 2137	Moderate	rare	–	–	3.0	**58.9**	37.2	0.9	–	27.0–28.0
	JG 12-23-61	Mod-weak	rare	–	–	3.3	**72.1**	23.9	0.7	–	27.0–30.6
-*Alnus sinuata*	P 2054	Mod-weak	–	–	–	32.3	**67.0**	0.7	–	–	19.8–25.2
	JG 12-14-67	Weak	–	–	–	8.0	**87.3**	4.7	–	-	21.6–25.2
	JG 3-11-56	Weak	–	–	–	17.3	**75.3**	7.3	–	–	18.0–21.6
	JG 2-14-68	Weak	–	–	–	33.7	**65.7**	0.7	–	–	23.4–27.0
	JG 8-1-67	Mod-weak	–	–	–	8.0	**87.0**	5.0	–	–	21.6–25.2
	P 770	Weak	–	–	–	38.4	**60.9**	0.7	–	–	27.0–32.4

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22–23); some other species do not have a clear vestibule and are less aspidate as *Alnus sinuata* (Plate 1, figures 27–28) or *Alnus crispa* (Richard 1970; Mayle et al. 1993). In surface view, the outer pore opening is round to oblong and colpus-like depending on the species. Below this is the vestibule, which is elliptical in shape. The innermost aperture of the pore is generally circular (Blackmore et al. 2003; Punt et al. 2003). According to Monoszon (1971), the outer pore in *Alnus glutinosa* has a diameter of 3.0–4.5 μm and the inner pore diameter is 4.3–4.5 μm; at pore lips the ektexine is variously thickened in different species. The outline of the grain is rounded to angular in polar view.

Erdtman (1943) described the main features of *Alnus* pollen morphology and suggested that the dominant pore number might be an important diagnostic character for differentiating between two alder species in Europe. Monoszon (1971) described the difference in prevailing pore numbers among four different species of the genus *Alnus* in Russia, including '*Alnaster*'. Russian palynologists have long recognized the *Alnobetula* subgenus known as *Alnaster* (Kontrimavichus 1976). Erdtman (1963) may have been the first to point out the polar arci in *Alnus* pollen.

Furlow (1979) thoroughly investigated the gross morphology of *Alnus* species, including photographs of pollen from a number of alder reference collections. These photographs appear to be of fresh material that had not been acetolyzed (i.e. treated with acetic anhydride and sulfuric acid to remove the cellulose), so unfortunately the arcus thickenings (if present) cannot be seen. His SEM photographs are useful in discerning the surface sculpture, and he tallied the numbers of pores that dominate in several modern collections.

2.2. *Key developments in* Alnus *palynology*

In ecologic studies of Quaternary pollen floras, it has been useful to identify major morphotypes of fossil *Alnus* pollen based on grain size and dominant pore number. For example, Bryan (1954) showed that large counts of modern pollen material from Greenland, including *Alnus serrulata* and *A. rugosa* var. *americana*, had around 65–69% 4-pored pollen, whereas *Alnus viridis and Alnus crispa* collections were dominated by 5-pored pollen (between 69 and 86%). Her cold-climate fossil alder collections were dominated by 5-pored grains. Similarly, Leopold (1955) found that counts of 200–500 grains in reference collections of *Alnus viridis* and *Alnus crispa* are dominated by 5-pored grains ($\sim$ 71%); in contrast, the more southerly taxon *Alnus rugosa* type (including *Alnus incana* ssp. *rugosa*, *Alnus maritima* and *Alnus serrulata*) were dominated by 4-pored grains (between 70 and 84%). In the late-glacial sediments, she noted that alder pollen at two sites in Connecticut were predominantly P5; in mid Holocene sediments, alder pollen at these sites were dominantly P4. In their studies of Late Pleistocene floras of western Washington, Barnosky (1985), Cwynar (1987; oral communication, 2009), Grigg and Whitlock (1998) and R. Nickman in Leopold et al. (2009) have identified fossil alder pollen as the montane *Alnus sinuata*-type (sitka alder), which is dominantly 5-pored. Typically, Sitka alder pollen have weakly developed arci and a fragile wall. This is in contrast to the lowland form, *Alnus rubra*-type (red alder) pollen, which has a somewhat larger and more robust morphology, thicker wall and strong arci and which is also dominantly 5-pored. They are not difficult to distinguish.

Monoszon (1971) described differences in the pores of modern pollen from four species of *Alnus* from central Asia: three species of subgenus *Alnus* (*A. incana*, *A. glutinosa* and *A. hirsuta*) and one species of subgenus *Alnobetula* ('*Alnaster*' *fruticosa*). He showed that *Alnus glutinosa* pollen are aspidate with protruding pore lips and definite thickenings of the outer wall along the pore rim, a feature also seen in Erdtman's (1963) tetraploid material as suggested in Plate 1, figure 3. In contrast, '*Alnaster*' *fruticosa* had very little protuberance of the pore lips, and thinner walls at the pore rim. All four of these taxa have well-developed vestibulate pores. Richard (1970) was able to distinguish between pollen from *Alnus crispa* var. *mollis* (arci weak or not apparent) versus pollen from *A. rugosa* var. *americana*-type (strong arci, protruding pore lips and sturdy construction of pollen walls).

In previous taxonomic studies of the genus *Alnus* (reviewed by Chen and Li 2004), the subdivision of the genus has been based on fruiting, flowering and leaf morphology and each study divided the genus differently into subgenera and sections. Navarro et al. (2003) were the first to use ribosomal DNA data (nrDNA ITS) to estimate phylogenetic relationships in the *Alnus* genus (19 species). Chen and Li (2004), who were the second to use ribosomal DNA data, reported somewhat similar results; their nrDNA ITS tree for the genus *Alnus* (Figure 1) was based on the 34 species they examined.

Thus far this paper has reviewed the morphology of *Alnus* pollen and discussed how distinctive pollen features have been used for taxonomic subdivision and species identification. The next section investigates whether these features are consistent within each species, and explores additional taxonomic implications of distinctive *Alnus* pollen morphology.

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3. Methods

In this study the authors investigated the morphology of pollen derived from three modern reference collections (pollen herbaria): (1) pollen prepared by E.B. Leopold at the US Geological Survey (Denver Federal Center); (2) pollen from Jane Gray's reference collection now housed at the University of Washington in Seattle; and (3) pollen prepared in E.B. Leopold's laboratory as part of the Washington State modern pollen slide collection. Slide numbers from these three sources are preceded by the letter P, JG and S, respectively. Jane Gray was a paleobotanist at the University of Oregon where she built a large modern pollen reference collection (= pollen herbarium). The Jane Gray reference collections were transferred to the University of Washington after her untimely passing in 2000.

