American Journal of Botany 91(6): 943–952. 2004.

NORTHERN HEMISPHERE BIOGEOGRAPHY OF *CERASTIUM* (CARYOPHYLLACEAE): INSIGHTS FROM PHYLOGENETIC ANALYSIS OF NONCODING PLASTID NUCLEOTIDE SEQUENCES[1]

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Phylogenetic relationships and biogeography of the genus *Cerastium* were studied using sequences of three noncoding plastid DNA regions (*trnL* intron, *trnL-trnF* spacer, and *psbA-trnH* spacer). A total of 57 *Cerastium* taxa was analyzed using two species of the putative sister genus *Stellaria* as outgroups. Maximum parsimony analyses identified four clades that largely corresponded to previously recognized infrageneric groups. The results suggest an Old World origin and at least two migration events into North America from the Old World. The first event possibly took place across the Bering land bridge during the Miocene. Subsequent colonization of South America occurred after the North and South American continents joined during the Pliocene. A more recent migration event into North America probably across the northern Atlantic took place during the Quaternary, resulting in the current circumpolar distribution of the Arctic species. Molecular clock dating of major biogeographic events was internally consistent on the phylogenetic trees. The arctic high-polyploid species form a polytomy together with some boreal and temperate species of the *C. tomentosum* group and the *C. arvense* group. Lack of genetic variation among the arctic species probably indicates a recent origin. The annual life form is shown to be of polyphyletic origin.

Key words: biogeography; *Cerastium*; cpDNA; molecular phylogeny; Northern Hemisphere; *psbA-trnH*; support weighting; *trnL-trnF*.

The historical biogeography of the Northern Hemisphere has long been of interest to botanists and zoologists. Evidence for different biogeographic patterns has increased in recent years with the increasing number of molecular phylogenetic studies being published. A recent synthesis of non-marine animal phylogenies, for example, suggests that the Northern Hemisphere has a complex biogeographic history involving vicariance events that span time intervals from the Cretaceous onwards (Sanmartin et al., 2001).

Biogeographic studies of the Northern Hemisphere floras have generally focused on taxa with restricted and disjunct distributions that are thought to have been broader in the past (i.e., the eastern Asian–eastern North American disjunctions, e.g., Wen, 1999; Donoghue et al., 2001; Xiang and Soltis, 2001; Milne and Abbott, 2002). Those few taxa studied that have wider distributions throughout the Northern Hemisphere are mostly evergreen or deciduous angiosperms adapted to a warm temperate, subtropical, or tropical climate (e.g., *Paeonia*, Sang et al., 1997; *Quercus*, Manos et al., 1999; *Arbutus*, Hilman et al., 2001; *Lycium*, Tatsuya et al., 2001; but see Lindqvist and Albert, 2002). Thus far, little is known about the biogeographic history of more cold-adapted Northern Hemisphere taxa.

Two major migration routes have been discussed with regard to the historical biogeography of the Northern Hemisphere floras: the North Atlantic land bridge (NALB) and the Bering land bridge (BLB). The NALB is thought to have been most important during the early Tertiary, connecting the floras of North America and southern Europe via Scotland and southern Greenland, or via northern Greenland and Fennoscandia (Tiffney, 1985). It is generally accepted that the southernmost connection was severed during the early Eocene, approximately 50 million years ago (Mya), when the climate was subtropical at these latitudes (Tiffney, 1985; Tiffney and Manchester, 2001). The northernmost connection is thought to have persisted until ca. 40 Mya (Milne and Abbott, 2002). This second connection may have been less important because Fennoscandia was separated by seaways from southern Europe as well as from Asia at the time (Tiffney, 1985).

The connection between Asia and North America via the Bering land bridge persisted throughout most of the Tertiary and was severed at approximately 5.5–4.8 Mya (Marincovich and Gladenkov, 1999). It is thought that the BLB was most important for intercontinental migration after the NALB dis-

[1] Manuscript received 17 July 2003; revision accepted 30 January 2004.

The authors thank the curators at the ILLS, MO, O, W, WU herbaria, Clive Stace, and Danny Gustafson for providing material, Matthew Gitzendanner and Charlotte Lindqvist for help in the lab, and Steve Farris for permission to use the XAC parsimony jackknifing application and the ZAC support weighting application. Most of the laboratory work was performed at the Soltis laboratory, University of Florida, and some sequencing was performed at the MegaBACE-laboratory, University of Oslo. This project was initiated and supported in part by a Fulbright Fellowship to A. C. S. Additional funding was provided by the National Centre for Biosystematics at The Natural History Museums and Botanical Garden, University of Oslo, and by the University of Florida Research Foundation.

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appeared (Tiffney, 1985; Tiffney and Manchester, 2001) and that it was more important for deciduous angiosperms than for evergreen angiosperms because the high latitude of the region would have served as a filter to the migration of evergreen angiosperms due to winter darkness (Tiffney and Manchester, 2001; Milne and Abbott, 2002). The BLB reappeared during the Quaternary glacial epochs (Elias et al., 1996). During the Quaternary glaciations, migration across the BLB was only possible for taxa of arctic or boreal affinity (Hultén, 1937; Murray, 1981). The Bering area was an important refugium for the arctic flora during the Quaternary glaciations (e.g., Hultén, 1937; Abbott et al., 2000; Abbott and Brochmann, 2003), and the BLB must have influenced the biogeography of the current circumpolar flora.

Divergence time estimates may become the most reliable method for distinguishing between migration via the BLB and via the NALB (Donoghue et al., 2001). Even when an assumption of a molecular clock cannot be calibrated with fossil data, age estimates of divergence times based on well-studied geological events are better than none at all. Furthermore, internal consistency of several divergence time estimates within the same molecular phylogeny, i.e., several independent geologically correlated migration/vicariance events, provide self-corroboration even in the absence of a strict molecular clock.

The genus *Cerastium* L. (Caryophyllaceae) can be used for testing different hypotheses of biogeographic relationships in the Northern Hemisphere. *Cerastium* is a genus of approximately 100 perennial or annual, herbaceous, or rarely slightly woody species (e.g., Jalas et al., 1993; Dequan and Morton, 2001), with an almost cosmopolitan distribution. The genus is most abundant in temperate and cold regions, especially at high elevations, with a center of diversity in Eurasia (Dequan and Morton, 2001). A few species are cosmopolitan weeds (e.g., *C. fontanum* and *C. glomeratum*), but most species have a restricted distribution.

