Evaluating the taxonomy of elms (*Ulmus*) using DNA sequence data

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Introduction

The elms (*Ulmus* L: Ulmaceae) number approximately 45 species worldwide (Buchel 2000), distributed primarily in the north temperate zone. The nomenclature of the genus is confusing, with more than 250 published names, of which many are not in obvious synonymy (Hipp, Gog, and Weber in prep.), and estimates of the number of species range from 20 to 45 (Sherman-Broyles 1997). The sectional classification was revised in 1994 using chloroplast DNA (cpDNA) restriction site data (Wiegrefe et al. 1994). In that classification, two subgenera were recognized comprising five sections. In this study, we utilize cpDNA and nuclear ribosomal (nrDNA) sequences to (1) evaluate relationships among species in the genus and (2) provide a framework for evaluating alpha-taxonomic and nomenclatural issues. We focus on specimens in the living collections at The Morton Arboretum, which is one of the largest living *Ulmus* collections in the world (more than 30 species, in addition to numerous infraspecific taxa, hybrids, and cultivars; Figure 1) and has the source of numerous elm cultivars.

Methods

Sampling—We sampled 34 taxa with a strong emphasis on section *Ulmus,* which constitutes the majority of the genus (approximately 30 species) and is particularly well represented in the Morton Arboretum living collections. Many of our accessions were wild-collected in China by botanists from the Chinese Academy of Forestry, Beijing for George Ware's research on elm cultivars. Because one focus of our work includes evaluation of taxonomic segregates, names on the accessions in our study largely follow the taxonomy recognized by regional workers, which, like the Morton Arboretum's living collections (redwood.mortonarb.org/PageBuilder?cid=2), includes a larger number of taxonomic segregates than the most current published checklist of species (Buchel 2000). In a very few cases, identifications were incorrect, and these have been corrected in the data shown. Vouchers are deposited at The Morton Arboretum herbarium (MOR). Outgroups *Zelkova serrata* and *Hemiptelea davidii* were selected based on Ueda et al. (1997).

Data collection and analysis—DNA was isolated from fresh or frozen leaf tissue using DNeasy kits (QIAGEN, Valencia, CA). PCR was conducted on three species for 19 regions (Table 1; thirteen regions successfully amplified out of the 19 attempted). Three regions were selected for the study: the nrDNA internal transcribed spacer (ITS) region and two cpDNA spacers (3'rps16–5'trnK and psbD–trnT). Cycle sequencing was conducted using BigDye (Applied Biosystems, Foster City, CA) and sequencing was conducted on an ABI 3730 capillary sequencer. Data partitions were analyzed separately and in combination using maximum parsimony in PAUP* v4.0b10 (Swofford 2002). Data congruence was assessed by visually inspecting trees and using the incongruence length difference test (Farris et al. 1994). The cpDNA regions appear to be congruent (ILD P = 0.046), but the three-way test involving ITS suggests incongruence (P = 0.002) that appears to be due to differences in the placement of a few species that do not strongly affect overall topology of the combined tree. Bootstrap values reported on the combined analysis tree are consequently based on both the combined (3-gene) analysis and the cpDNA analysis only (Figure 2).

Results and Discussion

The two cpDNA regions in combination with ITS provide moderate support for a number of species groups, but poor support for relationships among species groups within section *Ulmus* (Figures 2, 3). ITS analyzed alone (not shown) provides relatively little information. Not surprisingly, our work confirms the subgenera of Wiegrefe et al. (1994) with strong support (1.00 bootstrap). These subgenera are supported by several biogeographic and morphological characters. Subgenus *Oreoptelea* is distributed primarily in the New World and is characterized by elongated pedicels (with secondary reduction in *U. crassifolia*) and ciliate samara margins (Figure 1a; some species in subgenus Ulmus have independently derived ciliation). Subgenus *Ulmus*, much the larger of the two, is a predominantly Old World group. Our sampling was not adequate to evaluate Wiegrefe et al.'s sectional classification, but numerous clades recovered within section *Ulmus* are worth discussing.