Pollen material from all three of these reference collections was prepared using a 3-minute acetolysis treatment (Faegri et al. 1989) to remove cellulose from the pollen cell. Acetolysis is necessary in order to be able to observe arci thickenings on pollen grains. Most of this pollen had been pre-treated with 10% potassium hydroxide solution to remove cell contents (Faegri et al. 1989) prior to acetolysis. All pollen samples were mounted in glycerin jelly and sealed with clear plastic nail polish. To determine pore-number percentages in particular reference collections, 300-grain tallies were made of grains in polar view.

The size of pollen grains commonly changes over time or as an artifact of the preparation treatment. For example, specimens mounted in glycerin jelly are prone to swelling with time (Faegri and Deuse 1960), particularly if any potassium hydroxide solution treatment is not thoroughly washed out and the pollen slurry returned to a neutral pH before mounting (E.B. Leopold, unpublished data). Pollen size is also affected by the mounting medium: glycerin-jelly-mounted grains tend to be 1.25 times larger than those mounted in silicone oil (Moore et al. 1991).

The 59 reference collections embrace 29 species that include previous 'subspecies' now elevated to species level by Chen and Li (2004) (Figure 1, Table 1, Appendix 1). This paper follows the taxonomic name conventions used on the Global Biodiversity Information Facility website (GBIF 2010). Most collections reported here are represented by only one or two collections per species, although some species are represented by as many as four, five or six collections. Because of this variable sampling, the value of this study lies mostly in the wide number of species considered.

Of the *Alnus* species studied by Chen and Li (2004), one (*Alnus inokunae*) is no longer a valid species according to GBIF (2010), and the present study lacked pollen material for five others. *Alnus lanata* was omitted by Chen and Li (2004) but is included in this study as it is a species recognized by GBIF (2010).

4. Results

The main aim of this paper is to discover how *Alnus* pollen types fit with the nrDNA ITS data of Chen and Li (2004), or with other similar taxonomic treatments (Navarro et al. 2003). Chen and Li's (2004) strict consensus tree based on DNA ITS data that appears in Figure 1 has grouped species into Clades A through E. Information on the dominant pollen-pore number for each species is added in parentheses on the right of each species in Figure 1, with traditional classifications shown on the right. Table 1 presents pollen features for the 59 reference collections. Table 2 shows the consistency by species of the dominant pore number and the range. Table 3 summarizes the data by clades showing dominant pore number, range and some major features of the different groupings, including in which species polar arci are common. Table 4 summarizes the characteristics of presumed hybrid collections of *Alnus rugosa*.

Table 1 lists the observed dominant pore number (the number of pores in percent in tallies of 300) for

Table 2. Consistency of pollen features within *Alnus* species. For those species of which more than one pollen collection was studied, this table shows consistency in dominant pore number. Three taxa are inconsistent, as were the apparently hybrid specimens of *Alnus rugosa* (see Figure 3, Table 4).

ALNUS Species	No. of Collections	Dominant Pore No.	Range Pore No.
SUBGENUS *ALNUS*			
***Alnus incana* complex (21 collections)**			
Alnus incana	5	4-pored	3–6
"	1	5-pored	3–6
Alnus glutinosa	2	5-pored	
Alnus rubra	6	5-pored	*2–6*
Alnus rugosa (selected)	3	4-pored	3–5
Alnus tenuifolia	3	4-pored	3–5
"	2	5-pored	4–6
Alnus rhombifolia	5	5-pored	3–7
Alnus japonica	1	4-pored	4–5
"	1	5-pored	4–6
Alnus serrulata	2	4-pored	2–5
SUBGENUS *CLETHROPSIS*			
Alnus maritima	2	4-pored	3–5
SUBGENUS *ALNOBETULA*			
Alnus pendula	2	5-pored	4–6
***Alnus viridis* complex (12 collections)**			
-*Alnus crispa*	2	5-pored	3–6
-*Alnus fruticosa*	2	5-pored	3–6
-*Alnus maximowiczii*	2	5-pored	4–7
-*Alnus sinuata*	6	5-pored	4–6

Table 3. Pollen features of the genus *Alnus* by subgenera and clades of Chen and Li (2004). Data are from Table 1. Table shows prevailing pollen pore number (P). Geographic distributions of taxa are listed in the final column. Data include the species examined here or from published references on *Alnus* pore numbers.

Taxon	Dominant Pore No.	Range	Distribution of species
GENUS *ALNUS*			
SUBGENUS *ALNUS*; dominantly 4- or 5-pored			
E CLADE: Arci moderately to strongly developed. Chiefly 4- or 5-pored.			
Alnus acuminata	P4	3–6	Mexico (Andean alder)
Alnus jorullenisis	P5	3–7	Mexico (Mexican alder)
Alnus rhombifolia	P5	3–7	West North America (white alder)
Alnus incana Complex; Arci weak to strongly developed.			
-*Alnus incana*	P4, 5	3–6	Europe (grey alder)
-*Alnus hirsuta*	P4	4–5	East Asia (Manchurian alder)
-*Alnus glutinosa*	P5	3–6	South Europe, West Asia (black alder)
-*Alnus rugosa*	P4	3–5	East North America (speckled alder)
-" *'hybrid colls'*	P3, 4 or 5	0–9	See text; Figure 3
-*Alnus rubra*	P5	2–6	West coastal North America (red alder)
-*Alnus tenuifolia*	P4, 5	3–6	West US to AK (thin leaf alder)
"	P4, 5	–	Wang et al. (1995)
-*Alnus sibirica*	P4	3–5	E. Asia,. This study; Ikuse (1956)
D CLADE: including Section *Cremastogyne*; chiefly P4 and one P5.			
Alnus cremastogyne	P5	4–6	East Asia, West China; this study; and Wang et al. (1995)
Alnus ferdinandi-coburgii	P4	3–5	East Asia; this study and Wang et al. (1995)
Alnus lanata	P4	2–6	East Asia
Alnus nepalensis	P4	3–5	East Asia (Nepalese alder); this study; and Wang et al. (1995)
Alnus trabeculosa	P4	3–5	China and Japan; Wang et al. (1995)
C CLADE:			
Alnus serrulata	P4	2–5	East North America (hazel alder)
Alnus japonica	P4, 5	4–6	Japan; this study
"	P4	3–5	Wang et al. (1995)
"	P5	4–6	Ikuse (1956) Huang (1972)
B CLADE: no data; 2 species, *Alnus orientalis*, *Alnus subcordata*			
SUBGENUS *CLETHROPSIS*. No clade assigned. Dominantly 4- or 5-pored; strong arci.			
Alnus maritima	P4	3–5	E. N. Am. (seaside alder)
Alnus formosana	P5	4–6	Taiwan, E. Asia (Formosan alder)
Alnus nitida	P5	3–5	Himalayas, Vishnu-Mittre and Sharma 1963
SUBGENUS *ALNOBETULA* (formerly *Alnaster*): Dominantly 5-, 6- or 7-pored. Polar arci common in two taxa of A Clade. Arci strength and pollen size variable; arci weak in several taxa.			
Alnus viridis Complex			
-*Alnus crispa*	P5	3–6	Northern N. Am. (green alder)
-*Alnus fruticosa*	P5	3–6	E. & W. N. Am.
-*Alnus maximowiczii*	P5	4–7	Japan
-*Alnus mandshurica:* Arci medium to weak, small pollen	P5	3–6	E. Asia
-*Alnus sinuata:* Arci moderately to weakly developed; most are small grains, fragile	P5	4–6	W. N. Am., Siberia (Sitka alder)
A CLADE			
Alnus firma: Pollen large; polar arci common	P7	5–8	Kyushu, Japan
Alnus sieboldiana: Pollen large; arci moderately developed; polar arci common	P6	5–6	Honshu, Japan
Alnus pendula	P5	4–6	Japan, Korea
GENUS *BETULA* (selected spp.)			
Betula glandulosa	P5	3–6	North America. Arctic. Scrub birch
Betula nana	P3	3	Livingston slide, Scotland (dwarf alpine birch)
Betula chinensis	P3	2–5	Chinese birches; Wang et al. (1995, p. 90–92)
Betula platyphylla	P3	2–4	Wang et al. (1995, p. 90–92)