In this paper, we provide a molecular phylogeny of the genus *Cerastium* based on sequences of noncoding plastid DNA regions. We use the phylogenetic relationships discovered to elucidate the biogeography of the genus with emphasis on the Northern Hemisphere. Divergence time estimates for three independent migration/vicariance events are made using a simple molecular clock assumption. The molecular phylogeny is also compared to currently recognized infrageneric relationships within the genus.

MATERIALS AND METHODS

Materials—Leaf material for DNA extraction and sequencing was collected in the field and in botanical gardens and dried in silica (Appendix; see Supplemental Data accompanying the online version of this article). Additional material was sampled from herbarium specimens (in ILLS, MO, O, W, and WU) or from vouchers from other recent studies on *Cerastium* (Khalaf and Stace, 2000; Gustafson et al., 2003; Appendix).

DNA extraction, amplification, and sequencing—Total DNA was extracted from dried leaf material (herbarium specimens or silica-dried material) using the cetyltrimethylammonium bromide (CTAB) miniprep method of Doyle and Doyle (1987). The *trnL* intron and *trnL-trnF* intergenic spacer (hereafter, the *trnL-trnF* region) were amplified using the universal primers of Taberlet et al. (1991). For most specimens, the *trnL-trnF* region was amplified as one fragment using the c and f primers, but for some of the herbarium specimens the *trnL* intron and *trnL-trnF* intergeneric spacer had to be amplified separately using primers c and d and e and f, respectively. The *psbA-trnH* region was amplified using the primers *psb*AH and *trn*HR of Sang et al. (1997).

Polymerase chain reactions (PCR) were performed in volumes of 25 μL containing 1× PCR buffer (0.05 mol/L KCl, 0.02 mol/L Tris [pH 8.0], 1.5 mmol/L $MgCl_2$, 0.001% Tween), 200 μmol/L of each dNTP (USB, Cleveland, Ohio, USA), 0.5 μmol/L of each primer, 5% DMSO, and 1.25 units *Taq* DNA Polymerase (Eppendorf, Hamburg, Germany), and 2.5 μL of unquantified genomic DNA. In a few cases, PCR reactions were performed in volumes of 25 μL using the AmpliTaq DNA polymerase buffer II kit (Applied Biosystems, Foster City, California, USA) containing 0.2 mmol/L of each dNTP, 0.04% bovine serum albumen (BSA), 0.01 mmol/L tetramethylammonium chloride (TMACl), 0.8 μmol/L of each primer, and 2 μL unquantified genomic DNA. Amplifications were performed in a Biometra T3 thermal cycler (Whatman Biometra Biomedizinishe Analytik GmbH, Göttingen, Germany) or in a Gene Amp PCR System 9700 (Applied Biosystems) using a program consisting of 4 min at 95°C followed by six cycles of 20 s denaturation (95°C), 1 min annealing (starting at 58°C; temperature decreasing by 1°C per cycle) and 1 min extension (72°C), and 35 (in a few cases only 25) cycles of 20 s denaturation (95°C), 1 min annealing (52°C) and 1 min extension (72°C), ending with a final 5 min extension (72°C). Successful PCR reactions were purified with exonuclease I and shrimp alkaline phosphatase (ExoSAP).

Cycle sequencing, using the same primers as in the PCR, was performed with CEQ 2000 Dye Terminator Cycle Sequencing kit (Beckman Coulter, Fullerton, California, USA) in quarter reactions (i.e., 2 μL DTCS Quick Start Master Mix, 3.2 pmol primer and 1 μL cleaned PCR product), using the program suggested by the manufacturer on an Eppendorf Mastercycler (Brinkmann Instruments, Westbury, New York, USA). Sequenced products were precipitated in ethanol and sodium acetate to remove excess dye terminators before running them on a CEQ 8000 Sequencer (Beckman Coulter, Fullerton, California, USA). In a few cases, cycle-sequencing reactions were prepared with DYEamic ET dye terminator kit for MegaBACE (Amersham Biosciences, Piscataway, New Jersey, USA) following the manufacturer's recommendations and were run on a MJ Dyad 96-block thermal cycler. In these cases, the products were purified using Sephadex and run on a MegaBACE 500 (Amersham Biosciences, Piscataway, New Jersey, USA).

Alignment and indel coding—Sequences were assembled and edited using Sequencher 4.1.4 (Gene Codes, Ann Arbor, Michigan, USA). The sequences were aligned manually using Se-Al (Rambaut, 2002). Most of the alignments were straightforward, but the many gaps made some sections of the alignments ambiguous. Solving such ambiguities was attempted by following the advice of Kelchner (2000). Thus, gaps resulting from variable-length strings of a mononucleotide repeat unit were not considered as potential phylogenetic characters. Also, insertions of equal length that differed in being a repeat unit of a sequence at either side of an insertion were treated as separate insertion and deletion (indel) events. Only one section, a hypervariable AT-rich region of the *trnL* intron, had to be excluded because of alignment ambiguity (see Results). Indels were coded as present/absent (coded as A/T because NONA cannot read numeric characters in combination with nucleotide characters) and added to the matrices as additional, unordered characters following the ''simple indel coding'' of Simmons and Ochoterena (2000). Only indels of two or more base pairs were considered as potentially informative characters.

In a previous analysis of subfamilial relationships within Caryophyllaceae, *Stellaria* L. was recognized as sister to *Cerastium* (Smissen et al., 2002). Accordingly, *S. longipes* and *S. graminea* were used as outgroups in the current analysis, following the outgroup criteria of Farris (1972, 1980). Aligning the *psbA-trnH* sequences of *Cerastium* with those of *Stellaria* proved difficult. Therefore, *C. cerastoides* and *C. dubium*, which formed a sistergroup to the rest of the ingroup taxa in the analyses of the *trnL-trnF* region (see Results), were used as an alternative outgroup in the analysis of the *psbA-trnH* data.

