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POLYPLOID EVOLUTION AND BIOGEOGRAPHY IN *CHELONE* (SCROPHULARIACEAE): MORPHOLOGICAL AND ISOZYME EVIDENCE[1]

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Chelone is a genus of perennial herbs comprising three diploid species (*C. cuthbertii*, *C. glabra*, and *C. lyonii*) and a fourth species (*C. obliqua*) that occurs as tetraploid and hexaploid races. To assess patterns of isozyme and morphological variation, and to test hypotheses of hybridization and allopolyploidy, we analyzed variation among 16 isozyme loci from 61 populations and 16 morphological characters from 33 populations representing all taxa and ploidy levels. Based on morphological analyses using clustering (unweighted pair group method using an arithmetic average) and ordination (principal components analysis and canonical variance analysis) methods, we recognize three diploid species without infraspecific taxa. Polyploids in the *C. obliqua* complex were most similar morphologically to diploid populations of *C. glabra* and *C. lyonii*. Patterns of isozyme variation among polyploids, which included fixed heterozygosity and recombinant profiles of alleles present in diploids, suggested polytopic origins of tetraploids and hexaploids. Our data indicate independent origins of polyploids in or near the southern Blue Ridge, Interior Highlands and Plains, and Atlantic Coastal Plain regions from progenitors most similar to *C. glabra* and *C. lyonii*. Extant tetraploids were not implicated in evolution of hexaploids, and plants similar to *C. cuthbertii* appeared unlikely as diploid progenitors for polyploids. We propose multiple differentiation and hybridization/polyploidization cycles in different geographic regions to explain the pattern of allopatry and inferred polytopic origins among polyploids.

Key words: biogeography; *Chelone*; evolution; isozymes; phenetics; polyploidy; Scrophulariaceae.

Recent systematic and evolutionary investigations in plants utilizing DNA and isozyme markers have documented several examples of allopolyploidy which arose several times independently (Roose and Gottlieb, 1976; Werth, Guttman, and Eshbaugh, 1985a, b; Soltis and Soltis, 1989, 1993; Brochmann, Soltis, and Soltis, 1992; Wyatt, Odrzykoski, and Stoneburner, 1992). These studies generally supported hypotheses that multiple and polytopic origins of polyploids were not uncommon and that diverse and multiple origins contributed to the genetic heterogeneity and evolutionary potential of polyploids (Soltis, Doyle, and Soltis, 1992; Soltis and Soltis, 1991, 1993). Previous research also demonstrated that the potential for hybridization and polyploidy was influenced greatly by ecohistorical factors, such as the pronounced geoclimatic changes and subsequent plant migrations during the Quaternary Period (Ehrendorfer, 1959; Stebbins, 1971). In eastern North America, the repeated expansion, contraction, fragmentation, and amalgamation of species' ranges during the Pleistocene and Holocene epochs, which began two million years ago (Cain, 1944; Holt and Paterson, 1970; Jacobs, Werth, and Guttman, 1984; Delcourt and Delcourt, 1987; Delcourt, 1991), undoubtedly resulted in new contacts between previously isolated populations (Qiu and Parks, 1994; Kuser et al., 1997), increased the probability of interlineage hybridization and introgression (Parks et al., 1994; Parker et al., 1997), and led to extinctions of progenitor taxa (Werth, 1991).

Chelone is a useful model for investigating the pattern and process of hybrid and polyploid evolution among eastern North American plants. The genus *Chelone* is a morphologically distinctive group of perennial herbs whose monophyly is defined by a galeate corolla, subspicate inflorescence, a bracteate inflorescence, and a reticulate-rugulate pollen exine surface (Nelson, 1995). It comprises three diploid ($2n = 28$) species and one species complex with tetraploid and hexaploid races (Nelson, 1995), is subdivided into a series of morphological and geographic variants (Pennell, 1935), has several purported examples of inter- and intraspecific hybridization (Fernald, 1950; Radford, Ahles, and Bell, 1968; Bittman, 1980), and is distributed both north and south of the boundary of the last glacial maximum.

Species and varietal determinations in *Chelone* are notoriously difficult because of inconsistency and intergradation among characters (Pennell, 1935; Gleason, 1952; Crosswhite, 1965; Radford, Ahles, and Bell, 1968). Corolla color, beard color, staminode tip color, petiole length, and leaf base have been used to delimit species in *Chelone* (Nelson, 1995). *Chelone glabra* has some white in the corolla and beard, a green-tipped staminode, and cuneate leaf bases. Both *C. cuthbertii* and *C. lyonii* have purple corollas, yellow beards, and rounded leaf bases. Whereas *C. cuthbertii* has sessile leaves and a purple-tipped staminode, *C. lyonii* has petiolate leaves and a white to pink-tipped staminode. The polyploid *C. obliqua* has a distinctive combination of morphological charac-

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TABLE 1. Numbered populations of *Chelone* and abbreviations of taxa and physiographic regions (Brouillet and Whetstone, 1993) sampled for isozyme and morphological divergence. All populations were examined for isozyme and morphological variation except those indicated by an asterisk (*), which were only examined morphologically. Numbers of individuals examined (*N*) for isozyme divergence given in parentheses.

CUTH *C. cuthbertii* Small (*N* = 66)
Piedmont Plateau/Valley & Ridge (P/VR): 1 = NC: Stokes Co., Danbury, *Nelson 185* (*N* = 1). **Southern Blue Ridge (SBR): 2 =** NC: Jackson Co., Panthertown Valley, *Nelson 200* (*N* = 26); **3 =** Yancey Co., Celo, *Nelson & McLeod 196** (*N* = 19). **4 =** VA: Carroll Co., Blue Ridge Parkway, *Nelson 194** (*N* = 5). **Atlantic Coastal Plain (ACP): 5 =** VA: Chesterfield Co., Chesterfield Seeps, *Nelson 189* (*N* = 4); **6 =** *Nelson 190** (*N* = 11).

GLAB *C. glabra* L. (*N* = 453)
GLAB CHLO var. *chlorantha* (Pennell & Wherry) Cooperrider (*N* = 23)
Southern Blue Ridge (SBR): 7 = NC: Yancey Co., Celo, *Nelson & McLeod 195** (*N* = 12); **8 =** Pensacola, *Nelson & Benesh 227** (*N* = 11). **9 =** TN: Sevier Co., Double Spring Gap, *Jennison 1376 & 1376a.*
GLAB DILA var. *dilatata* Fern. & Wieg. (*N* = 61)
New England & Maritime (NEM): 10 = MA: Barnstable Co., West Barnstable, *Fernald 7507**. **11 =** NS, Canada: Grand Narrows, *Nelson 169* (*N* = 25). **12 =** PEI, Canada: Bloomfield, *Nelson 167** (*N* = 3). **13 =** PQ, Canada: Gaspe Peninsula, *Nelson 166* (*N* = 28). **Superior Uplands (SU): 14 =** ON, Canada: Cochrane, *Nelson 163* (*N* = 5).
GLAB ELAT var. *elatior* Raf. (*N* = 120)
Appalachian Plateau (AP): 15 = KY: Powell Co., Daniel Boone National Forest, *Nelson 178* (*N* = 6). **16 =** OH: Harrison Co., Anapolis, *Nelson 180* (*N* = 2); **17 =** Vinton Co., Lake Hope State Park, *Nelson 179** (*N* = 7). **New England & Maritime (NEM): 18 =** NH: Grafton Co., Franconia Notch State Park, *Nelson 172* (*N* = 4); **19 =** *Nelson 173* (*N* = 4). **20 =** VT: Windham Co., Wilmington, *Nelson 175** (*N* = 27). **Southern Blue Ridge (SBR): 21 =** GA: Union Co., Wolfpen Gap, *Nelson & Benesh 231* (*N* = 44). **22 =** TN: Polk Co., Farner, *Nelson 201* (*N* = 26).
GLAB ELON var. *elongata* Pennell & Wherry (*N* = 9)
Appalachian Plateau (AP): 23 = OH: Pickaway Co., Wayne Twp., *Bartly 31424 & 31425**; **24 =** Ross Co., Betsh Fen Preserve, *Nelson 181** (*N* = 5); **25 =** Liberty Twp., *Bartly 31424 A & B**; **26 =** Ross Lake Wildlife Area, *Nelson 182* (*N* = 4).
GLAB GLAB var. *glabra* (*N* = 55)
Appalachian Plateau (AP): 27 = OH: Ross Co., Betsh Fen Preserve, *Nelson 184** (*N* = 5). **New England & Maritime (NEM): 28 =** ME: Waldo Co., Mt. Hope Cemetery, *Nelson 171** (*N* = 11). **29 =** NB, Canada: Deer Island, *Nelson 170** (*N* = 8). **30 =** NH: Cheshire Co., Shaddock, *Nelson 174** (*N* = 8). **31 =** ON, Canada: Morrisburg, *Nelson 164* (*N* = 3). **32 =** PEI, Canada: Alberton, *Nelson 168* (*N* = 9). **33 =** PQ, Canada: Montmagny, *Nelson 165** (*N* = 3). **Piedmont Plateau/Valley & Ridge (P/VR): 34 =** AL: St. Clair Co., Bowman Cemetery, *Nelson & Benesh 233* (*N* = 4). **35 =** SC: Anderson Co., Savannah River, *Nelson & Benesh 232* (*N* = 4).
GLAB LINI var. *linifolia* Coleman (*N* = 161)
Mississippi Embayment (ME): 36 = AR: Greene Co., Glory Hole Bog, *Nelson 143* (*N* = 16); **37 =** Pine Hill Cemetery Bog, *Nelson 142** (*N* = 27). **38 =** MS: Marshall Co., Coldwater River, *Nelson, Huneycutt, & Benesh 234* (*N* = 8). **Superior Uplands (SU): 39 =** MN: Lake Co., Two Harbors, *Nelson 162** (*N* = 27). **Ozark Plateau (OP): 40 =** MO: Butler Co., Military Crossing Cemetery Bog, *Nelson & Hudson 144* (*N* = 8); **41 =** Poplar Bluff State Forest, *Nelson & Hudson 145* (*N* = 28); **42 =** Poplar Bluff, *Nelson & Hudson 146* (*N* = 13). **Central Lowlands (CL): 43 =** IA: Franklin Co., Spring Creek, *Nelson 224* (*N* = 16). **44 =** MI: Delta Co., Garden Corners Twp., *Hils & Nelson 176* (*N* = 4). **45 =** OH: Champaign Co., Cedar Bog State Memorial, *Nelson & Jaworski 183* (*N* = 14); **46 =** Fulton Co., Braily, *Pennell & Wherry 13518**.