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Table 4. A comparison of different collections of *Alnus rugosa* pollen; the presumed "hybrid" condition is based on pollen features. See Figure 3.

Pollen Collection	Sample No.	Pore No. range	Equatorial diameter μm	Mean range μm
Normal examples:				
Alnus serrulata	EL 106; P3729	2–5	25.5–31.1	8.5
Alnus rugosa var. *americana*	EL157; P3726	3–6	15.6–25.5	9.9
Alnus rugosa var. *americana*	JG 11-18-55	3–5	21.6–28.8	7.2
Presumed hybrids:				
Alnus rugosa var. *americana*	EL 109; P3727	0–6	18.2–52.0	33.8
Alnus rugosa	JG 8-8-67	0–6	13.0–29.9	16.9
Alnus rugosa	EL 7-23-53	0–9	10.4–31.2	20.8

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pollen of each species, together with certain other features such as the strength of the arci on the pollen wall, the frequency of polar arci (i.e. circular arci at the pole) and the size range of pollen grains in each collection. Table 1 also lists the range in pollen diameters. Because the general pollen size varies greatly between *Alnus* species and commonly among collections of the same species, grain-size in *Alnus* pollen is not particularly useful as it may be influenced by methods of preparation (Faegri and Deuse 1960; Vishu-Mittre and Sharma 1963).

The dominant number of pore apertures in the pollen grain of *Alnus* is a basic feature of species (Bryan 1954; Erdtman 1963). Grains with a strongly dominant pore number typically comprise more than 60% of the pollen count, but in 7 out of 59 collections the dominant pore number was below 60% of the count (Table 1). Three of these occurred in the subgenus *Alnus* collections (*A. acuminata*, *A. japonica* and *A. lanata*), and three were in *Alnus viridis* complex (*A crispa*, *A. mandshurica* and *A. maximowiczii*). The range in pore number in most *Alnus* species is generally ± one or two pores (Table 1). Exceptions are graphed in Figure 3 where the range in pore number in *Alnus rugosa* is unusually extended. In these cases hybrid conditions are suspected. Table 2 tests the consistency of the dominant pore number within a species. Three species were found to be inconsistent in predominant pore numbers in different collections (Table 2).

4.1. *Subgenus* Alnus

Many of the 21 species in subgenus *Alnus* are trees except for those growing at high latitudes such as the shrub *Alnus hirsuta*, Manchurian alder of northeast Asia. This study includes collections from 17 taxa (Tables 1, 3); among these the dominant pore numbers are four or five. All but 5 of the 39 collections in subgenus *Alnus* have pollen with moderately to strongly developed arci. Chen and Li (2004) recognized four clades in the subgenus *Alnus*, which they designated Clade E, D, C and B (Figure 1). These clades are described in the order of their appearance in Figure 1.

4.1.1. *Clade E*

Clade E is the largest clade and contains 12 species, of which the present study includes materials for 10. Species of the subgroup *Alnus incana* complex (eight taxa) are widespread in the Northern Hemisphere, and these have a dominant pore number of four or five. However, *Alnus rugosa* of eastern North America shows anomalous numbers of pores and a very wide range of pore numbers within a single collection (a feature that suggests these are probably hybrid collections). In the *A. incana* complex all but three taxa have pollen arci that are moderately to strongly developed.

The three other taxa in Clade E include two species of Central America and one of western USA. Interestingly, the Mexican alder (*Alnus jorullensis*) and the Western North American species *A. rhombifolia* have P5 as the dominant pore number while the Andean alder (*A. acuminata*) has P4. Collections of *Alnus tenuifolia* (thin-leaf alder, Pacific Northwest) and *Alnus incana* (grey alder of Europe) are inconsistent in dominant pore number (each having P4 and P5). Three member species occasionally have polar arci, but only in certain collections (*Alnus glutinosa*, *A. incana* and *A. rugosa*). *Alnus inkumae* was not sampled. In Clade E, particularly aspidate pores were observed in *A. acuminata*, *A. glutinosa*, *A. rhombifolia* and *A. rugosa*. Pollen features in this clade do not seem to fit any general patterns.

4.1.2. *Clade D*

Clade D material represents three out of the five taxa, two of which comprise the *Cremastogyne* section: *Alnus cremastogyne* (P5 occurring in Southeast Asia) and *Alnus ferdinandi-coburgii* (P4 of China). *Alnus nepalensis* of Nepal and China (P2135) is mainly P4,