Phylogeny reconstruction—The *psbA-trnH* spacer, the *trnL* intron, and *trnL-trnF* spacer matrices were subjected to parsimony analyses using NONA (Goloboff, 1999), equal character weights, gaps as missing data, 100 random entry-order replicates, and tree bisection-reconnection (TBR) branch swapping, followed by 100 parsimony ratchet iterations (Nixon, 1999a). Initially, parsimony analyses using both PAUP* (Swofford, 2002) and NONA were attempted, but when analyzing the *trnL-trnF* spacer matrix PAUP* hit the

cutoff point of 30 500 most parsimonious trees (MPTs; 93 steps) while swapping on the first tree. The same analysis in NONA gave six MPTs (93 steps; see Results). NONA does not consider branches with minimum optimized length equal to zero, but stores trees as dichotomous. When using the default settings, PAUP* considers such potential zero-length branches, thus increasing the number of most parsimonious trees. Therefore, only NONA was used for the remaining analyses. Alternative MPTs were imported from NONA into Winclada (Nixon, 1999b), where actual and potential zero-length branches were negated (the ''hard collapse'' option) and strict consensus trees calculated.

Parsimony jackknife support for internal branches was estimated using the program XAC (J. S. Farris, unpublished program). One thousand replicates were conducted, each performing subtree pruning-regrafting (SPR) branch swapping with five random entry orders per replicate. With XAC, approximately 63% or higher jackknife frequency (63% corresponding to the complement of the character removal rate, e^{-1}) represents (with sampling error) support by the equivalent of one or more uncontradicted synapomorphies (Farris et al., 1996); values between approximately 63% and 50% (ambiguity) should have some robustness to extra steps. Parsimony jackknife trees were subjected to support weighting (Farris, 2001) using the program ZAC (J. S. Farris, unpublished program). Use of this application follows the findings of Källersjö et al. (1999; see also Savolainen et al., 2002) in that characters are directly reweighted (successively) according to their performance with respect to branch support rather than via homoplasy measures, which show no necessary correlation with branch support. For example, Källersjö et al. (1999) found that the retention index (Farris, 1989) was higher for a subset of characters (third positions of codons) that supported groups better on average, but that the retention index for these same sites varied consistently with decreasing data matrix size. As such, support must not be conflated with homoplasy for an accurate evaluation of positive support present in data. Here, we take the support-weighted tree to represent best those groups with positive support; jackknife values from equally weighted data (mapped onto these trees) provide a more quantitative estimate of support, but one that also includes the influence of homoplasy.

For a rough estimate of the minimum age of some clades, a simple molecular clock assumption was employed (cf. Sanderson, 1998; Lindqvist and Albert, 2002) based on the earliest opening of the Bering Strait, approximately 7.4–4.8 Mya (Marincovich and Gladenkov, 1999). Simple proportions of the minimum path lengths of unambiguously optimized substitutions, as calculated by Winclada (Nixon, 1999b), gave maxima and minima of 2.29 and 1.35 substitutions per million years. Estimates of other minimum ages were made by dividing number of changes by the above rates. Internal consistency was checked by comparing the estimated age with the known age of the formation of the Isthmus of Panama (3.5–1.9 Mya; Coates and Obando, 1996; Haug and Tiedemann, 1998).

RESULTS

Sequence divergence and alignments—The *trnL* intron matrix included 48 taxa: 46 species of *Cerastium* and two species of *Stellaria*. The length of the intron varied from 489 base pairs (bp) (e.g., *C. arcticum*) to 559 bp (*C. lithospermifolium*; see Appendix for GenBank accessions in Supplemental Data accompanying the online version of this article). The first 65 characters of the 5′ end of the *trnL* intron were excluded from the matrix due to difficulties with reading the beginning of the sequences. In addition, up to 82 bp in an AT-rich area of variable length (position 325–407) were also excluded from the matrix. After alignment and the exclusion of the two segments just described, the *trnL* intron had an aligned length of 559 bp. Twenty indels were coded as present/absent (A/T). The resulting matrix had 579 characters, of which 38 were parsimony informative.

The *trnL-trnF* spacer matrix included 46 taxa: 44 species of *Cerastium* and two species of *Stellaria*. The length of the spacer varied from 322 bp (*C. uniflorum*) to 432 bp (e.g., *C. latifolium*). No part of the *trnL-trnF* spacer was excluded, and the aligned length was 482 bp. Twenty-six indels were coded as present/absent (A/T), resulting in a matrix of 508 characters of which 57 were parsimony informative.

The *psbA-trnH* matrix included 55 taxa of *Cerastium*. The sequences of the two species of *Stellaria* were not included because they deviated strongly from the sequences of the *Cerastium* species, making the alignment ambiguous. For the *Cerastium* species, the length of the spacer varied from 266 bp (*C. subpilosum*) to 333 bp (*C. cerastoides*). After alignment and the exclusion of 29 bp of the beginning (5′ end) and 21 bp of the end (3′ end) of the fragment due to difficulties in reading the sequence, the *psbA-trnH* spacer had an aligned length of 316 bp. Nineteen indels and three inversions were coded as present/absent (A/T), resulting in a matrix of 338 characters of which 55 were parsimony informative.

Phylogeny reconstruction—The maximum parsimony analysis of the *trnL* intron resulted in a single most parsimonious tree (MPT; tree not shown) of 71 steps with a consistency index (CI) of 0.94 and a retention index (RI) of 0.97. The maximum parsimony analysis of the *trnL-trnF* spacer resulted in six MPTs (not shown) of 93 steps (CI = 0.94, RI = 0.98). The parsimony analysis of the combined matrix (i.e., the *trnL-trnF* region; 1087 characters of which 95 were parsimony informative) resulted in 17 MPTs of 164 steps (CI = 0.95, RI = 0.98; strict consensus shown in Fig. 1). In all these analyses, the ingroup formed a monophyletic group with 100% jackknife support. *Cerastium cerastoides* and *C. dubium* formed a strongly supported clade in all three analyses (99% or 100% jackknife support; Fig. 1), thus justifying using them as alternative outgroups in the analysis of the *psbA-trnH* spacer. The maximum parsimony analysis of the *psbA-trnH* spacer resulted in three MPTs of 110 steps (CI = 0.74, RI = 0.92; strict consensus shown in Fig. 2). The reduced ingroup (excluding *C. cerastoides* and *C. dubium*) was monophyletic (100% jackknife support).