TABLE 1. Continued.

GLAB OCHR var. *ochroleuca* Pennell & Wherry (*N* = 24)
Atlantic Coastal Plain (ACP): 47 = NC: Martin Co., Jamesville, *Nelson 192** (*N* = 6); **48 =** Northampton Co., Jordan Mill Pond, *Nelson 186* (*N* = 9). **49 =** VA: Charles City Co., Courthouse Creek, *Nelson 188** (*N* = 9).
LYON *C. lyonii* Pursh (*N* = 153)
Southern Blue Ridge (SBR): 50 = NC: Caldwell Co., Grandfather Mt., *Nelson 139* (*N* = 31); **51 =** Mitchell Co., Roan Mt., *Nelson 138** (*N* = 30); **52 =** Swain Co., Clingman's Dome, *Nelson 141* (*N* = 19); **53 =** Transylvania Co., Brevard, *Nelson & Benesh 230** (*N* = 16); **54 =** Yancey Co., Mt. Mitchell, *Nelson 140** (*N* = 39). **55 =** TN: Unicoi Co., Mt. Unaka, *Nelson 202* (*N* = 18).
OBLI *C. obliqua* L. (*N* = 167)
OBLI ERWI var. *erwiniae* Pennell & Wherry (*N* = 74)
Southern Blue Ridge (SBR): 56 = NC: Buncombe Co., Pisgah Campground, *Nelson 197* (*N* = 27); **57 =** McDowell Co., Pisgah Campground, *Nelson 198** (*N* = 10); **58 =** Transylvania Co., Brevard. *Nelson & Benesh 229** (*N* = 37).
OBLI OBLI var. *obliqua* (*N* = 15)
Atlantic Coastal Plain (ACP): 59 = NC: Gates Co., Cole Creek Swamp, *Nelson 187** (*N* = 3); **60 =** Pender Co., Long Creek, *Nelson 193** (*N* = 9). **61 =** VA: Southhampton Co. Carey Bridge, *Nelson 191* (*N* = 3).
OBLI SPEC var. *speciosa* Pennell & Wherry (*N* = 78)
Ozark Plateau (OP): 62 = MO: Butler Co., Poplar Bluff, *Nelson & Hudson 147** (*N* = 24). **Central Lowlands (CL): 63 =** MO: Lincoln Co., Prairie Slough Wildlife Management Area, *Nelson 150* (*N* = 16); **64 =** Pike Co., Ted Shank Wildlife Management Area, *Nelson 148* (*N* = 13); **65 =** *Nelson 149** (*N* = 22). **Interior Low Plateaus (ILP): 66 =** IN: Martin Co., McBryde's Bluff, *Nelson 177** (*N* = 3).

ters, but individual characters are found in one or more of the diploid species.

Two species, *C. glabra* and *C. obliqua*, have been subdivided into seven and three varieties, respectively (Pennell and Wherry, 1928; Pennell, 1935) (Table 1). *Chelone cuthbertii*, which is disjunct between the southern Blue Ridge region of North Carolina and the coastal plain of Virginia, is exclusively diploid ($2n = 28$) (Pennell, 1935; Nelson, 1995) as is *C. lyonii*, a southern Blue Ridge endemic that occurs in Tennessee, North Carolina, and South Carolina. *Chelone glabra*, ranging from the Carolina coast to Iowa and from Mississippi to Newfoundland, is diploid with one reported tetraploid population (Pennell, 1935; Cooperrider and McCready, 1970; Nelson, 1995). Populations of *C. obliqua* are either tetraploid endemics of the southern Blue Ridge or occur as hexaploids in the Interior Highlands and Plains region (Arkansas to Michigan to Tennessee) or the Atlantic Coastal Plain from South Carolina to Maryland (Pennell, 1935; Cooperrider and McCready, 1970; Nelson, 1995). *Chelone glabra* is the most widespread and polymorphic species in the genus; several of its varieties are hypothesized to hybridize with other diploid and polyploid taxa of the genus (Radford, Ahles, and Bell, 1968; Bittman, 1980).

In an unpublished Master's thesis using flavonoid, morphological, and isozyme data, Bittman (1980) inferred an autoploid origin of *C. obliqua* tetraploids (var. *erwiniae*) from *C. glabra*, an alloploid origin of the hexaploids of *C. obliqua* (vars. *obliqua* and *speciosa*) between *C. lyonii* and tetraploid populations of *C. obliqua*, and

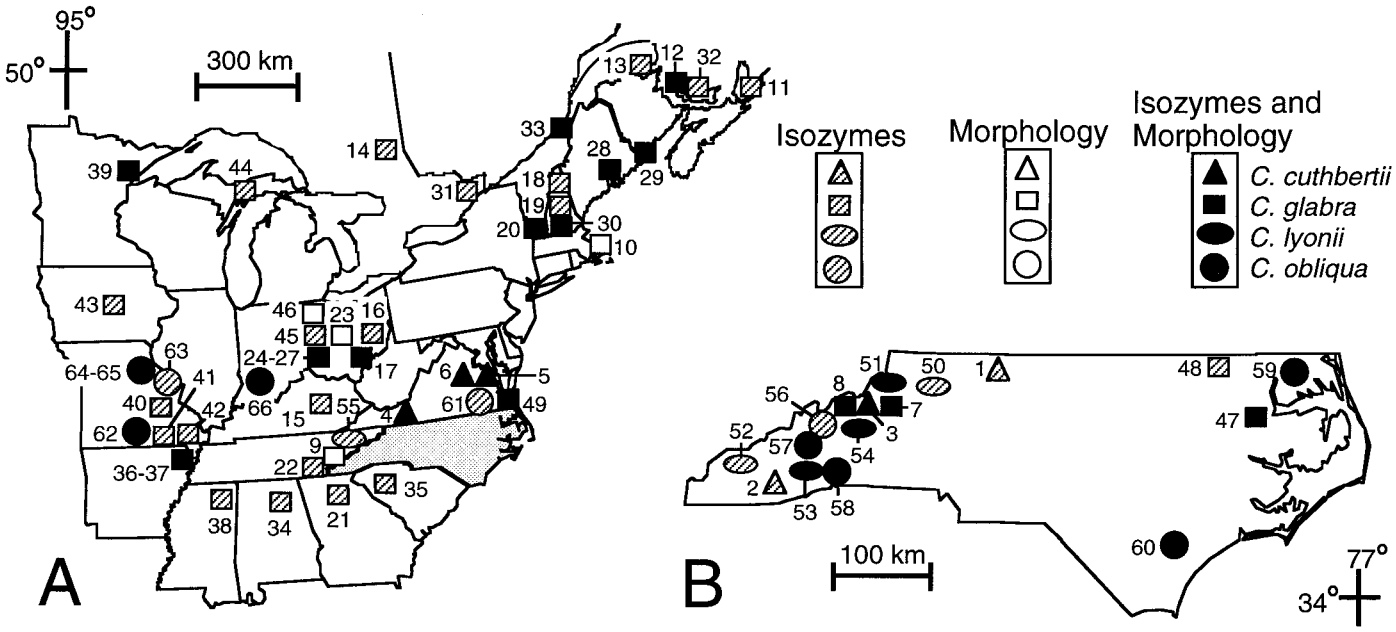

Fig. 1. Collection localities and types of data obtained for four species of *Chelone* (Table 1) from: (A) eastern North America except North Carolina; (B) North Carolina. See Nelson (1995) for distribution maps of species.

possible interspecific hybridization between *C. lyonii* and *C. glabra*.

We initiated this investigation to quantify the amount and pattern of variation for isozymes and morphological characters within the genus, to assess the species and varietal delimitations of Pennell (1935), to test hypotheses of hybridization and polyploid origins proposed by Bittman (1980), and to examine the effect of historical biogeographic factors on the pattern and process of evolution in the genus.

MATERIALS AND METHODS

We examined 839 individuals from 61 populations representing 12 taxa in *Chelone* for isozyme variation (Table 1). Sampling was done throughout the range of species and varieties (Nelson, 1995) and in as many physiographic regions (Fenneman, 1938; Brouillet and Whetstone, 1993) as possible (Fig. 1). Population sizes of *Chelone* were generally less than ten individuals, although a few populations greater than 100 individuals were observed. Sample sizes for each taxon and population also are indicated in Table 1. For isozyme studies, leaf material was collected from individual plants in the field and stored on ice until used. Populations 2, 3, 7, 22, 40, 41, 50–52, 54–57, and 65 contained some individuals grown from seed collections. Vouchers were deposited at the Robert Bebb Herbarium (OKL) at the University of Oklahoma.

TABLE 2. Morphological characters used in phenetic analysis of species of *Chelone*. Characters showing significant ($P < 0.05$) variation among all species in ANOVA indicated by a plus sign (+). Characters identified for differentiating species in canonical variance analysis (CVA) indicated by an asterick (*).