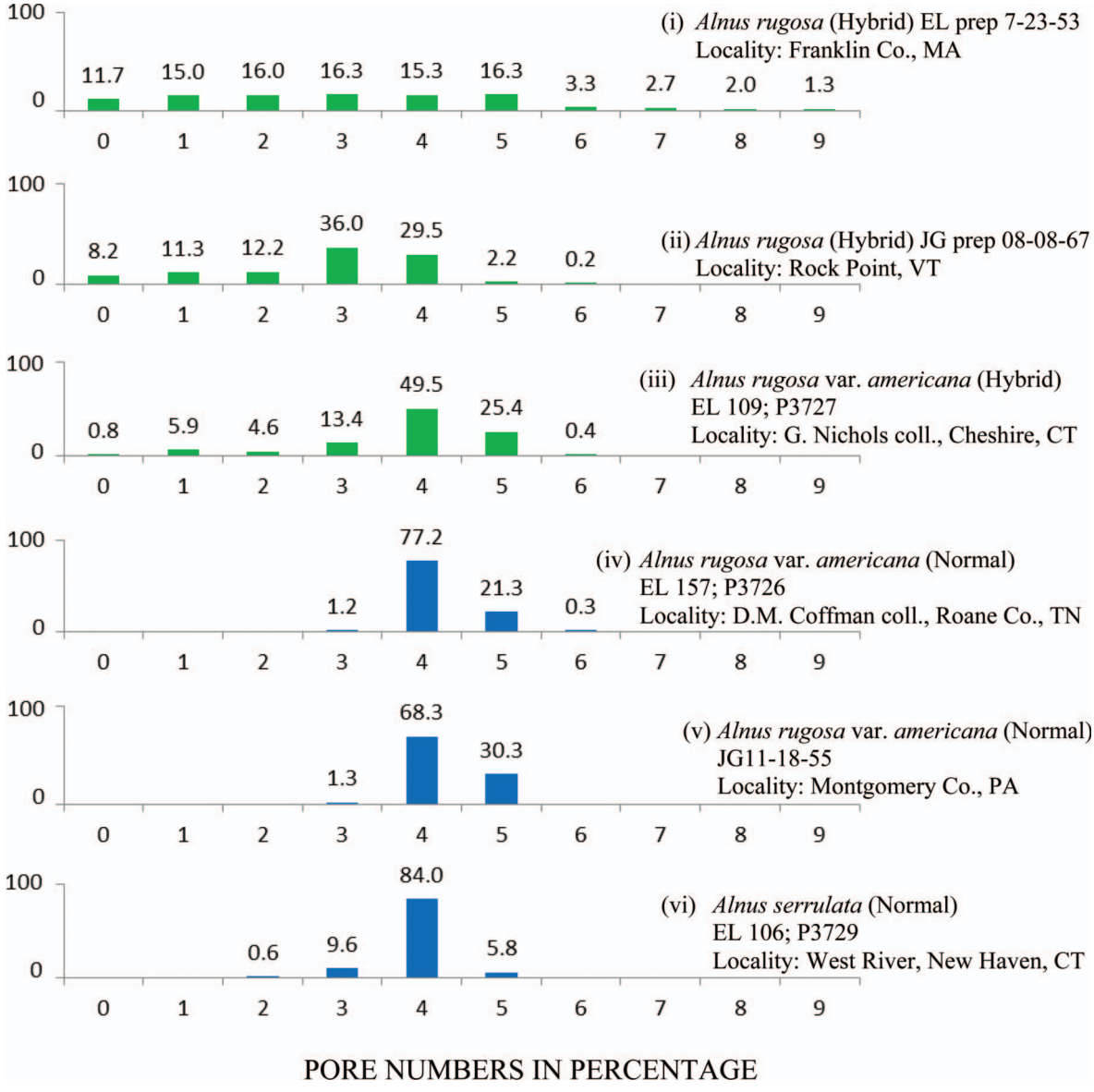

Figure 3. Distribution of pore numbers in selected pollen collections of certain *Alnus* species. The data reveal that the diverse pore frequency in three presumed hybrid collections of *Alnus rugosa* (shown in green) contrasts with that in three collections (shown in blue) having more typical (more limited) distribution of pore numbers in each pollen slide; these include two 'normal' *Alnus rugosa* var. *americana* collections and one normal *Alnus serrulata* collection. Furlow (1979, 1990) reported field evidence that *Alnus rugosa* has frequent hybrids with *Alnus serrulata* along the east coast of the USA (see Table 4).

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which is also confirmed by Wang et al. (1995). The Asian species *A. lanata* (P4) is included as part of this clade as it is closely related to *A. cremastogyne* (P5). The Japanese species *A. trabeculosa* was not sampled, but Wang et al. (1995) mentioned two collections of this species that are dominantly 4-pored. Pollen of *Alnus cordata* of northwest Europe was not sampled. No polar arci are reported in pollen of Clade D taxa. Pollen morphology in Clade D follows no pattern, even though all species in this clade are linked by their Old World geography.

4.1.3. *Clade C*

Two of the three species in Clade C were sampled: *Alnus serrulata* and *Alnus japonica*. Of these, *Alnus serrulata* (hazel alder, occurring in Canada and eastern North America) is dominantly 4-pored. The species *Alnus japonica* (of Japan and China) exhibits inconsistent dominant pore numbers: one collection (JG12–21–61) was dominantly P4 and one (JG11–14–55) was weakly dominated by P5. Both Ikuse (1956) and Huang (1972) reported P5 for this

species, but Wang et al. (1995) reported P4 as the dominant pore number (Tables 2, 3); this species seems to be intrinsically inconsistent in pore number. Our collections have weak arci, a feature that was also reported by Wang et al. (1995, p. 90) in China. We did not sample *Alnus matsumurae*, which occurs in Japan.

4.1.4. *Clade B*

Clade B contains two species, *Alnus orientalis* and *Alnus subcordata*, neither of which were sampled. The latter has a restricted distribution, occurring only in eastern Sweden.

4.2. *Subgenus* Clethropsis

The subgenus *Clethropsis*, which contains three species, is named for its arboreal habit (Murai 1968, *klethra* Gr. alder tree). All three taxa bloom in the fall which, in addition to *A. nepalensis* of Clade D, are the only known taxa of *Alnus* to do so (Chen and Li 2004, p. 327). Of the three *Clethropsis* species, *Alnus maritima* of eastern North America is 4-pored, and *Alnus nitida* of Pakistan and the Himalayas (Vishnu-Mittre and Sharma 1963) and *Alnus formosana* of Taiwan are dominantly 5-pored. None of these species has any polar arci; both *A. formosana* and *A. maritima* have strongly developed arci. Our collections only contain small grains < 27 μm. Murai (1968) and Chen and Li (2004) group these species together, but Navarro et al. (2003) using nrDNA ITS data find no support for the existence of this subgenus which their data suggest is much like other taxa of subgenus *Alnus*.

4.3. *Subgenus* Alnobetula

Navarro et al. (2003) who sampled 19 species divide this subgenus into two groups: Group 1 includes the *Alnus viridis* complex and Group 2 includes *Alnus firma* and *Alnus pendula*, which were recognized as part of Clade A by Chen and Li (2004).

4.3.1. *Group 1:* Alnus viridis *complex*

The five species that we sampled in the *Alnus viridis* (green alder) complex are very consistent in having pollen with predominantly five pores (based on 13 individual collections); three collections have low dominance of 5-pored pollen (52–59%). The member taxa are closely related according to Chen and Li (2004; Figure 1). The *Alnus viridis* complex includes five Northern Hemisphere taxa and the European species *Alnus viridis*). We lacked collections of *A. viridis*, but because it is closely related to these taxa (Figure 1) it is likely that *A. viridis* is P5 also. Two taxa, *Alnus crispa* (in accord with Mayle et al. 1993) and *Alnus sinuata*, are distinctive in having somewhat thin-walled fragile pollen with less developed arci and relatively thin pore lips. Several taxa in this group have weak to moderately thickened arci, but pollen of *A. fruticosa* has strongly developed arci. Two taxa, *A. fruticosa* and *A. maximowiczii*, show occasional polar arci (doughnuts). The latter species shows prominent vestibulate pores (Plate 1, figures 24–25), which are especially well illustrated by Reinink-Smith (2010, plate 1, figures 17–19).