Two total (available) evidence analyses were performed, one including all taxa (orphans included; orphans being taxa for which one or two but not all three fragments had been sequenced) and one including only those taxa for which all three fragments had been sequenced with success (orphans excluded). Maximum parsimony analysis of the matrix including taxa with missing values for one or two of the three sequenced fragments (orphans included; 1425 characters of which 150 were parsimony-informative) resulted in more than 1000 MPTs (cutoff point) of 277 steps (CI = 0.85, RI = 0.94; not shown). When orphans were excluded, the number of parsimony-informative characters was reduced to 112, the number of MPTs was reduced to 4 (217 steps, CI = 0.87, RI = 0.94), and both resolution and jackknife support were increased (strict consensus shown in Fig. 3).

Two major clades and three subclades were resolved in the analyses (Figs. 1 and 3). *Cerastium cerastoides* and *C. dubium* formed a clade that received 100% jackknife support (Fig. 1). The other species formed a monophyletic group that received high jackknife support in all analyses except that based on the *trnL* intron alone (not shown). Within this ''eucerastium'' clade, three subclades were resolved. In the first subclade (the ''strephodon'' clade), species from Russia and Georgia were grouped with one species from Kyrgyztan with a jackknife support of 90% (Fig. 3; see also Fig. 1). In the analysis of

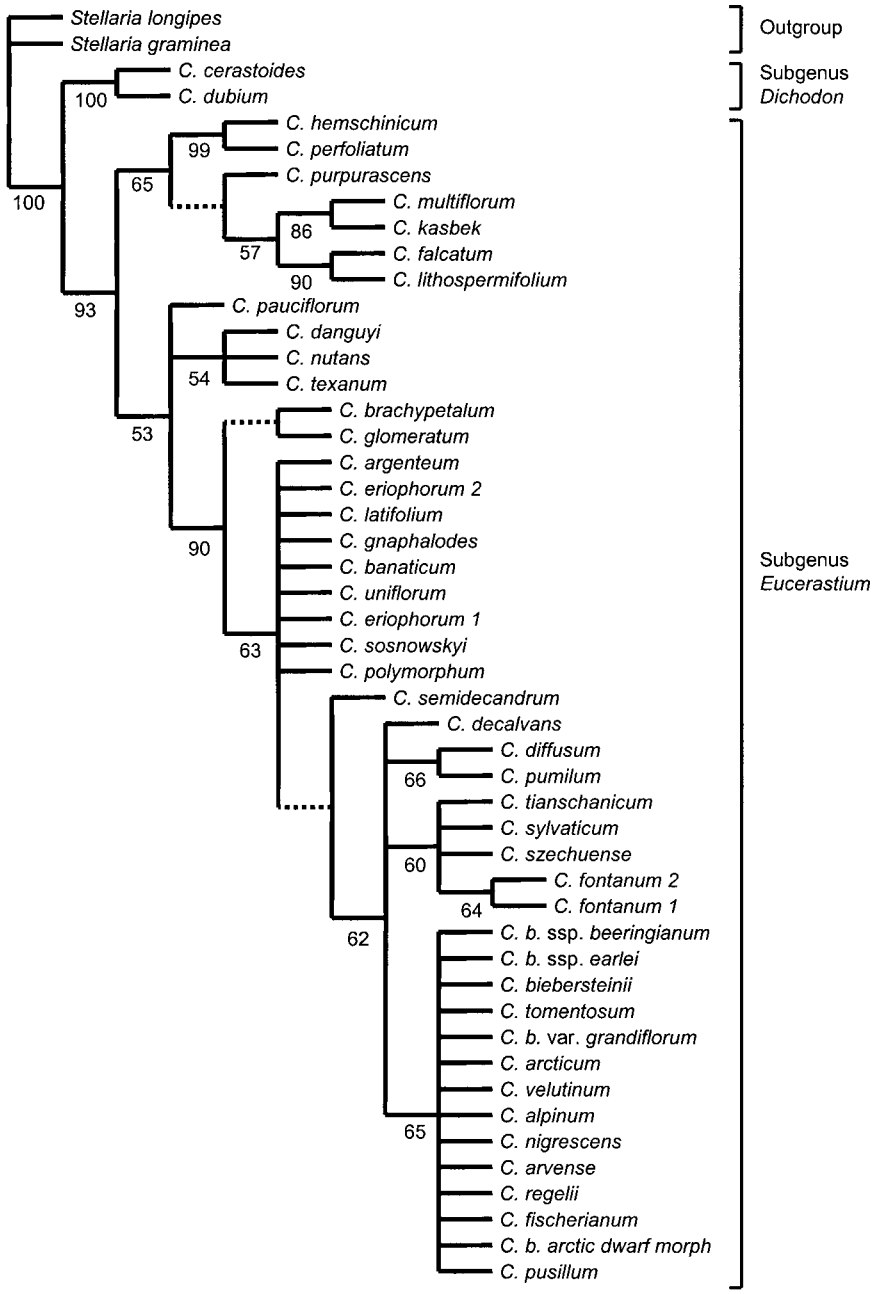

Fig. 1. Strict consensus of 17 most parsimonious trees found using the combined data of the *trnL* intron and the *trnL-trnF* spacer from *Cerastium* species. Parsimony jackknife values above 50% are shown below branches. Classification follows Schischkin (1970). Branches that collapsed in the support weighting analysis are shown with stippled lines. Subspecies and varieties of *C. beeringianum* are abbreviated thus: *C. b.* ssp. *beeringianum*, *C. b.* ssp. *earlei*, *C. b.* var. *grandflorum*, *C. b. arctic dwarf* morph. *Cerastium eriophorum 2* was collected as *C. laniferum*; *C. fontanum 2* was collected as *C. holosteoides* (see Appendix).

psbA-trnH, this clade occurred in both the strict consensus tree and the support weighting analysis and included two additional species from Russia and one species from Turkey, but did not receive jackknife support >50% (Fig. 2).

In the second subclade (the ''American'' clade), species from North and South America were grouped. In the *psbA-trnH* analysis, four South American species grouped with three of the North American species in a moderately supported clade (66% jackknife support, Fig. 2). This clade of American taxa was also present in the other analyses, although comprising fewer taxa. This clade received weak (54%, Fig. 1) to high (100%, Fig. 3) jackknife support, receiving a support value of 93% in the analysis of the *trnL* intron alone (tree not shown).