1. Average ratio of mid-leaf width to width where leaf begins to curve toward the petiole (+).
2. Average ratio of mid-leaf width to length (+).
3. Leaf base shape (+*).
4. Average number of leaf margin serrations/cm.
5. Average petiole length (*).
6. Average inflorescence length.
7. Average ratio of bract width to length.
8. Sepal pubescence type (+*).
9. Average ratio of sepal width to length.
10. Average corolla tube length.
11. Average ratio of lower lip width to length (+*).
12. Upper corolla and lip color.
13. Lower lip beard color (+*).
14. Ratio of staminode length to filament length (*).
15. Staminode color (+*).
16. Average style length (+).

Analyses of morphological variation were based on collections from 33 populations representing all species and varieties of *Chelone* (Table 1; Fig. 1). The morphological data set for each of the 33 populations can be obtained from the first author. Using clustering, ordination, and statistical methods, 11 quantitative and five qualitative morphological characters (Table 2) were examined for variation from two to six individuals per population using herbarium specimens or collections of the first author. Twelve of the 16 vegetative and floral characters (1–8, 10, 12–13, and 15) were used in previous taxonomic treatments (Pennell and Wherry, 1928; Pennell, 1935), whereas characters 9, 11, 14, and 16 had not been used previously. In addition, a number of characters designated qualitatively in previous studies (Pennell and Wherry, 1928; Pennell, 1935) were quantified in the present study. We obtained a minimum of ten measurements for each quantitative character for populations except when material was limited by number of herbarium sheets or small population sizes. Characters 1, 2, 7, 9, 11, and 14 were scaled using ratios to eliminate size differences that might result from environmental factors. NTSYS-pc (Rohlf, 1990) was used to standardize a data matrix of populational averages for each character (available upon request from the first author), calculate a dissimilarity matrix using the average taxonomic distance coefficient, compute a cophenetic correlation coefficient, and generate a phenogram with the unweighted pair-group method using an arithmetic average (UPGMA) (Fig. 2). A correlation matrix of characters was constructed to calculate eigenvectors used in principal components analysis (PCA). Three-dimensional models of populations (Fig. 3) were constructed and used to analyze characters involved in groupings.

A one-way analysis of variance (ANOVA) was used to calculate

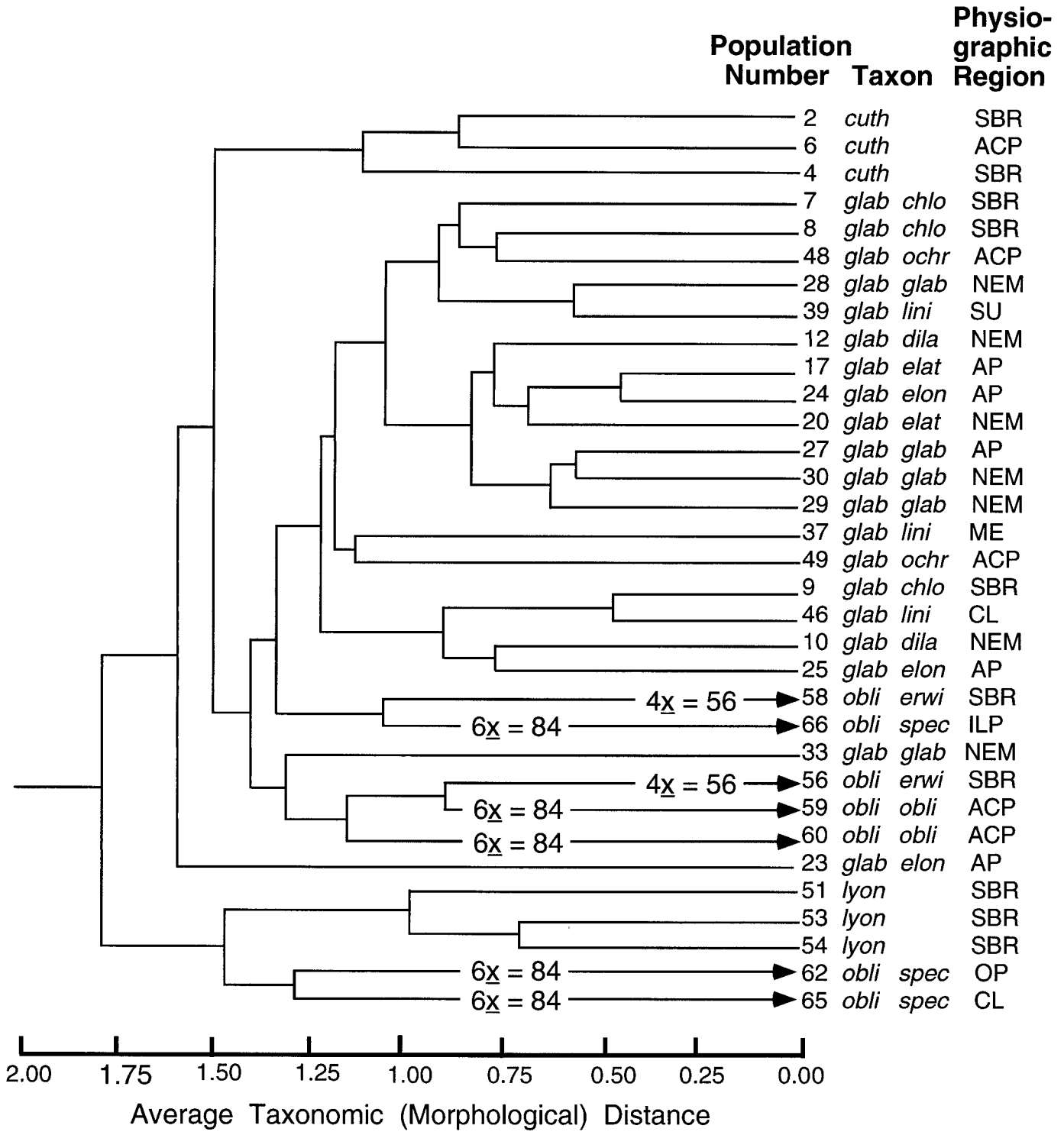

Fig. 2. UPGMA phenogram derived from average taxonomic distance coefficients using 16 morphological characters (Table 2) from 33 populations of *Chelone*. Population numbers and abbreviations for physiographic regions and texa are listed in Table 1. Arrows (→) indicate position of polyploids. Cophenetic correlation was 0.818.

group means and standard deviations, to compute *F* values and significance levels for each quantitative character, and to conduct Scheffé's test of significance for variation among groups (SAS, 1990). A Canonical Variance Analysis (CVA) (Dixon and Brown, 1979) was done to determine which combination of characters provided maximal discrimination among species, relative to the variation within species. Both a standard classification and a jackknifed classification (Dixon and Brown, 1979) were produced in the CVA to test group relationships. All populations were included in analyses using UPGMA clustering, PCA, CVA, and ANOVA. UPGMA cluster analyses were done with and without polyploid populations (numbers 56–66) to determine clustering patterns of diploids and polyploids.

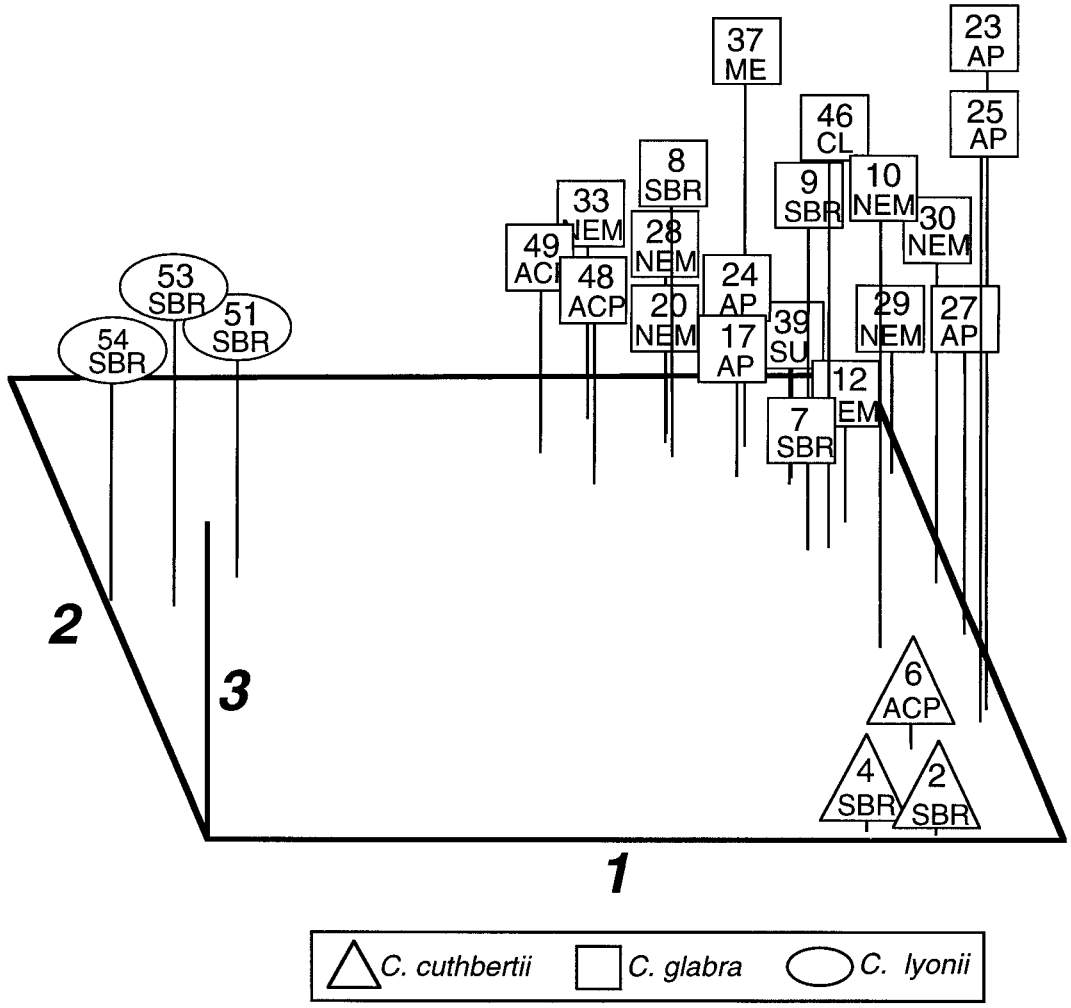

Fig. 3. Three-dimensional model of populations derived from principal components analysis (PCA) of 16 morphological characters (Table 2) in *Chelone*. Population numbers and abbreviations for physiographic regions and taxa are listed in Table 1. The first three components accounted for 61% of total variance (31, 17, and 13%, respectively).