4.3.2. *Group 2: Clade A*

Clade A by DNA data is a well-supported group of three species native to Japan (*Alnus firma*, *Alnus pendula* and *Alnus sieboldiana*). As noted, this division is similar to that recognized by Navarro et al. (2003). In our four collections of this group, the pollen size is definitely large: two are between 30 and 45 μm, the greatest among our collections.

In two species the prevailing pore number tends to be the highest in the genus *Alnus*: *Alnus firma* is dominantly P7 and *Alnus sieboldiana* is predominantly P6 (Table 1). Pollen from these have strongly vestibulate pores with thickened pore lips, and polar arci are common (16% and 40%, respectively) which is an outstanding feature as depicted in Figure 2 (Plate 1, figures 24–25; see also Reinink-Smith, 2010, plate 1, figures 16–17). In contrast, the third species of Clade A (*A. pendula*) does not have polar arci. *Alnus maximowiczii* (of the *Alnus viridis* complex) closely resembles these and has rare polar arci.

The most important pollen characteristic in *Alnobetula* taxa is the high (≥ 5) dominant pore number, but also useful are the pollen characters in Clade A that are not easily mistaken for other parts of the genus (common polar arci).

4.4. *Other Betulaceae*

Pollen morphological features, such as pore dominance and polar arci, may also be important in identifying and characterizing other taxa within the birch family. With regard to pore dominance, in a sample of *Betula glandulosa* from the Yale Herbarium, an anomalous *Alnus*-like pollen collection that showed real arci and pore numbers from three to six with a dominant pore number of five was observed. A repeat sample from the herbarium sheet yielded the same. A similar pore condition in *Betula glandulosa* was reported by Erdtman (1943, p. 72–73). Similarly,

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Wang et al. (1995, p. 90–92) reported that collections of *Betula platyphylla* and *B. chinensis* have dominantly (58%) 3-pored pollen, but with a wide range in pore numbers between 2 and 5. Erdtman (1943, p. 75) illustrates arci on *Carpinus chinensis* pollen. The photographs of Bassett et al. (1978, p. 124, figure 50) with interference contrast light microscopy suggest that arci-like creases in pollen of *Betula populifolia* may be folds, and not thickenings.

Although the polar arcus feature is rarely observed in pollen of the Family Betulaceae, a few reported occurrences suggest that polar arci are not restricted to the genus *Alnus*. For example, Huang (1972, p. 74) reported polar arci in *Carpinus rankanensis* pollen from Taiwan (no arci occurred between the pores). Polar arci have also been observed in pollen grains from the genus *Betula*. In Plate 1, figure 31, such a grain from *Betula nana* is illustrated (see Table 3).

5. Discussion

Based on this study, morphological differences in pollen features within the genus *Alnus* can be grouped into four categories: (1) number of pores (dominance and range); (2) strength (thickness) of the arci; (3) presence or absence of polar arci; and (4) size of the pollen grains.

5.1. Dominant pore number and range

Although individual pollen grains of the genus *Alnus* exhibit pore numbers in the range 2–8 (Table 1), generally one pore number is dominant in any given sample and the range in pore numbers is smaller within each of the three subgenera. Specifically, species within the subgenus *Alnus* are dominantly 4- or 5-pored, with a range in pore numbers of 2–7 within individual samples. Species within the subgenus *Clethropsis* are also dominantly 4- or 5-pored, with a range in pore numbers of 3–6 within individual samples. In contrast, species within the subgenus *Alnobetula* are dominantly 5-, 6-, or 7-pored with a range in pore numbers of 3–8 within individual samples.

Of the 59 samples of the genus *Alnus* from the three reference collections, the dominant pore number is strongly dominant ($> 70\%$) in $29/59 = 49\%$ of the samples, and the dominant pore number is at least moderately dominant ($> 60\%$) in $50/59 = 85\%$ of the samples. The dominance percentage is inversely correlated with the range in pore number in most samples. For example, in samples with strongly dominant ($> 70\%$) pore number, the range in pore number is typically 3 (i.e. the dominant pore number $\pm$ 1). Samples with lower dominant pore-number percentages (50–60%) exhibit a wider range (between 4 and 5) in pore numbers, such as in *Alnus acuminata* (sample P1239), *Alnus lanata* (P2138), *Alnus rhombifolia* (JG 3–12–56), *Alnus crispa* (JG 3–17–56a), *Alnus mandshurica* (P2139) and *Alnus maximowiczii* (P 2137). Samples exhibiting the lowest dominant pore-number percentages (49%, 36% and 16%, Figure 3) also exhibit the broadest range (7–10); these are probably hybrids with a large number of imperfect pollen grains, as discussed in Section 7 below.

5.2. Arci

Strength (thickness) of the arci is a feature that varies between species and is not a character that separates the subgenera or sections (Table 1).

5.3. Polar arci

The presence of circular arci at the poles (Figure 2; Plate 1, figures 24–25; see Reinink-Smith, 2010, plate 1) is a feature occasional in species with large many-pored *Alnus* pollen, as in *A. glutinosa* (Erdtman 1963). Polar arci occur in low frequency in three species in subgenus *Alnus* and in two species of the *A. viridis* complex, but are common (16% and 40%) in two Asian species of subgenus *Alnobetula* (Table 1; Reinink-Smith 2010, table 1). This feature is an informative taxonomic character, singular in the genus (see especially *A. sieboldiana* and *A. firma*).

5.4. Pollen size

The size of the pollen grain differs between species but all are smaller than ~ 45 μm. Pollen sizes in tetraploid specimens of *Alnus glutinosa* tend to be somewhat larger than diploid collections (Erdtman 1963). Pollen size does differ between collections of the same species, in some cases probably related to preparation treatment. Of these pollen features, the dominant pore number, the strength of the arci, aspidate condition and the thickness of the pore lip seem the most significant taxonomically.

Among the presumed hybrid examples of *Alnus rugosa*, three not only have a wide range of pore numbers but they also have a very wide range in pollen cell size. For example, the two 'normal' *A. rugosa* collections have a range in pore numbers of $\pm$ 1 or 2 and the range in pollen size is narrow (21.6–28.8 μm and 15.6–25.5 μm). However, the hybrid specimens have at least double the range of pore numbers and quadruple the cell sizes. It appears that the ranges in cell size are directly correlated with the pore number in these collections. The cell size with the narrower range will probably have a greater number of fertile pollen cells.