In the third subclade (the ''orthodon'' clade), most species formerly described as belonging to section *Orthodon* (Appendix, Fig. 3) were grouped with high jackknife support (85–100%). Within this clade, there was little resolution, especially in the *psbA-trnH* analysis (Fig. 2), but resolution was increased with the total available evidence approach (Fig. 3). The low-ploid ($2n = 36$) taxa from Southeast Europe grouped with two species from Georgia and one species from Turkey in a clade with a jackknife support of 69% (Fig. 3). The high-polyploid

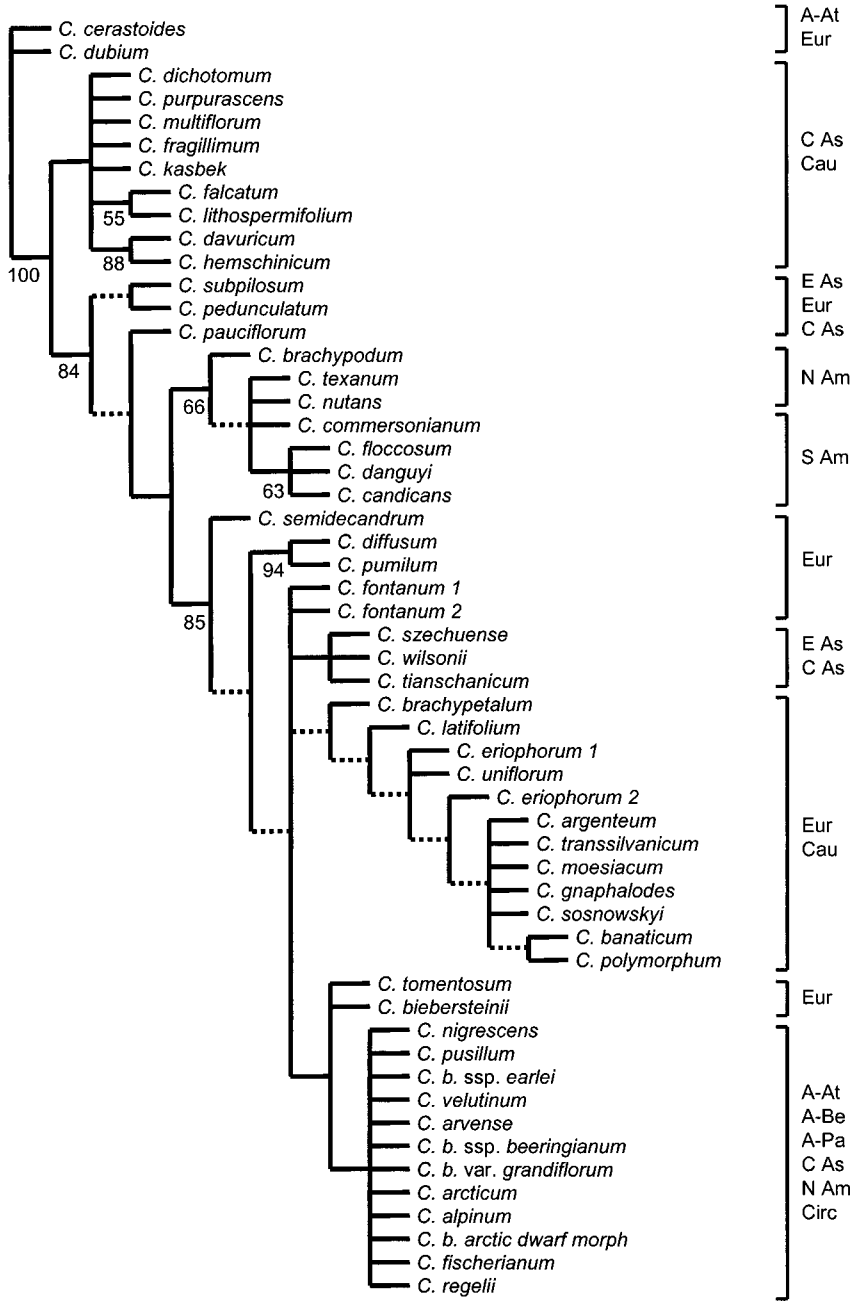

Fig. 2. Strict consensus of three most parsimonious trees found using the *psbA-trnH* data from *Cerastium* species. Parsimony jackknife values above 50% are shown below branches. Geographic distribution is shown to the right of the tree; A-At = Amphi-Atlantic, Eur = European, C As = Central Asian, Cau = Caucasian, S Am = South American, N Am = North American, E As = East Asian, A-Be = Amphi-Beringian, Circ = circumpolar/circumboreal, A-Pa = Amphi-Pacific. Branches that collapsed in the support weighting analysis are shown with stippled lines. Abbreviation: *C. b.*, *C. beeringianum*. *Cerastium eriophorum 2* was collected as *C. laniferum*; *C. fontanum 2* was collected as *C. holosteoides* (see Appendix).

species of the *C. alpinum* group were grouped with species of the *C. tomentosum* group and the *C. arvense* group with a jackknife support of 81% (Fig. 3).

DISCUSSION

Biogeographic inferences—The cpDNA phylogeny suggests an Old World origin of the genus *Cerastium*. A Eurasian center of diversity may suggest a Eurasian origin; however, none of the African taxa were included in the current analysis. The topology of the cpDNA phylogeny is correlated with geographic distributions of the taxa sampled. The North American taxa are divided, some grouping with the South American taxa and some forming a clade with the arctic taxa. This divergence probably corresponds to two separate migration events that can be explained by different Earth history events (Fig. 4).