Enzyme extraction followed established protocols (Gottlieb, 1981; Elisens and Nelson, 1993) except that 120 mg/mL PVP-40 was added to the extracting buffer. Leaf extracts were centrifuged in 1.5-mL tubes and the supernatant was absorbed onto wicks of Whatman 17 MM chromatography paper. Ten enzyme systems coded by 16 putative loci in diploids were resolved on 12% starch gels utilizing two buffer systems. System I was histidine citrate buffer, pH 6.5 (Soltis et al., 1983) with a gel buffer obtained from a 1 : 3 aqueous dilution of the electrode buffer and system II was tris-EDTA buffer (Soltis et al., 1983) with a gel buffer obtained from a 1 : 3 aqueous dilution of the electrode buffer. System I was used to resolve one locus each for [NADP] glyceraldehyde 3-phosphate dehydrogenase (*G3pd*), 6-phosphogluconate dehydrogenase (*6Pgd*), and menadione reductase (*Mnr*). System II was employed to resolve one locus each for alcohol dehydrogenase (*Adh*), fluorescent esterase (*Fest*), glucose dehydrogenase (*Gdh*), and malic enzyme (*Me*), two loci for triosephosphate isomerase (*Tpi*), three loci for phosphoglucoisomerase (*Pgi*), and four loci for phosphoglucomutase (*Pgm*). Enzyme-activity staining protocols and agarose overlays generally followed Soltis et al. (1983) and Wendel and Weeden (1989), except for *Mnr*, which was stained using a modification of Conkle et al. (1982). Loci were numbered consecutively beginning with the most anodal form. Alleles at each locus were labeled alphabetically from the most anodal form.

Allele and genotype frequencies were determined for each diploid species and allele presence or fixation was noted for polyploids of *C. obliqua* (Table 3). Three pairs of putative duplicated loci were observed (*Pgi-1* and *-2*, *Pgm-1* and *-2*, and *Pgm-3* and *-4*) but were not included in quantitative analyses. Dosage effects were apparent for duplicated loci in polyploid taxa, but polyploid genotypes were not determined. The GENESTAT2 program (Whitkus, 1988) was used to calculate Nei's (1972) genetic identity and distance coefficients (Table 4), and gene diversity statistics within and among diploid species and varieties (Table 5), and for physiographic regions (Table 5). The BIOSYS-1 program (Swofford and Selander, 1981) was used to calculate Nei's (1972) genetic distance and to generate UPGMA phenograms (Sneath and Sokal, 1973) (Fig. 4). Values for the estimated number of migrants (N_m) were calculated from G_{ST} (Wright, 1951), which was based on patterns among common alleles. Alleles found among polyploids that were present in only a few diploid populations were tabulated (Table 6) and mapped (Fig. 5). The clustering pattern of populations on the isozyme UPGMA (Fig. 4, clusters I and II) was mapped to illustrate geographic location of populations, values for total genetic diversity (H_T), and relation to the approximate southern boundary of the Wisconsinian glacial maximum (Fig. 6).

RESULTS

The UPGMA phenogram based on morphological data for diploid and polyploid populations (Fig. 2) had a co-

TABLE 3. Summary allele frequencies for ten polymorphic loci among three diploid species in *Chelone*. The symbol "+" denotes presence of an allele and "fix" indicates an allele fixed within numbered populations (Table 1) of *C. obliqua*.

Locus/frequency in diploid species				Presence (+) or fixation (fix) among *C. obliqua* polyploid populations and varieties										
				erwiniae (4*x*)			*obliqua* (6*x*)			*speciosa* (6*x*)				
allele	*cuthbertii*	*glabra*	*lyonii*	56	57	58	59	60	61	62	63	64	65	66
Adh-1														
a	0.05	0.13	0.03	—	—	—	—	—	—	+	+	+	+	+
b	0.95	0.86	0.77	fix	fix	fix	fix	fix	fix	+	+	+	+	+
c	—	0.01	0.20	—	—	—	—	—	—	—	—	—	—	—
Fest-1														
a	—	0.03	—	—	—	—	—	—	—	—	—	—	—	—
b	1.00	0.96	0.97	fix	fix	fix	fix	fix	fix	+	fix	fix	fix	fix
c	—	0.01	0.03	—	—	—	—	—	—	+	—	—	—	—
G3pd-1														
a	—	—	0.04	—	—	—	—	—	—	—	—	—	—	—
b	0.98	0.28	0.39	fix	fix	fix	fix	fix	fix	—	—	—	—	—
c	0.02	0.72	0.57	—	—	—	—	—	—	fix	fix	fix	fix	fix
Gdh-1														
a	0.08	0.01	—	—	—	—	—	—	—	—	—	—	—	—
b	0.20	0.90	0.72	+	+	fix	fix	fix	fix	fix	+	fix	+	fix
c	—	0.01	0.21	—	—	—	—	—	—	—	—	—	—	—
d	0.51	0.08	0.07	+	+	—	—	—	—	—	+	—	+	—
e	0.21	—	—	—	—	—	—	—	—	—	—	—	—	—
Me-1														
a	—	—	0.01	—	—	—	—	—	—	—	—	—	—	—
b	1.00	0.96	0.98	fix	fix	fix	fix	fix	fix	fix	fix	fix	fix	fix
c	—	0.04	0.01	—	—	—	—	—	—	—	—	—	—	—
Mnr-1														
a	0.04	0.07	0.01	—	—	—	—	—	—	—	—	—	—	—
b	0.88	0.91	0.84	fix	fix	fix	fix	fix	fix	+	+	+	+	fix
c	0.08	0.02	0.15	—	—	—	—	—	—	+	+	+	+	—
6pgd-1														
a	—	0.02	0.02	—	—	—	—	—	—	—	—	—	—	+
b	0.58	0.57	0.84	+	+	fix	+	+	+	+	+	+	+	+
c	0.42	0.41	0.14	+	+	—	+	+	+	+	+	+	+	—
Pgi-3														
a	—	—	0.01	—	—	—	—	—	—	—	—	—	—	—
b	—	—	0.01	—	—	—	—	—	—	—	—	—	—	—
c	—	0.01	0.01	—	—	—	—	—	—	—	—	—	—	—
d	0.02	0.02	0.01	—	—	—	—	—	—	—	—	—	—	—
e	0.56	0.73	0.58	+	+	+	+	+	+	+	+	+	+	+
f	0.25	0.04	0.01	—	—	—	—	—	—	—	—	—	—	—
g	0.07	0.18	0.32	+	+	+	+	+	+	+	+	+	+	+
h	0.08	0.01	0.03	—	—	—	—	—	—	—	—	—	—	—
i	0.02	0.01	—	—	—	—	—	—	—	—	—	—	—	—
j	—	—	0.02	—	—	—	—	—	—	—	—	—	—	—
Tpi-2														
a	—	—	0.01	—	—	—	—	—	—	—	—	—	—	—
b	—	0.03	0.01	fix	fix	—	—	—	—	—	—	—	—	—
c	0.27	0.10	0.24	—	—	—	—	—	—	—	—	—	—	+
d	0.01	—	0.01	—	—	—	—	—	—	—	—	—	—	—
e	0.60	0.82	0.64	—	—	fix	fix	fix	fix	fix	fix	fix	fix	+
f	0.12	0.05	0.09	—	—	—	—	—	—	—	—	—	—	—
Tpi-3														
a	0.03	0.01	0.03	—	—	—	—	—	—	—	—	—	—	—
b	0.04	0.01	—	—	—	—	—	—	—	—	—	—	—	—
c	0.93	0.98	0.97	fix	fix	fix	fix	fix	fix	fix	fix	fix	fix	fix

phenetic correlation coefficient of 0.818, compared to a value of 0.905 when only diploids were analyzed. In Fig. 2, polyploid populations clustered with diploid species: some 6*x* populations of *C. obliqua* clustered with *C. lyonii* and *C. glabra*, whereas 4*x* and other 6*x* populations of *C. obliqua* clustered within *C. glabra*. No polyploids clustered with *C. cuthbertii*. In morphological analyses among diploid populations, UPGMA (figure not shown) and PCA (Fig. 3) both indicated three major clusters corresponding to *C. glabra*, *C. cuthbertii*, and *C. lyonii*. Addition of polyploids in PCA portrayed similar groupings as UPGMA phenograms (Fig. 2). Neither UPGMA nor PCA approaches using morphological characters segregated populations of any variety of *C. glabra* or *C. obliqua* as proposed by Pennell (1935) or others.

Results of ANOVA analyses showed significant variation ($P < 0.05$) among species for the morphological characters 1–3, 8, 11, 13, and 15–16 (Table 2). The most

TABLE 4. Mean values for Nei's genetic identity and distance coefficients within and among species and varieties of *Chelone*.