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5.5. Other concerns

One question of concern is the faithfulness (i.e. consistency) of pollen features within a species. Individual taxa are fairly consistent in showing the same predominant pore numbers in different collections of the same species, but because the sample size is low this feature is hard to evaluate here (Tables 1, 4). Three taxa were inconsistent. In five collections of *Alnus tenuifolia*, three were dominantly 4-pored $\pm$ 1 (76–83%) and two were dominantly 5-pored $\pm$ 1 (67% and 86%). In *Alnus japonica*, one collection from Honshu was P4 (68%) and one from Hokkaido was P5 (59%; Table 1); in two collections from China, Wang et al. (1995) report one as P5 (56%) and one as P4 (80%). In the case of *Alnus japonica*, there may be geographical factors (local races) affecting these examples. In *Alnus incana* one collection from Hungary was dominantly 5-pored $\pm$ 2 (71.9%) while five collections from the USA and Sweden were dominantly 4-pored. While identification problems at the Herbarium level could have occurred, it is of interest that the inconsistency of *Alnus tenuifolia* specimens (Table 1) appeared to be repeated in collections from two continents.

Fundamental vegetative characteristics separate subgenus *Alnobetula* taxa from other parts of the genus; the samara seeds have well-developed wings (Murai 1967) and *Alnobetula* is the only part of the genus that has both short and long shoots bearing leaves and sessile winter buds, a character shared with some species of the genus *Betula* (Chen and Li 2004). As a group, the subgenus *Alnobetula* taxa (formerly *Alnaster*) are consistent in having predominantly 5-, 6- or 7-pored pollen; in this feature, pollen data fits well with the vegetative uniqueness of *Alnobetula*. *Alnus crispa* (green alder) placed in this subgenus by Chen and Li (2004) and by Furlow (1979) are shrubs that are circumpolar in distribution. Five Japan/Korea-based native species of this group (*Alnus firma*, *A. mandshurica*, *A. maximowiczii*, *A. pendula* and *A. sieboldiana*) are more local and not circumpolar. Navarro et al. (2003) consider this subgenus to be basal in the phylogeny, and Murai (1968) states that this group is the more primitive and perhaps the more ancient part of the genus.

In terms of pollen morphology, *Alnobetula* taxa make a tightly knit morphological pollen group. All sampled taxa have dominant pore numbers of less than five. Some *Alnobetula* species (Clade A) show unusually large pollen with many pores and a common occurrence of polar arci (Figure 2), and others are simply many-pored. The Clade A group is Asian in distribution, which is of interest because they closely resemble some of the many-pored *Alnus* pollen reported from the Miocene of Alaska (Reinink-Smith 2010).

In the subgenus *Alnus*, all sampled taxa have dominant pore numbers ranging from P4 to P5. Individual species inconsistently display different dominant pore numbers in different collections (Table 2). This was not the case among the eight species (17 collections) of subgenus *Alnobetula*. Chen and Li (2004) state that the eight species of subgenus *Alnus* ''form a clade with weak support''. Table 1 shows that the dominant pore number feature is mostly scattered like a checkerboard in subgenera *Alnus* and *Cremastogyne*, but is consistent ($\geq$five) in *Alnobetula*.

Both Chen and Li's (2004) DNA-based taxonomy of *Alnus* and the vegetative subcategories of Furlow (1979) fit equally well with the pollen features, mainly because both systems strongly recognize the uniqueness of subgenus *Alnobetula*. In important aspects including pollen features, this subgenus stands out from the rest of the genus *Alnus*.

6. How these features of alder pollen may be helpful for identification

Shifts in dominant pore number of alder within a stratigraphic section alone may be significant, as illustrated by Reinink-Smith (2010). Depending on the geographic area, as mentioned in New England, pollen of living species of alder can permit recognition of some local *Alnus* taxa based on pore number. It is very useful for Quaternary work in the Pacific Northwest area if two common species of *Alnus* can be easily distinguished from their pollen. In this area the montane species *A. sinuata*, with its weak arci, simple pores and thin walls, permit differentiation from the lowland form *A. rubra* which has larger, more robust strongly vestibulate pollen.

Circular (doughnut-shaped) polar arci are a feature that by itself is not very helpful in distinguishing subgenera. However, the frequency of polar arci with the large number of pores makes the pollen of two Japanese taxa stand out from others; these features are especially obvious in the Miocene samples of Reinink-Smith (2010) in Alaska. Based on the present study, her specimens identify the appearance of Clade A in the Miocene. Reinink-Smith's (2010) data demonstrate that these pollen characteristics have important biogeographic implications in helping distinguish Old World Asian taxa in the Late Cenozoic pollen record. The simultaneous appearance of *Alnobetula* in the Miocene–Pliocene of Japan (Tanai 1961) and Alaska (Wolfe 1966) can only have happened via the existence of a Bering Land Bridge. These features are now documented by megafossils and late Miocene pollen in Alaska.

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7. Pollen morphology as an index of hybridization

The presence of malformed pollen cells is typically an indicator of infertile pollen and seems to mark certain hybrids (Stebbins 1959). Furlow (1979, 1990) described vegetative evidence of hybridization between *Alnus serrulata* and '*Alnus incana* ssp. *rugosa*' in the mountains of West Virginia and coastal plain of Maryland where their ranges overlap. Woodworth (1929) described apomixis in populations of *A. serrulata* (which he called *A. rugosa*), but in a later paper (1930) he showed that an *A. rugosa* collection had normal cytological characteristics. Our collections of these species were therefore of special interest.

While the usual pore number in a pollen collection from subgenus *Alnus* may be 3 to 6 as in *A. incana* and *A. hirsuta* (Table 3; Appendix 1), the range is very different and greatly expanded in certain presumably hybrid specimens (Figure 3; Table 4). In *A. rugosa*, certain collections have a much greater variance than others. In pollen from New England three collections may demonstrate the hybrid character of *Alnus rugosa* pollen. Two collections of *Alnus rugosa* and one of *Alnus rugosa* var. *americana* (EL 7–23–53; JG 8–8–67 and EL 109 P3727) show highly aberrant pollen with pore numbers ranging from 0 to 8 or 9 and cell sizes ranging from 10.4 to $\sim$ 50 μm in diameter. Many cells are misshapen; 2- and 3-pored and inaperturate cells are tiny (Plate 1, figures 15–20) and have the same wall structure and color as all the other pollen in the collection, so we take these to be cytological aberrant pollen cells suggesting only partial fertility. However, in another *Alnus rugosa* var. *americana* (EL 157 P3726, Figure 3) as well as two further collections of *A. rugosa* (Table 1; and figured by Reinink-Smith 2010, plate 1, figures 28–29) show normal pore numbers (3–5) and size range (15.6–28.8 μm). Clearly some east-coast specimens of *Alnus rugosa* seem to have normal characteristics and some seem to be hybrids.