• ***The Ulmus davidiana complex***—*Ulmus japonica, U. wilsoniana*, and *U. propinqua* (Figure 1c) have all been held as synonyms of *U. davidiana* var. *japonica*. Our work recovers two clades comprising these taxa: one comprising *U. propinqua* and *U. davidiana* var. *davidiana* (bootstrap = 0.99); and one comprising *U. davidiana* var. *mandshurica*, hybrids involving *U. davidiana* var. *japonica* (including *U.* 'Morton', Accolade Elm), and a few unidentified accessions received from China (bootstrap support = 0.76). A group of specimens accessioned as *U. wilsoniana* form a separate clade, though these do not appear to have the leaf size or pubescence character used by Rehder (1940) to distinguish *U. wilsoniana* from *U. davidiana* s.s. It is consequently not clear whether these clusters are phylogenetically significant. It appears that the taxonomy of this group—which has been particularly important to the development of Dutch elm disease-resistant cultivars—bears further investigation, to evaluate whether the clades we are detecting in this study reflect taxonomic differences or intraspecific genetic differentiation.

• ***The Ulmus glabra – Ulmus minor clade***—A clade comprising *Ulmus glabra*, *U. procera* [= *U. minor*], *U.* × *hollandica* (*U. glabra* × *U. minor*), and one of our *U. pumila* accessions is strongly supported (bootstrap = 0.97). This clade falls sister to a clade comprising *U. foliacea, U. sukaczevii* [= U. *glabra], U. elliptica* [= *U. glabra*], *U. wallichiana* (perhaps sister to *U. elliptica*, though this bears investigation with additional material; see note [1] on Figure 2), *U. pumila, U. microcarpa, U. glaucescens* var. *lasiocarpa*, *U. lamellosa*. Within this latter clade, *Ulmus glaucescens* var. *lasiocarpa* and *U. lamellosa* (Figure 1d) come out sister to one another, which is supported by a very distinctive bark character. It is somewhat troubling to see *U. pumila* falling in these two separate clades. However, Wiegrefe (1992) also found molecular diversity in the *U. pumila* accessions she sequenced, and it may be that a long history of hybridization with numerous species will make it difficult to place *U. pumila* reliably on the tree. In fact, *U. pumila* has been used in numerous breeding experiments and is a component of several cultivars.

It is worth noting that *U. glabra* has been held to have the following taxa as synonyms: *U. elliptica, U. campestris, U. sukaczevii* (among others). In our tree, both *U. elliptica* and *U. sukaczevii* fall separate from the *U. glabra* clade. This taxonomic issue bears further study.

• ***Ulmus castaneifolia***—*Ulmus bergmanniana* and *U. castaneifolia* form a clade in our analysis separate from *U. multinervis*. This is interesting on two accounts. First, *U. bergmanniana* and U. *castaneifolia* both have a very distinctive woolly pubescence on the abaxial leaf surface, though they do appear to be distinct from one another in leaf shape and margin. However, *U. multinervis* also shares this pubescence character and has been subsumed into *U. castaneifolia* in recent works (Fu et al. 2002), but in our analysis it falls with *U. gaussenii* and *U. prunifolia*. The taxonomic distinctness of *U. multinervis* and *U. castaneifolia* bears investigating with additional material.

• ***Nikko elm***—The Japanese endemic *Ulmus laciniata* var. nikkoensis (Figure 1e) comes out among two accessions of *U. davidiana* var. *japonica*. This entity is a local form collected near Nikko in central Honshu, Japan. The variety was not recognized by Owhi (1965) at all, and in fact the variety may represent a solitary hybrid between *U. laciniata* and *U. davidiana* var. *japonica* (G. Ware, pers. comm.); as the plants grown in the U.S. all appear to be progeny of a single (1925) introduction, the Nikko elm may be better thought of as a cultivar than as a true variety; additional fieldwork will be needed to assess this. Our finding that this plant falls among the *U. davidiana* var. *japonica* accessions in the cpDNA tree supports the argument that one of its parents is *U. japonica*, despite the horned appearance of the leaves, which suggest the influence of *U. laciniata*.