Species/variety	Identity (range)	Distance
Within species of ***Chelone***		
C. cuthbertii	0.848 (0.740–0.935)	0.168
C. glabra	0.846 (0.440–1.000)	0.176
C. lyonii	0.767 (0.562–0.895)	0.273
Within varieties of ***C. glabra***		
chlorantha	0.643 (0.643–0.643)	0.442
dilatata	0.964 (0.924–1.000)	0.037
elatior	0.867 (0.676–0.998)	0.149
elongata	0.998 (0.998–0.998)	0.002
glabra	0.873 (0.728–1.000)	0.143
linifolia	0.857 (0.732–0.985)	0.157
ochroleuca	0.901 (0.860–0.951)	0.105
Between species of ***Chelone***		
C. cuthbertii ×		
C. glabra	0.792 (0.531–0.974)	0.136
C. lyonii	0.799 (0.614–0.956)	0.113
C. glabra ×		
C. lyonii	0.791 (0.622–0.989)	0.028
Between varieties of ***C. glabra***		
chlorantha ×		
dilatata	0.622 (0.449–0.770)	0.499
elatior	0.760 (0.549–0.955)	0.290
elongata	0.794 (0.639–0.951)	0.250
glabra	0.691 (0.463–0.978)	0.395
linifolia	0.694 (0.440–0.950)	0.389
ochroleuca	0.752 (0.545–0.946)	0.304
dilatata ×		
elatior	0.822 (0.680–0.992)	0.203
elongata	0.787 (0.753–0.804)	0.240
glabra	0.904 (0.720–1.000)	0.108
linifolia	0.858 (0.732–0.998)	0.157
ochroleuca	0.877 (0.784–0.984)	0.133
elatior ×		
elongata	0.897 (0.781–0.976)	0.112
glabra	0.857 (0.674–0.993)	0.162
linifolia	0.840 (0.690–0.993)	0.179
ochroleuca	0.900 (0.701–0.986)	0.109
elongata ×		
glabra	0.849 (0.763–0.984)	0.168
linifolia	0.835 (0.783–0.930)	0.183
ochroleuca	0.905 (0.812–0.962)	0.102
glabra ×		
linifolia	0.850 (0.727–0.998)	0.167
ochroleuca	0.894 (0.766–0.984)	0.115
linifolia ×		
ochroleuca	0.871 (0.723–0.980)	0.141

useful characters (and their numbers) for discriminating among species using CVA were: lower lip width-to-length ratio (character 11), sepal pubescence (8), staminode-to-filament length ratio (14), staminode color (15), beard color (13), leaf base (3), and petiole length (5). Using these characters, it was possible to separate 100% of the populations in each of the species' groups. In the jackknifed classification, 97% of the four species groups were discriminated. When 12 groups were designated (corresponding to *C. cuthbertii*, seven named varieties of *C. glabra*, *C. lyonii*, and three named varieties of *C. obliqua*), upper corolla and lip color (12), sepal pubescence (8), beard color (13), leaf base (3), and petiole length (5) discriminated 66.7% of the populations (45.5% in the jackknifed classification). Means for quantitative characters in CVA overlapped among all intraspecific taxa in *C. glabra* and *C. obliqua*.

Sixteen loci, coding for ten putative enzymes, were scored: one locus each for *Adh*, *Fest*, [NADP] *G3pd*, *Gdh*, *Mnr*, *6Pgd*, *Me*, two for *Tpi*, three for *Pgi*, and four for *Pgm*. The number of isozymes detected was similar to those reported for diploid plants (Wendel and Weeden, 1989; Murphy et al., 1996) except for *Pgi* and *Pgm*. These loci exhibited isozyme profiles indicating duplicated loci in both diploid and polyploid populations. The apparent sets of duplicated loci were not included in quantitative analyses but, because *Pgm-1* was polymorphic, it was compared among populations (Table 6; Fig. 5).

Summary allele frequencies for ten polymorphic loci among diploid species were calculated and compiled in Table 3. Allele and genotype frequencies for each population are available from the first author. All species shared the same highest frequency allele at all but two loci, *G3pd*-1 and *Gdh*-1. Although each diploid species had unique alleles (Table 3: *C. cuthbertii* and *C. glabra* had one, *C. lyonii* had six), all occurred at low frequencies and were confined to one or two populations. Polyploids had no unique alleles. Several low-frequency alleles (summary frequencies <0.21) were shared between two species: one between *C. cuthbertii* and *C. lyonii*, two between *C. cuthbertii* and *C. glabra*, and four between *C. glabra* and *C. lyonii*. Patterns that appeared to be fixed heterozygosity occurred in all 11 polyploid populations at *Pgi-3*, in ten populations for *6Pgd-1*, in five populations for *Adh-1*, in four populations each for *Gdh-1* and *Mnr-1*, and in one population for *Tpi-3*.

Five alleles had a differential occurrence among diploid and polyploid populations (Table 6; Fig. 5). *Adh-1a* occurred in hexaploids of *C. obliqua* in the Central Lowland, Interior Low Plateau, and Ozark Plateau regions and in all three diploid species. Both *6Pgd-1a* and *Fest-1c* occurred in populations of *C. glabra* and *C. lyonii* in the southern Blue Ridge region and were both present in *C. obliqua* hexaploids and in *C. glabra* either in the Ozark Plateau (*6Pgd-1a*) or Interior Low Plateau regions (*Fest-1c*). Although the *Tpi-2b* allele was found only in the southern Blue Ridge area in *C. glabra*, *C. lyonii*, and tetraploids of *C. obliqua*; *Pgm-1a* was observed in the Ozark Plateau (*C. glabra*) and again in Blue Ridge populations of *C. glabra* and tetraploids of *C. obliqua*.

Table 4 contains coefficients for Nei's (1972) genetic identity (*I*) and distance (*D*) within and among diploid species and varieties of *Chelone*. Mean *I* values within species were 0.848 in *C. cuthbertii*, 0.846 in *C. glabra*, and 0.767 in *C. lyonii*, whereas average *I* values among species ranged from 0.791 to 0.799. Mean *I* values among convarietal populations of *C. glabra* ranged from 0.643 to 1.000, whereas pairwise comparisons among *C. cuthbertii*, *C. lyonii*, and varieties of *C. glabra* ranged from 0.725 to 0.895.

UPGMA trees did not differentiate among diploid species or varieties of *Chelone* but did exhibit a geographic pattern. The UPGMA tree generated from genetic distances (Fig. 4) among diploid populations consisted of two primary clusters and two outliers. The two most divergent populations were found in the southern Blue Ridge and consisted of populations of *C. glabra* var. *chlorantha* and *C. lyonii*. Two large groups occurred that generally corresponded to populations in unglaciated

TABLE 5. Mean values for proportion of polymorphic loci (P), mean number of alleles per polymorphic locus (K_p), total gene diversity (H_T), and number of migrants exchanged between populations (N_m) in three diploid *Chelone* species and the physiographic regions where they occur.

Species/varieties	Physiographic region	P	K_p	H_T	N_m
C. cuthbertii		36.7	1.52	0.291	0.52
	Atlantic Coastal Plain	30.0	1.40	0.194	1.41
	Southern Blue Ridge/Piedmont	40.0	1.58	0.263	1.06
C. glabra		26.3	1.29	0.224	0.16
var. *chlorantha*		30.0	1.40	0.411	0.06
var. *dilatata*		22.5	1.23	0.087	0.46
var. *elatior*		27.5	1.30	0.192	0.15
var. *elongata*		40.0	1.45	0.152	0.00
var. *glabra*		17.8	1.19	0.180	0.16
var. *linifolia*		31.0	1.34	0.233	0.23
var. *ochroleuca*		26.7	1.30	0.153	0.44
C. glabra	Southern Blue Ridge/Piedmont	28.3	1.35	0.227	0.20
	New England and Maritime	17.5	1.18	0.077	0.44
	Central Lowland/Superior Upland	33.3	1.37	0.188	0.30
	Appalachian Plateau	28.0	1.32	0.116	2.80
	Mississippi Embayment	26.7	1.27	0.242	0.19
	Ozark Plateau	40.0	1.43	0.209	0.44
	Atlantic Coastal Plain	26.7	1.30	0.180	0.30
C. lyonii	Southern Blue Ridge	48.3	1.73	0.374	0.28

(cluster I) and glaciated regions (cluster II) (Figs. 4, 6). Cluster I contained populations of *C. glabra*, *C. cuthbertii*, and *C. lyonii* from the Appalachian Plateau, Atlantic Coastal Plain, Piedmont/Valley and Ridge, southern Blue Ridge, and the southern Mississippi Embayment. Cluster II contained populations of *C. glabra* from the formerly glaciated regions of eastern New England and Superior Uplands as well as populations from the unglaciated Ozark Plateau, Atlantic Coastal Plain, Mississippi Embayment, and Central Lowland regions, and populations of *C. lyonii* from the southern Blue Ridge.

Genetic diversity statistics were computed for diploid taxa at 16 loci unbiased for sample size and population number (Table 5). The proportion of polymorphic loci (P), mean number of alleles per polymorphic locus (K_P), and total gene diversity (H_T) were consistently lowest in *C. glabra* and highest in *C. lyonii*. The estimated number of migrants exchanged between populations (N_m) was highest in *C. cuthbertii* and lowest in *C. glabra*. The southern Blue Ridge populations of *C. cuthbertii* had greater levels of gene diversity than populations on the Atlantic Coastal Plain, although coastal populations had higher N_m values. For populations of *C. glabra*, P, K_P, and H_T were lowest in the New England and Maritime Province and highest in the Ozark Plateau (P and K_P) and Mississippi Embayment (H_T) regions.