In the two collections of *Alnus serrulata*, only a small variation ($\pm$ 1) in pore number range exists and sizes seem in the normal range (25–32 μm; 2- to 5-pored in EL 106 P3729; Appendix 1). Available collections of this species do not show hybrid characteristics. Curiosity is therefore focused on the extreme range in pore numbers in three specimens of *A. rugosa* plotted in Figure 3. The geographic locality of these presumed-hybrid specimens is central east coast USA, which is the region where Furlow (1979, 1990) stated that hybrids of these taxa are occasionally found. Murai (1968) also describes areas of hybridization in other *Alnus* species, for example between *A. crispa* and *A. sinuata* where their ranges overlap and among Asian species of *Alnus*.

Pollen of the sister genus *Betula* in certain cases shows morphological variance. Leopold found one *Betula nana* collection from Scotland (Dan Livingstone, Duke Collection 537) with not only strong arci (or folds) on a 3-pored pollen grain, but the grain also had a highly developed polar arcus (Plate 1, figure 31). E.B. Leopold and J.A. Wolfe have both seen such grains in the Neogene fossil pollen preparations from Alaska (oral communications 2000). In the Miocene of Poland, Stuchlik et al. (2009) figured a wide number of *Betula* grains with polar arci (plate 26, figures 1–6, 8–9; plate 34, figures 10–19). The apparently strange occurrence of *Alnus* pollen from a collection of *Betula glandulosa* in the Yale Herbarium (EL 118) might suggest a misidentification problem. However, Wang et al. (1995) provide examples in China of two *Betula* species having from 2- to 5-pored and 2- to 4-pored (hence *Alnus*-like) pollen. These observations clearly illustrate the genetic proximity of the sister genus *Betula* to *Alnus* (*Alnobetula*) as discussed by Chen and Li (2004).

Using allozyme data, Bousquet et al. (1987) placed the divergence of '*A. viridis* ssp. *crispa*' from '*A. incana*/*rugosa*' as only about one million years ago (see Furlow 1979, p. 26) marking the presumed first evolution of *Alnobetula*, but the subgenus is probably older. From leaf characters and venation patterns, Wolfe (1966) indicated that *Alnus viridis*/*crispa* (*Alnobetula*) appeared in the Pliocene (Clamgulchian) of Alaska. Wolfe (1966, p. B18) also reported that ancestral species of both *A. incana* and *A. crispa* appear in the Miocene. *Alnus* fruits (subgenus *Alnobetula*) described by Fyles and Mathewes (2000) and Fyles et al. (1994) are reported in the Pliocene of Banks Island and Neogene of Ellesmere Island. Because taxa of the *Alnus viridis* complex are chiefly northern and some are arctic (Murai 1968, figure 4), these fossil records might mark the development of cold-adapted members of the genus. Reinink-Smith's (2010, plate 1, figures 11–14) pollen examples from the Late Miocene of Alaska seem to record *Alnobetula* phenotypes (6-pored grains with polar arci; see Reinink-Smith, 2010, plate 1, figures 16–18). Miocene species based on leaves in a wide number of localities in Japan and Korea were reported by Tanai (1961) and by Tanai and Suzuki (Wakamatsu flora, 1963 p.73) as ancestral to the *Alnobetula* species *A. maximowiczii*.

8. Conclusions

Pollen of the genus *Alnus* is morphologically unique among angiosperms being breviaxial, stephanoporate with protruding, aspidate multiple pores and usually arci thickenings between the pores. The pollen data in this study uphold the fundamental dichotomy between the subgenus *Alnaster* (subgenus = *Alnobetula*) and the rest of the genus, as described by the gross morphology and by nrDNA ITS data. Within the subgenus

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Alnobetula (= *Alnaster*), the range of pollen morphology based on 17 reference slides (collections from 9 taxa) demonstrate that the subgenus *Alnobetula* is well defined by the dominance of 5- to 7-pored pollen. Two Asian taxa of *Alnobetula* that are closely related according to DNA ITS data (*Alnus firma* and *Alnus sieboldiana*) stand out by having dominantly 6- or 7-pored pollen grains that are large; these features are not seen elsewhere in the genus as sampled here. The common occurrence of circular polar arci in these two taxa is also an important phenotypic character, useful in tracing the genetic relationships and heritage of Miocene pollen along the Pacific Rim as shown by Reinink-Smith (2010). These features of modern alder pollen are linked to the occurrence of winged seeds in *Alnobetula*. Within the subgenus *Alnus*, however, the pollen morphological features show little relation to the groupings based on gross morphology or taxonomy. Of the 16 component taxa studied, ten species are dominantly 4-pored and three others are 5-pored; three are inconsistent in dominant pore number. Neither the subgenus *Clethropsis* (3 species) nor the section *Crematstogyne* (3 species) of Chen and Li (2004) is identifiable in terms of pollen characters alone, or in fact by ITS data according to Navarro (2003). The subgenus *Alnus* contains a mix of predominantly P4 and P5 taxa, although the P4 condition is most common.

Based on fossil leaves and venation evidence provided by Wolfe (1966), Wolfe and Tanai (1980) and fruits described by Fyles et al. (1994) and Fyles and Mathewes (2000) in Alaska, the subgenus *Alnobetula* may have appeared by the middle Miocene (Seldovia Point) and in later Neogene deposits. Reinink-Smith (2010) provided new pollen evidence that the subgenus *Alnobetula* (probably Clade A, now restricted to Japan) makes a distinct appearance in Alaska in the Late Miocene. The Miocene megafossils of *Alnobetula* ('*Alnaster*'; Tanai 1961; Wolfe and Tanai 1980) on both sides of the Pacific Rim are now also corroborated by pollen evidence that indicates the presence of the Bering Land Bridge by the middle Miocene.

The pollen features useful for tracking geographic occurrences of *Alnobetula* lineages of the Old World are the common occurrence of circular polar arci in large-sized pollen grains with many (5–8) pores. Depending on the geographic region (where a limited number of *Alnus* taxa are present in the flora), the difference in pollen morphology of subgenera provides a tool to help identify subfossil pollen of present-day alder taxa.

Cytologically aberrant pollen found in three out of six collections of *Alnus rugosa* suggest the existence of hybrids, as predicted by Furlow's (1990) field observations of hybrids in the eastern seaboard of the USA. Certain features typical of alder pollen (presence of arci, variance in pore number) are seen as occasional features of other Betulaceae genera, *Carpinus* and *Betula*. The authors find that Chen and Li's (2004) DNA-based taxonomy of *Alnus* fits well with the pollen features as the vegetative subcategories of Furlow (1979), mainly because both systems strongly recognize the separateness of subgenus *Alnobetula* (formerly *Alnaster*) within the genus. Neogene records of both macrofossil and pollen data are in accord with the suggestions from taxonomists Chen and Li (2004), Nararro (2003) and Furlow (1979, 1990) that *Alnobetula* may represent the basal (more ancient) section of the genus *Alnus*.