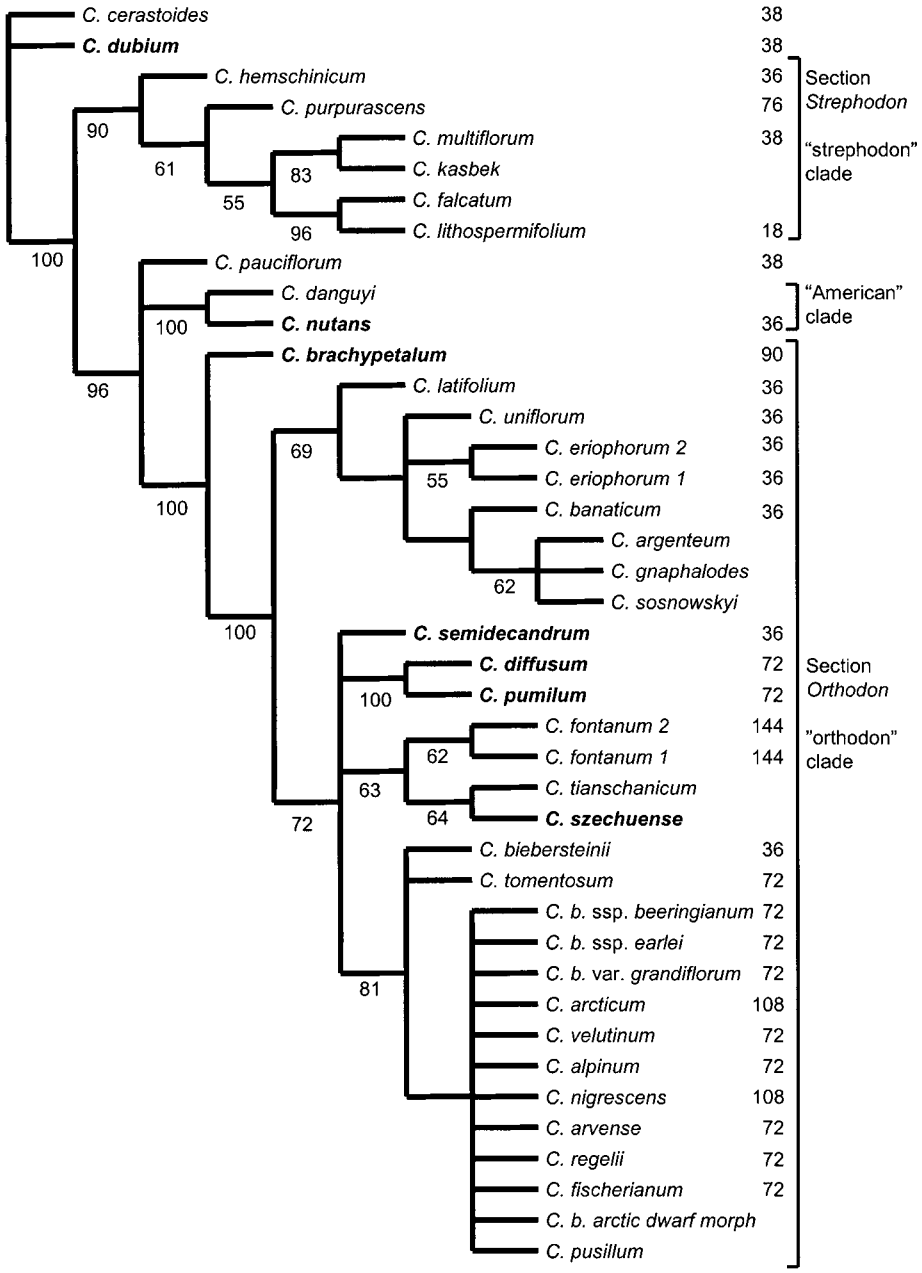

Fig. 3. Strict consensus of four most parsimonious trees for *Cerastium* species found using total available evidence, i.e., including only taxa sequenced for all three DNA regions (*trnL* intron, *trnL-trnF* spacer, and *psbA-trnH* spacer; orphans excluded). Parsimony jackknife values above 50% are shown below branches, and known chromosome numbers are shown to the right (e.g., Löve and Löve, 1975; Goldblatt, 1984, 1985; Goldblatt and Johnson, 1990, 1994). Sectioned classification follows Schischkin (1970) and Sokolova (1996). Annuals are in boldface type. The annuals of section *Orthodon* have previously been placed in subsection *Fugacia*; see Discussion: Infrageneric classification. The strict consensus and the support weighting analysis did not deviate topographically. Abbreviation: *C. b.*, *C. beeringianum*. *Cerastium eriophorum 2* was collected as *C. laniferum*; *C. fontanum 2* was collected as *C. holosteoides* (see Appendix).

Biogeography in the Americas—The South American taxa (ploidy level unknown) are grouped together with the tetraploid North American taxa ($2n = 34, 36$) with high jackknife support. The South American taxa are derived from within a temperate North American clade (Fig. 2). For a rough estimate of the minimum age of the South American taxa, a simple molecular clock assumption was employed (cf. Sanderson, 1998; Lindqvist and Albert, 2002) based on the earliest opening of the Bering Strait, approximately 7.4–4.8 Mya (Marincovich and Gladenkov, 1999; event 1 in Fig. 4). The Central Asian *C. pauciflorum* is sister to the ''American'' clade in all the analyses and the species of the ''strephodon'' clade from Central Asia or Caucasus are sister to *C. pauciflorum* and the ''American'' clade (Fig. 3). Minimum path lengths of unambiguously optimized substitutions as calculated by Winclada (Nixon, 1999b) amounted to 10 changes between *C. nutans* and *C. multiflorum* of the ''strephodon'' clade, and to 11 changes between *C. nutans* and *C. pauciflorum* (Fig. 4). Simple proportions yield maxima and minima of 2.29 and 1.35 substitutions per million years. In the same tree, the South