DISCUSSION

Pattern of morphological and isozyme differentiation among diploids—Phylogenetic analyses in *Chelone* based on morphological characters and undertaken using *Chionophila* Benth. and *Nothochelone* (A. Gray) Straw as outgroups (Nelson, 1995) identified three diploid species each characterized by unique characters. Phenetic approaches such as clustering and ordination analyses (Figs. 2, 3) similarly delineated clusters of populations corresponding to three diploid species. Diploid species were delimited morphologically based on phylogenetic (sensu Nixon and Wheeler, 1990), as well as phenetic criteria (Stuessy, 1990).

Unlike morphological data, isozyme profiles of *Chelone* provided no fixed differences among diploid species. Mean genetic identity values between species (range 0.791–0.799; Table 4) were greater than the average interspecific *I* values of 0.67 reported by Gottlieb (1981) and overlapped with those among conspecific populations of *Chelone* (range 0.440–1.00). Genetic distance-based UPGMA clustering did not discriminate clusters of populations corresponding to three primary morphotypes, but rather grouped populations according to geographic affinity (Fig. 4; evolutionary biogeography section). This pattern of relatively minor morphological differentiation accompanied by little divergence at isozyme loci is also common among diploid species from several other genera in eastern North America: *Antennaria* (Bayer and Crawford, 1986), *Coreopsis* (Cosner and Crawford, 1990), *Heuchera* (Soltis, 1985), *Marshallia* (Watson, Elisens, and Estes, 1991), *Quercus* (Manos and Fairbrothers, 1987), and *Sullivantia* (Soltis, 1982). Floral characters such as beard, staminode, and corolla color in the three diploid species of *Chelone* may be under selective pressures that provide structure in morphological analyses but do not when examining neutral molecular markers such as isozymes.

Our morphological and genetic data did not support delimitation of infraspecific taxa in *C. glabra*. The varieties of *C. glabra* delimited by Pennell (1935) were not defined by any constant and unique morphological or isozyme characters and did not appear as consistent groups in clustering and ordination analyses. Morphological character ranges and variation overlapped extensively in comparisons between varieties defined by Pennell (1935) and, although there were corolla color and leaf character variants in *C. glabra*, no consistent pattern emerged that facilitated recognition and delimitation of infraspecific taxa.

Low levels of isozyme divergence impeded elucidation of evolutionary processes responsible for observed patterns of differentiation. Testing alternative hypotheses of primary divergence vs. secondary contact and gene flow/

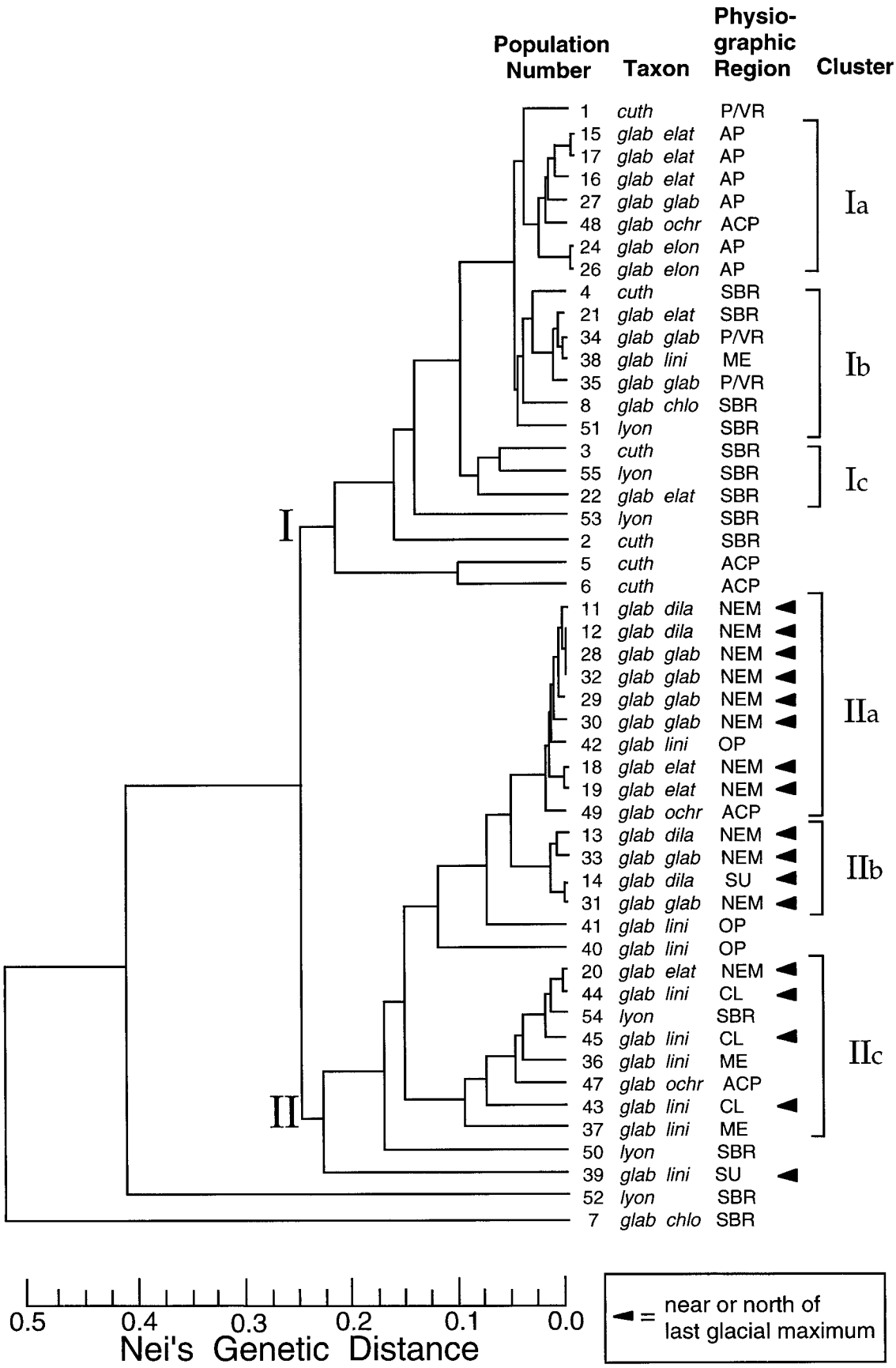

Fig. 4. UPGMA phenogram derived from Nei's (1972) genetic distance coefficient for 50 populations (Table 1) of three diploid species of *Chelone*. Population numbers and abbreviations for physiographic regions and taxa are listed in Table 1. Cophenetic correlation was 0.834. Clusters I a–c and II a–c mapped in Fig. 6. Triangles indicate populations near or north of last glacial maximum.

TABLE 6. Taxonomic and geographic distribution of alleles found in polyploids that are present only in a few diploid populations of *Chelone*. Allele summary frequencies in diploids and presence (+) or fixation (fix) in polyploids are presented for taxa and physiographic regions.

Species/variety	Physiographic region	Locus-allele				
		Adh-1a	*Fest-1c*	*6Pgd-1a*	*Pgm-1a*	*Tpi-2b*
Diploid species ($2n = 2x = 28$)						
cuthbertii	Atlantic Coastal Plain	—	—	—	—	—
	Southern Blue Ridge/Piedmont	0.06	—	—	—	—
glabra	Appalachian Plateau	—	—	0.17	—	—
	Atlantic Coastal Plain	—	—	—	—	—
	Central Lowlands/Superior Upland	0.16	—	0.05	—	—
	Mississippi Embayment	0.40	—	—	—	—
	New England and Maritime	0.04	—	—	—	—
	Ozark Plateau	0.32	0.03	—	0.02	—
	Southern Blue Ridge/Piedmont	0.10	0.01	0.02	0.54	0.13
lyonii	Southern Blue Ridge	0.03	0.03	0.02	—	0.02
Polyploid complex ($4x = 56$, $6x = 84$; varieties of *C. obliqua*)						
erwiniae ($4x$)	Blue Ridge (southern pops. 56–57)	—	—	—	—	fix
	Blue Ridge (northern pop. 58)	—	—	—	fix	—
obliqua ($6x$)	Atlantic Coastal Plain	—	—	—	—	—
speciosa ($6x$)	Central Lowlands	+	—	—	—	—
	Interior Low Plateau	+	+	—	—	—
	Ozark Plateau	+	—	+	—	—

hybridization ideally would require at least moderate levels of differences among species (Crawford, 1990; Rieseberg, Carter, and Zona, 1990; Wolfe and Elisens, 1993). Nevertheless, several aspects of the isozyme data were most concordant with a recent divergence of extant diploids from a common ancestor. First, the amount of allozyme differentiation was minimal within the genus. Second, a large number of alleles (23 of 42) and highest-frequency alleles (8 of 10) were shared among diploid species (Table 3). Finally, alleles unique to species or detected in two species were few in number (8 and 11, respectively), low in frequency (16 of 19 $<$ 0.08), and distributed generally in one to three populations (Table 3). Time and barriers to gene flow generally would be considered necessary prerequisites for genetic differentiation to occur (Crawford, 1990; Avise, 1994). Considering these criteria, we hypothesize that species of *Chelone* have diverged recently.

Although interspecific qualitative allelic differences were few, gene flow and interspecific hybridization appeared to be of limited occurrence. Alleles with discontinuous distributions generally were not clustered in contiguous populations and did not exhibit frequency or occurrence ''clines'' (Arnold and Bennett, 1993). Only *Me-2a* had a populational occurrence that was contiguous and crossed species boundaries in *C. lyonii* (population 53) and *C. glabra* (population 21) in the southern Blue Ridge. However, isozyme markers were not robust enough to better test purported hybridization between *C. glabra* and *C. lyonii* (Bittman, 1980). Common measures of genetic variation (e.g., P and H_T) were most similar to values observed in perennials with selfing to mixed mating systems (Hamrick, 1987; Hamrick and Godt, 1990), a reproductive mode that promotes localized patterns of differentiation. The estimated number of migrants (N_m) exchanged between populations (all but one taxon/region $\leq$ 1.41) also indicated low levels of gene flow (Table 5; Hamrick, 1987; Ellstrand, 1992). Because gene flow appeared to be limited among populations and species of *Chelone*, we suggest that the pattern and amount of isozyme divergence among species reflected local and minor variations of a widespread morphological and isozymic groundplan.