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Appendix 1. Locality data for 59 collections of *Alnus* taxa

Table A1. List of *Alnus* pollen modern reference material (alphabetical by subgenus) and localities of samples used here. Taxa are as in Chen and Li (2004). Selected *Betula* pollen reference material employed for this report are included. JG are Jane Gray preparations. EL are E. Leopold preparations from Yale Herbarium. P numbers are Leopold preparations of the US Geological Survey collections. S numbers are Leopold's Seattle preparations at the University of Washington.

Taxon	Herbarium Collection #	Sheet Number	Locality
GENUS *ALNUS*			
SUBGENUS *ALNUS*			
A. acuminata Kunth	P1239	UC 141949	Sonora, Mexico
A. cremastogyne Burk.	P2145	USNH 1529622	Szechuan, China
A. ferdinandi-coburgii (C.K. Schneider)	P2143	USNH 2244070	Yunnan, China
A. japonica Sieb. et Zuc	JG 11-14-55	UC365601	Hokkaido, Japan
	JG 12-27-61	K. Sohma coll.	Honshu, Japan
A. jorullensis H.B.K.	JG 3-17-56	UC 350582	Jalisco, Mexico
A. lanata Duthie ex Bean	P2138	USNH 539606	Szechuan, China
A. nepalensis D. Don.	P2135	USNH 1058217	Yunnan, China
A. rhombifolia Nutt.	JG 3-19-56	UC 718154	San Diego Co., CA
	JG 7-13-67	Sheldon 11772, U of OR	Coos Co., OR
	JG 3-12-56	UC	Santa Clara Co., CA
	JG 12-17-53	UC 630537	Del Norte Co., CA
	S272	UW 57973	Darlington, CA
A. serrulata (Aiton) Willd. var. *vulgaris* Spach.	JG 3-13-56	UC 806037	Bedford Co., PA
A. serrulata (Aiton) Willd.	EL 106, P3729	Yale Herbarium	New Haven, CT
	JG 5-24-67	U of OR	Rock Point, VT
***Alnus incana* Complex**			
-*A. glutinosa* (L.) Gaertn.	JG 1-5-68	OSU 113954	Corvallis, OR campus
	JG 8-2-67	U of OR	Upland, Sweden
-*A. hirsuta* Turcz. ex Rupr.	JG 3-12-56	UC 365600	Hokkaido, Japan
-*A. incana* (L) Moench.	JG 3-16-56	UC 253607	Hungary
-*A. incana* ssp. *incana* Moench**	JG 2-4-68	OSU 42178	Jackson Co., OH
	JG 7-27-67	U of OR	Sweden
	JG 11-56	U. of Texas	Hanover, NH
	P2052	J. Wolfe coll.	Alaska
	P2050	J. Wolfe coll.	McCarthy, AK
-*A. rubra* Bong.	P772	CU no. 64795	Marin Co., CA
	S19	D. Every 223	Seattle, WA
	JG 5-29-67	OSU 112736	Coos Co., OR
	JG 2-28-68	OSU 83985	Columbia Co., OR
	JG 3-10-56	UC 275559	Humboldt Co., CA
	JG 7-18-67	U of OR	Washington Co., OR
-*A. rugosa* (DuRoi) Spreng. var. *americana* (Regel) Fern.	JG 1-19-68	OSU 103418	Labrador, Canada
-*A. rugosa* "hybrid"	EL 7-23-53	Yale Herbarium	Franklin Co., MA
-*A. rugosa* var. *americana* Koch. "hybrid"	JG 8-8-67	U of OR	Rock Point, VT
-*A. rugosa* (DuRoi) Spreng.	JG 11-18-55	UC 588595	Montgomery Co., PA
-*A. rugosa* var. *americana* Koch. "hybrid"	EL 109; P3727	Yale Herbarium	Cheshire, CT
-*A. rugosa* (DuRoi) Spreng. var. *americana*	EL 157; P3726	Yale Herbarium	Roane Co, TN
-*A. sibirica* (Spach) Turcz. exKom.	P2140	USNH 2186298	Korea
-*A. tenuifolia* Nutt.	JG 7-25-67	U of OR	Pend Oreille, ID
-*A. tenuifolia* Rydberg	JG 11-14-55	UC 54759	Humboldt Co., CA
	P1388	R. Tschudy	Jamestown, CO
	S274	UWa 134673	Latah Co., ID
	P1373	E. Leopold coll.	Boulder, CO
SUBGENUS *CLETHROPSIS*			
A. maritima Nutt.	JG 11-15-55	UC 904033	Harden City, OK
	JG 1-22-68	OSU 116439	Nassau, Sussex Co., DE
A. formosana Burk.	JG 7-6-67	Taiwan Normal	Taiwan

(*continued*)

Table A1. (*Continued*).

Taxon	Herbarium Collection #	Sheet Number	Locality
SUBGENUS *ALNOBETULA*			
***Alnus viridis* Complex**			
-*A. crispa* (Ait.) Pursh.	JG 3-17-56b	UC 991802	Humber District, Canada
	JG 3-17-56a	UC 920645	Yellowknife, Canada
-*A. fruticosa* Rupr.	JG 8-7-67	U of OR	Lolo pass, MT
	JG 2-22-68	OSU 117598	Anchorage, AK
-*A. mandshurica* Callier	P2139	USNH 1052008	Korea
-*A. maximowiczii* Callier***	JG 12-23-61	K. Sohma coll.	Mt. Zao, Japan
-*A. maximowiczii**	P 2137	USNH 778285	Hondo, Japan
-*A. sinuata* (Regel.) Rydb.	JG 3-11-56	UC 545821	Del Norte Co., CA
	JG 2-14-68	OSU 90272	Hood River Co., OR
-*A. sinuata**	P2054	J. Wolfe	McCarthy, AK
-*A. sinuata**	P770	CU 86170	Snohomish Co., WA
-*A. sinuata** (Regel.) Löve et Löve	JG 12-14-67	OSU 88219	Latah Co., ID
-*A. sinuata* (Regel.) Rydb.	JG 8-1-67	U of OR	Cresent City, CA
Other: Clade A			
A. firma (S. and Z.) Murai	P2142	USNH 2244070	Oshima, Japan
A. pendula Matsum.	P2133	USNH 2188506	Hondo, Japan
	P2055	UCM082105	Honshu, Japan
A. sieboldiana Matsum.	P2132	USNH 2247505	Hiroshima, Japan
GENUS *BETULA*			
Betula glandulosa Michx.	EL 118	Yale Herbarium	Mount Washington, NH
"	EL 158	Yale Herbarium	Mount Washington, NH
Betula nana L.	Dan Livingstone 537	Duke University Herbarium	Scotland

* Herbarium collection is labeled *Alnus viridis* ssp. x_____

** Herbarium collection is labeled *Alnus incana* ssp. x_____

*** Herbarium collection is labeled *Alnus crispa* ssp. x_____

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