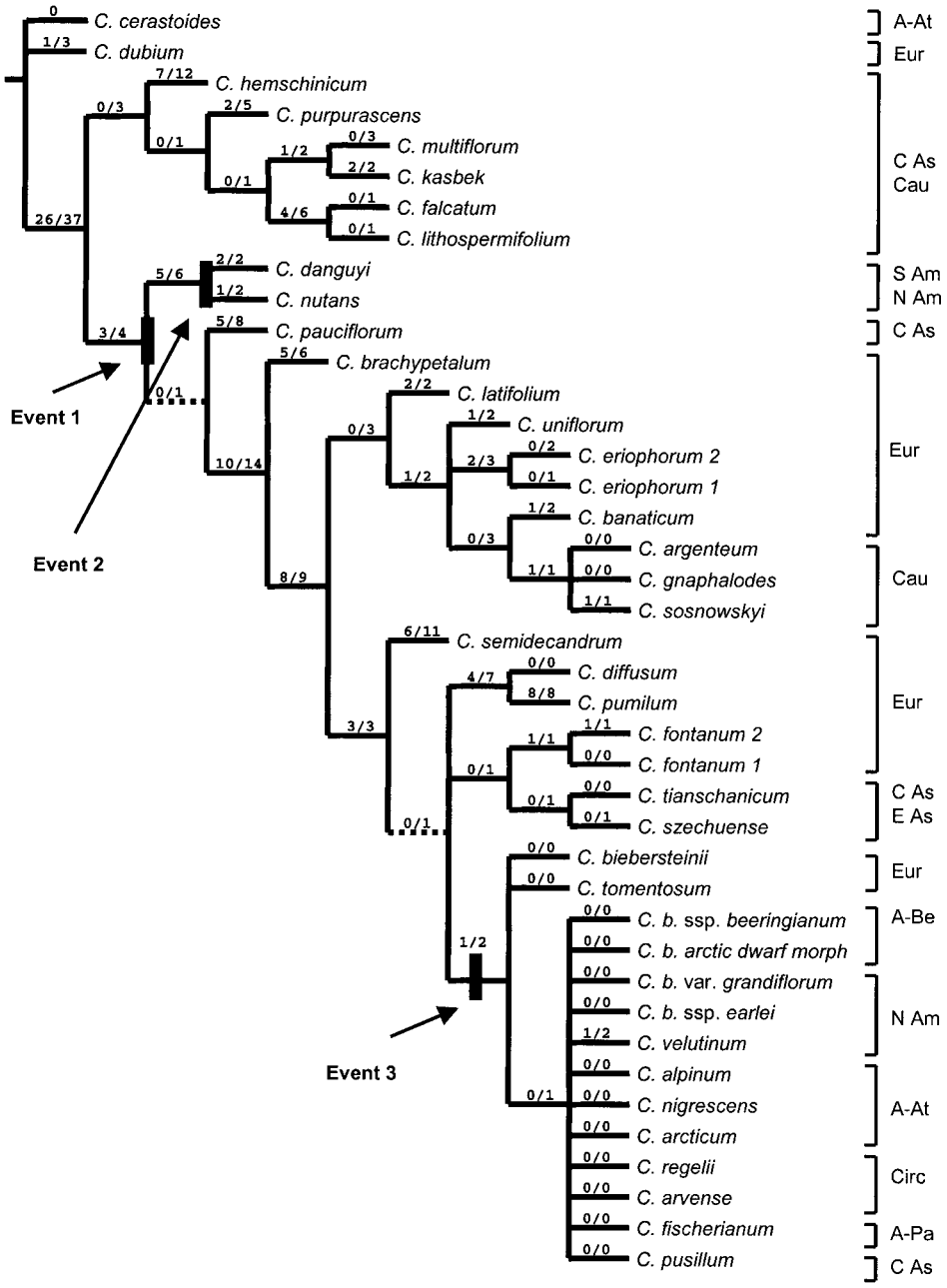

Fig. 4. One of the four most parsimonious trees for *Cerastium* species based on total available evidence (orphans excluded). Optimized unambiguous nucleotide changes (indels excluded)/optimized unambiguous changes (both nucleotide changes and indels) are shown above branches. The bold bars marking clades refer to biogeographic events discussed in the text: (1) the breakup of the Bering land bridge during the Pliocene, (2) the formation of the Isthmus of Panama, and (3) the invasion of the Arctic during the Pleistocene. Geographic distribution is shown to the right of the tree; for abbreviations see Fig. 2. Branches that collapse in the strict consensus are shown with stippled lines. Abbreviation: *C. b.*, *C. beeringianum*. *Cerastium eriophorum 2* was collected as *C. laniferum*; *C. fontanum 2* was collected as *C. holosteoides* (see Appendix).

American taxa are separated from the North American taxa by three changes. Dividing changes by the described substitution rates, South America was colonized by 2.22–1.31 Mya, an age estimate that is in rough accordance with the formation of the Isthmus of Panama (3.5–1.9 Mya; Coates and Obando, 1996; Haug and Tiedemann, 1998). Thus, it seems likely that the low-polyploid taxa of North America originated as a result of Central Asian taxa migrating across the BLB prior to its breakup and that the South American taxa originated as a result of North American taxa migrating across the Isthmus of Panama after this land connection was established. Favorable conditions for migrations of herbaceous montane plants across the Isthmus of Panama occurred from the Late Pliocene onward, when the climate was cooler and drier (Raven and Axelrod, 1975).

The arctic–alpine taxa—The cpDNA phylogeny confirms close relationships between the arctic and alpine high-polyploid species of the *C. alpinum* group. These taxa form a polytomy together with members of the boreal and temperate *C.*

tomentosum group (*C. tomentosum* and *C. biebersteinii*) and the *C. arvense* group (*C. arvense* and *C. velutinum*; see also Appendix). The low level of genetic variation observed within this group probably indicates a recent origin. Most of these taxa have identical cpDNA sequences, although *C. velutinum* is differentiated from the others by having one autapomorphic nucleotide change. Unambiguous optimized substitutions amounted to one and two changes between *C. tianschanicum*, of one of the sister groups, and *C. alpinum* and *C. velutinum*, respectively. Using the same simple molecular clock assumption as earlier, dividing changes by rate, the maximum–minimum age of this clade is estimated to be 1.48–0.44 Mya, thus placing the origin of the arctic high-polyploids during the Pleistocene.

In contrast to the genetic similarity demonstrated by the current results, these arctic, boreal, and temperate high polyploids can be distinguished morphologically (e.g., Hultén, 1956; Schischkin, 1970; Böcher, 1977), although the species groups are not always easily delimited (Khalaf and Stace, 2000), and some of the species have been shown to hybridize (Jonsell, 2001). Moreover, discriminatory molecular differences have previously been demonstrated for some of these species using other markers (i.e., isozymes, Brysting and Borgen, 2000; random amplified polymorphic DNA [RAPD] and sequence characterized amplified region [SCAR], Hagen et al., 2001; amplified fragment length polymorphism [AFLP], Gustafson et al., 2003). The different species have different geographic distributions, some being restricted, e.g., amphi-Pacific (*C. fischerianum*), North American (*C. beeringianum* subsp. *earlei*), or amphi-Atlantic (*C. alpinum*, *C. arcticum*, and *C. nigrescens*), others being more widespread to circumarctic (*C. beeringianum* subsp. *beeringianum* and *C. regelii*), possibly reflecting recurrent episodes of range expansions and contractions during the Quaternary glaciations. The North Atlantic was obviously not a barrier to dispersal in this group during the Pleistocene (Hagen et al., 2001).