Origin and relationships of polyploids—Cooperrider and McCready (1970) suggested a base number of $x = 14$ for *Chelone* and an euploid series with three diploid species (*C. cuthbertii*, *C. glabra*, *C. lyonii*; $2n = 2x = 28$) and tetraploid ($4x = 56$) and hexaploid ($6x = 84$) races of *C. obliqua* (Nelson, 1995). Phylogenetic analyses of morphological (Wolfe et al., unpublished data) and molecular data (Wolfe et al., 1997) in tribe Cheloneae indicated that *Chelone* was most closely related to (formed an unresolved trichotomy with) western North American genera *Chionophila* ($x = 8$) and *Nothochelone* ($x = 15$). The proposed phylogenetic relationships and base chromosome number of 14 in *Chelone* suggested a possible ancient polyploid event in an $x = 8$ lineage ($x = 8 \rightarrow 16$) followed by aneuploid reduction ($x = 16 \rightarrow 15 \rightarrow 14$) (Nelson, 1995). Observations of three pairs of duplicated loci in diploid species are concordant with hypotheses of paleopolyploidy and subsequent gene silencing rather than alternative hypotheses of three independent gene-duplication events (Haufler, 1987; Crawford, 1990). According to our hypothesis, a secondary cycle of polyploidy most likely resulted in tetraploid and hexaploid races and polyploid allozyme profiles observed in *C. obliqua*.

No unique morphological character or unique alleles delimited the *C. obliqua* polyploid complex. Tetraploid and hexaploid populations intergraded and overlapped morphologically with each other and appeared most similar to populations of *C. glabra* and *C. lyonii* (Fig. 2). Isozyme patterns indicated fixed heterozygosity at six loci. Even though *C. obliqua* was characterized by a recombinant genotype and phenotype, populations could be discriminated at a high level (85.7%) in canonical variance analyses and the complex could be delimited and identified by a unique combination of morphological characters. A purple corolla and petiolate leaves (both

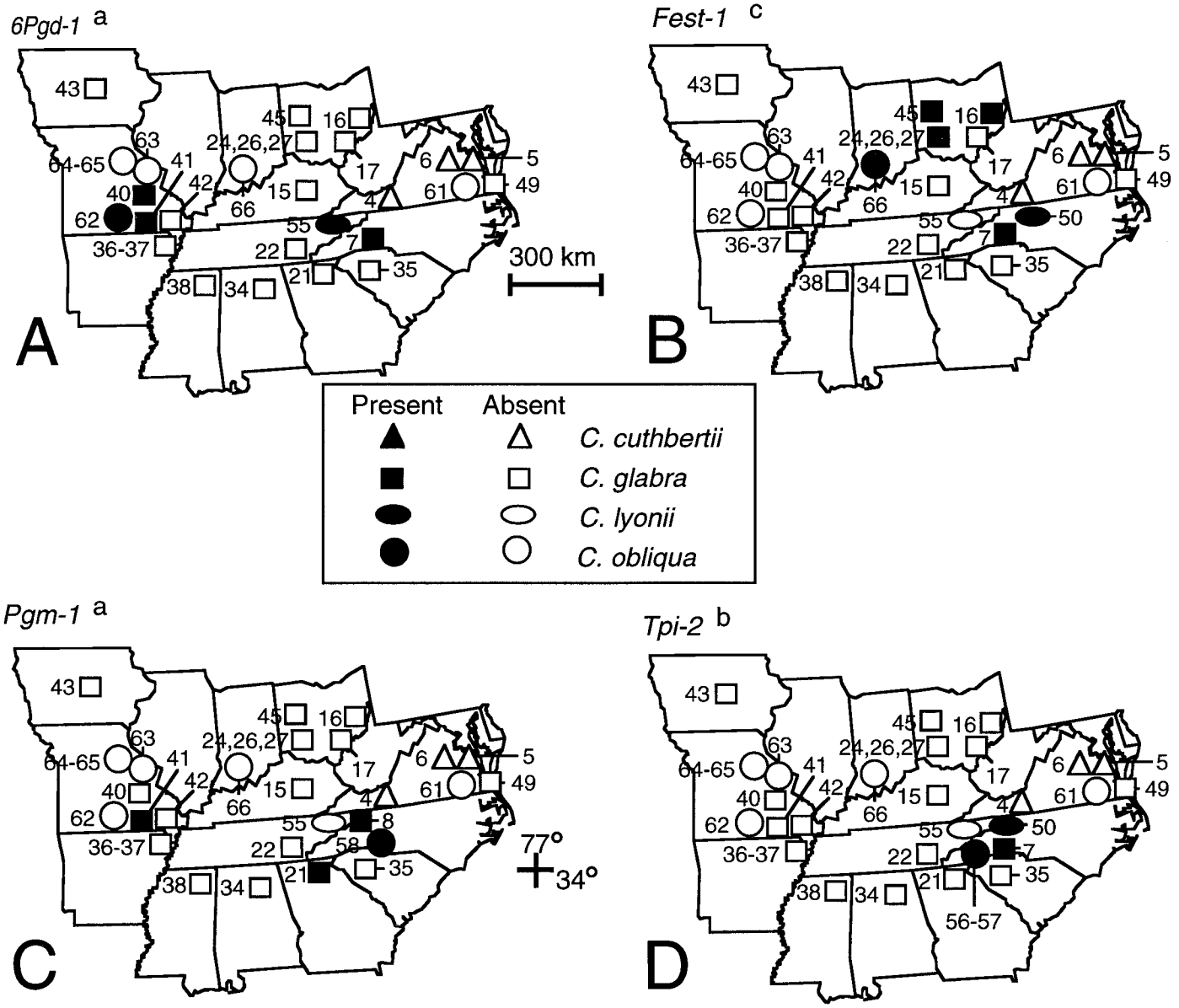

Fig. 5. Maps showing geographic distribution and alleles present in polyploid populations of *Chelone* that have a limited distribution among diploids: (A) *6Pgd-1a*; (B) *Fest-1c*; (C) *Pgm-1a*; (D) *Tpi-2b*.

present in *C. lyonii*) that narrow to the base (present in *C. glabra*) characterized all 4*x* and 6*x* populations of *C. obliqua*. Unlike species-level analyses, the recognition of varieties in *C. obliqua* was not supported by morphological or allozyme data. The 4*x* and disjunct 6*x* races had allopatric ranges, but none of Pennell's (1935) varieties of *C. obliqua* were defined by unique alleles or a unique set of morphological characters, and no varieties were discriminated consistently in phenetic analyses (Figs. 2, 3).

The recombinant patterns observed in *C. obliqua* are most concordant with hypotheses of alloploid origins for polyploid races. Among extant diploids, *C. cuthbertii* does not appear likely as a progenitor for tetraploids or hexaploids. No plants in 4*x* and 6*x* populations exhibit diagnostic morphological characters of *C. cuthbertii* and alleles at three loci lacking from isozyme profiles of *C. cuthbertii* are present in *C. glabra*, *C. lyonii*, and polyploids. Likely progenitors of the tetraploid race were similar to *C. glabra* but a contribution of plants similar to *C. lyonii* cannot be resolved. Our data suggested at least two independent origins for the tetraploids. Although tetraploids of *C. obliqua* were restricted to the southern Blue Ridge, 4*x* populations exhibited variation at several morphological characters, were internested among populations of *C. glabra* in morphological analyses (Fig. 2), and ''northern'' (56, 57) and ''southern'' populations (58) were differentiated by alternate fixed alleles at the *Tpi-2* locus. The *Tpi-2b* allele, which characterized northern populations, was found at frequencies as high as 1.00 in populations of *C. glabra* from the Blue Ridge region and at low frequencies in *C. lyonii*. Based on leaf flavonoid profiles, Bittman (1980) suggested autoploid origins of