The arctic high polyploids are thought to be allopolyploids that may have originated from different hybridization events. It has previously been suggested that *C. uniflorum* or a close relative has been involved in the origin of *C. nigrescens* (Böcher, 1977; Brysting and Borgen, 2000) and that *C. eriophorum* or a close relative has been involved in the origin of *C. alpinum* (Bosçaiu, 1996; Brysting and Borgen, 2000). The current phylogeny does not suggest a close relationship between the arctic species and their hypothesized alpine tetraploid progenitors, but the available haplotypic evidence does not exclude them as potential paternal progenitors.

Infrageneric classification—The two species *C. cerastoides* and *C. dubium* form a highly supported clade that is sister to the rest of the taxa (Fig. 1). These two species are also morphologically distinct, characterized by three (rarely four or five) styles and a capsule dehiscing by six teeth, whereas the normal pattern in *Cerastium* is five styles and 10 capsule teeth (e.g., Schischkin, 1970). Although this morphological difference is the result of variation in a single character (three vs. five carpels), the two species with three carpels have been placed in subgenus *Dichodon*, whereas the rest of the *Cerastium* species have been placed in subgenus *Eucerastium* (e.g., Schischkin, 1970; Hegi, 1979). Some authors have treated this morphological distinction at the genus level, placing the two species with three carpels in the genus *Dichodon* (e.g., Czerepanov, 1995; Sokolova, 1996). Löve and Löve (1975) also recognized the genus *Dichodon* but based their circumscription of the genus on a basic chromosome number of $x = 19$, thus including *C. davuricum* and *C. maximum* L. (both $2n = 38$). This circumscription based on cytological differences among species is not supported by the current data because *C. davuricum* does not group with *C. cerastoides* and *C. dubium* (Fig. 2). Considering their traditionally recognized morphological characteristics and the demonstrated genetic distinctiveness, the *Dichodon* group may deserve recognition at the genus level following Czerepanov (1995) and Sokolova (1996). However, *C. cerastoides* has been shown to hybridize with *C. alpinum* (Jonsell, 2001), and although intergeneric hybridization is known from other plant groups (e.g., Knobloch, 1972; Darbyshire et al., 1992; Bailey et al., 1993), we prefer to follow most current authors (e.g., *Flora Europea*, Jalas et al., 1993; *Flora of China*, Dequan and Morton, 2001; *Flora Nordica*, Jonsell, 2001) and treat the distinction at the subgeneric level, as subgenus *Dichodon* within the genus *Cerastium*.

Sister to the *Dichodon* group is a highly supported clade of the remaining *Cerastium* taxa (93% jackknife support, Fig. 1) that corresponds well to the subgenus *Eucerastium* as traditionally circumscribed (e.g., Schischkin, 1970). Within this clade, two subclades can be recognized that roughly correspond to two of three sections traditionally circumscribed within subsection *Eucerastium* (sections *Strephodon* and *Orthodon*; e.g., Schischkin, 1970; Fig. 3). However, *C. purpurascens* has traditionally been placed in section *Orthodon* (e.g., Schischkin, 1970) but is grouped within the ''strephodon'' clade in the current phylogeny. This cpDNA phylogeny accounts only for the maternal lineage which might explain the unexpected grouping of *C. purpurascens*. However, the relationship unraveled in this phylogeny supports a revised infrageneric classification of *Cerastium* sensu stricto (s.s.) (excluding *C. cerastoides* and *C. dubium*) based on investigations of seed and inflorescence morphology of *Cerastium* in the Caucasus (Sokolova, 1996). The ''strephodon'' clade corresponds well to subsection *Schizodon* sensu Sokolova (1996), which includes *C. purpurascens*.

Section *Orthodon* has traditionally been divided into two subsections based on habit (Schischkin, 1970). Annuals without sterile axillary branchlets have been referred to subsection *Fugacia*, whereas the perennials have been assigned to subsection *Perennia* (Schischkin, 1970). This distinction is not supported by the current phylogenetic tree. The annuals of subsection *Fugacia* are scattered throughout the ''orthodon'' clade (Fig. 3); thus, neither subsection *Fugacia* nor *Perennia* is monophyletic. Indeed, annuals are also found among the other species (Fig. 3), suggesting that the habit is not a useful character for infrageneric classification within this genus.

Cerastium pauciflorum was traditionally circumscribed as belonging in section *Strephodon* but does not group with the other species of the section (Fig. 3). The current results do not provide enough information to elucidate the infrageneric relationship of *C. pauciflorum*. Likewise, the North and South American taxa do not group within any circumscribed infrageneric groups, and, to our knowledge, no other information is available on their infrageneric classification. Only one of the two species traditionally included in section *Schizodon*, *C. fragillimum*, is studied here. This taxon is grouped with the species of section *Strephodon* as part of a polytomy (Fig. 2), and thus the status of section *Schizodon* cannot be inferred from the current results.

Differences in seed types and hair types have previously been used to separate taxa above the species level among the perennial species of genus *Cerastium* (Fenzl, 1842; Khalaf and Stace, 2000). A more thorough study considering both morphological and molecular data (including both nuclear and plastid markers) is needed before a proper revision of the infrageneric classification of the genus can be put forward.

Conclusions—The cpDNA phylogeny of *Cerastium* and a simple molecular clock assumption suggest an Old World origin and at least two migration events into North America from the Old World. The oldest migration event possibly took place across the Bering land bridge during the Miocene. A subsequent colonization of South America took place after the North and South American continents joined during the Pliocene. A more recent migration event into North America took place during the Quaternary, resulting in the current circumpolar distribution of the Arctic species.

Subgenus *Dichodon* and subgenus *Eucerastium* are monophyletic groups that may deserve to be recognized as separate genera. The classic circumscription of three sections within subgenus *Eucerastium* is not supported by the cpDNA phylogeny because none of the groups are monophyletic. An alternative infrageneric classification (Sokolova, 1996), although only considering the Caucasian species, divides *Cerastium* s.s. into the two subgenera *Schizodon* and *Cerastium*, which does find support in the current phylogeny. Further division, into subsections and series, for example, is not supported based on the present evidence.

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