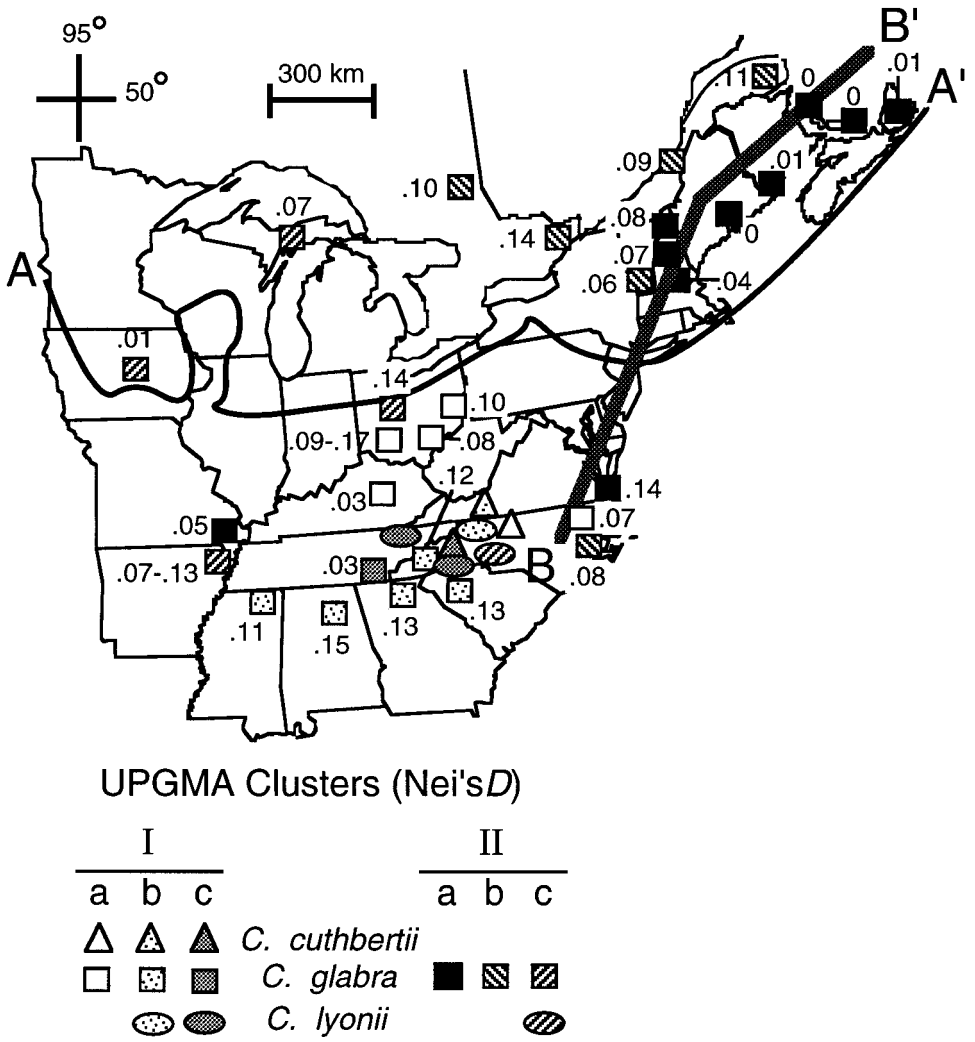

Fig. 6. Distribution of populations from clusters I a–c and II a–c (Fig. 4) in relation to approximate Wisconsinian glacial maximum (Line A-A′). Line B-B′ connects southern and northern population of cluster IIa. Total genetic diversity (H_T) values are given for each population of *C. glabra*.

tetraploid populations within *C. glabra*. Although the isozyme pattern of northern and southern tetraploids also could have resulted from differential gene silencing, our observations of morphological variation, fixed heterozygosity at six loci, and alternate fixed alleles at *Tpi-2*, are more consistent with hypotheses of alloploidy and polytopy for the tetraploid race.

Morphological data showed significant variation among coastal and interior hexaploid populations and suggested close relationships with *C. glabra*, *C. lyonii*, and tetraploids. Isozyme data were more robust and indicated that extant 4*x* populations were not recent progenitors for 6*x* populations of *C. obliqua*. Mutually exclusive alleles at five loci, including two monomorphic loci among tetraploids, characterized isozyme profiles of tetraploid and hexaploid populations. If tetraploid races were recent progenitors of hexaploids in a pillar complex (Stebbins, 1971), genomic additivity and the presence of alleles fixed for the tetraploids at *Pgm-1* and *Tpi-2* (Table 6) would be predicted among hexaploid populations (Roose and Gottlieb, 1976; Werth, 1989). Although it was possible that tetraploid progenitor genotypes were not sampled or that differential gene silencing caused this difference, a more likely explanation was the extinction of tetraploid progenitors of extant hexaploid populations.

Our data also suggest at least two independent origins for hexaploid populations of *C. obliqua*. Although morphological data are equivocal, coastal hexaploids had a unique isozyme profile among polyploids (Table 5) suggesting an origin separate from interior hexaploids and without the genomic contribution of extant tetraploids. The morphological and isozyme profile of interior hexaploids, which combined morphological characters and alleles at two loci found only in *C. glabra* and *C. lyonii*, also suggested multiple origins. Unique allozyme profiles at *Fest-1* and *6Pgd-1* allow discrimination among Central Lowland, Interior Low Plateau, and Ozark Plateau populations of interior hexaploids. With the forementioned proviso that inadequate sampling and differential gene silencing could produce these isozyme patterns, multiple origins of interior hexaploids from extinct tetraploid progenitors is suggested.

Evolutionary biogeographic patterns—Although the most closely related genera to *Chelone* (*Chionophila*, *Nothochelone*) occur in western North America, the four species of *Chelone* are restricted to eastern North America. Only the wide-ranging diploid *C. glabra* had populations found north of the last glacial maximum. Two of three ploidy levels and all four species occurred in the unglaciated southern Blue Ridge region. Hexaploid populations are known only from the Atlantic Coastal Plain and unglaciated areas of the Interior Highlands. Isozyme data also indicated greater genetic diversity in the Blue Ridge, among all species and within *C. glabra* and *C. cuthbertii* (Table 5; Fig. 6). All but one of 42 alleles detected were found in this region. Blue Ridge populations of *C. cuthbertii* and *C. glabra* had similar or higher proportions of polymorphic loci, observed heterozygosity, and total genetic diversity compared to populations outside the region (Table 5). Consequently, the Blue Ridge geographic region represents the center of morphological, cytological, and genetic (isozymic) diversity for the genus.

Based on Nei's (1987) formulas for stepwise mutation rates calculated from genetic identity coefficients, divergence estimates among three diploid species ranged from 1.25 to 1.53 million years BP (before present). These estimates and the low levels of morphological and isozyme divergence observed in *Chelone* indicated differentiation during the Pleistocene and Holocene epochs beginning two million years ago. Because paleoecological reconstructions of the eastern deciduous forest biome (Davis, 1983; Delcourt and Delcourt, 1987) suggested multiple north-south migrations during the Quaternary Period, we propose that areas south of or in the Blue Ridge region represented a recent center of diversification of the lineage.

Analyses of isozyme data consistently grouped diploid populations by geographic region and recent glacial history both within and among species (Figs. 4, 6). For example, among all diploid species, populations from the contiguous unglaciated areas of the Appalachian Plateau, Piedmont and Valley and Ridge, and southern Blue Ridge regions occurred together in the UPGMA phenogram (Figs. 4, 6). Populations of *C. glabra* from glaciated areas of the New England and Maritime, Central Lowlands, and Superior Upland regions also grouped together consistently (Figs. 4, 6). Isozyme divergence among diploid populations in *Chelone* apparently reflect geohistorical factors and migrational history rather than lineage differentiation.

The southern Blue Ridge, Ozark Plateau, and Atlantic Coastal Plain regions have been proposed as glacial refugia and as centers for revegetation and plant migration during interglacial periods (Cain, 1944; Davis, 1983; Delcourt and Delcourt, 1987; Delcourt, 1991). The infraspecific geographically based groupings observed in diploid populations of *C. glabra* and in polyploid races of *C. obliqua* generally are concordant with these hypotheses. Each of the three allopatric cytotypes of *C. obliqua* were confined to one of these regions (4*x* populations in the Blue Ridge, 6*x* populations disjunct in coastal and interior regions), and distance-based trees grouped diploid populations of *C. glabra* from each of these regions. Furthermore, populations of *C. glabra* from adjoining glaciated areas were associated with populations from purported refugial areas (Fig. 6). Regions with the highest overall genetic similarities were (1) the unglaciated Ozark Plateau and glaciated areas of the Interior Plains region, and (2) the coastal plain and glaciated New England and Maritime region. The isozymic similarities between the New England and Maritime and the Atlantic Coastal Plain populations were illustrated by the presence of *Tpi*-3b; the only allele not found among Blue Ridge populations.

Observed levels of populational genetic variation along an Atlantic coastal transect (B-B′, Fig. 6) were concordant with patterns expected from the central-marginal migrational model (Barrett and Husband, 1990; Avise, 1994), which predicted reduced levels of variation in marginal populations resulting from founding events, extinction, and genetic drift. Populations of *C. glabra* from the New England and Maritime region had lower values of polymorphic loci, observed heterozygosity, and total genetic diversity (H_T, Fig. 6) then those in populations of the coastal plain or in any other geographic region. Allelic diversity in the New England and Maritime region was a highly monomorphic subset of the genetic diversity observed in the Atlantic Coastal Plain populations of *C. glabra*. Similar patterns of reduced genetic variation resulting from putative postglacial range expansion also were observed in *Pinus* (Cwynar and MacDonald, 1987; Parker et al., 1997) and *Chamaecyparis* (Kuser et al., 1997). Based on our data, we propose that *C. glabra* migrated to the New England and Maritime region via an Atlantic coastal route. The geographical pattern of isozymes also suggests that populations of *C. glabra* in the glaciated interior migrated from an Ozark or southern Blue Ridge center.

Because the three allopatric cytotypes of *C. obliqua* were most similar genetically to diploid populations in their own or neighboring (unglaciated) regions, occurred in areas hypothesized to be glacial refugia, and lacked any novel alleles or morphological characters, we propose that polyploid evolution in *Chelone* was geologically recent and was facilitated by the vegetational changes effected during the Pleistocene and Holocene epochs. Similar chromosomal evolutionary scenarios have been postulated in angiosperms and ferns in eastern North America (Werth, Guttmann, and Eshbaugh, 1985a, b; Loveless and Hamrick, 1988; Haufler and Zhongren, 1991; Watson, Elisens, and Estes, 1991; Werth, 1991; Case, 1993). Plant migration and range contraction/expansion cycles during the Quaternary apparently increased the probabilities of multiple differentiation events and independent, allopatric polyploidization cycles such as those described in the European flora (Ehrendorfer, 1959, 1980). In the fern genus *Dryopteris*, Werth (1991) used isozyme data to propose a southward migration of diploid species, with subsequent sympatry and hybridization during glacial advances. We propose a similar scenario for polyploid evolution in *Chelone*. In *Chelone*, hybridization/polyploidization cycles apparently occurred in different refugial areas, resulting in the allopatric distribution of extant polyploid cytotypes and several putative examples of multiple, independent alloploid origins. Because there are few morphological and isozyme differences, plastid and additional nuclear molecular markers

will be examined to further test these evolutionary and biogeographic hypotheses.

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