Figure 1a. *Ulmus thomasi*. The ciliate samara margins that characterize subgenus Oreoptelea are obvious in this photograph. Morton Arboretum living collections 178-84*3.

Figure 1b. *Ulmus elliptica.* Morton Arboretum living collections 200-90*3.

Figure 1c. *Ulmus propinqua* var. *suberosa*. Morton Arboretum living collections 52-95*4.

Figure 1d. *Ulmus lamellosa.* Morton Arboretum living collections 317-90*1.

Figure 1e. *Ulmus laciniata* var. *nikkoensis*. Morton Arboretum living collections 180-84*1. Leaf detail (same specimen) by Bruce Marlin, www.cirrusimage.com.

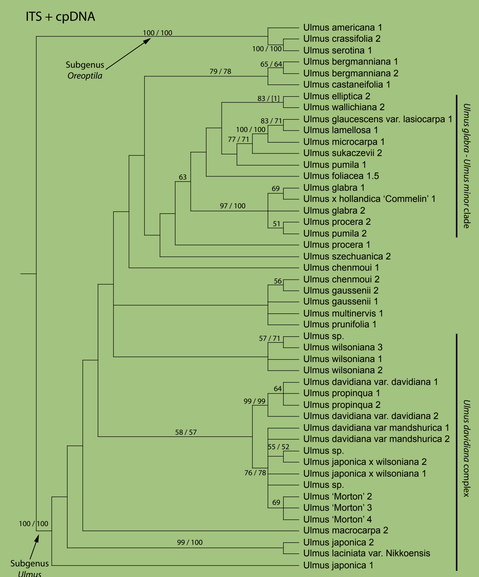

Figure 2. Maximum parsimony strict consensus, nrDNA and cpDNA regions analyzed in combination. Bootstraps are shown above branches for the combined analysis / cpDNA regions only. Tree is rooted using Zelkova and Hemiptelea (not shown). Tree length = 503 steps; CI = 0.726; RI = 0.762. [1] This sister relationship is found only in the ITS and combined analysis and its cause will be investigated further.

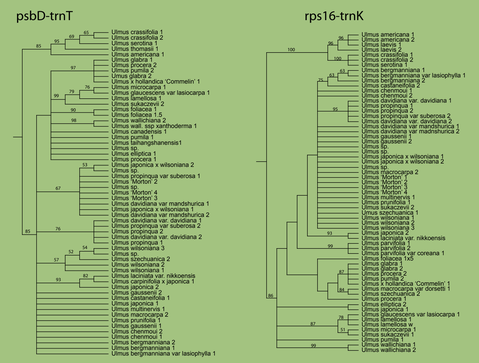

Figure 3. Maximum parsimony strict consensus, cpDNA regions analyzed separately. Bootstraps are shown above branches. Note that the ITS analysis is not shown because it provided very little resolution separate from the cpDNA analyses. *psbD-trnT*: 310 steps, CI = 0.813, RI = 0.845; *rps16-trnK*: 228 steps, CI = 0.746, RI = 0.879.

Region evaluated	Reference
atpI-atpH *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.
ndhA intron *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.
ndhF-rpl32 *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.
ndhJ-trnF *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.
petL-psbE *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.
psaI-accD *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.
psbD-trnT *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.
psbJ-petA *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.
rpl14-rps8-infA-rpl36 *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.
rpl16	Jordan, Courtney, & Neigel. 1996. American Journal of Botany 83(4): 430-439.
rpl32-trnL *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.
rpoB-trnC	Shaw et al. 2005. American Journal of Botany 92: 142-166.
3'rps16-5'trnK *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.
trn L-F	Taberlet et al. 1991. Plant Molecular Biology 17: 1105-1109.
trnD-trnT	Demesure, Sodzi, & Petit. 1995. Molecular Ecology 4: 129-131.
trnH-psbA	*psbA*: Sang, Crawford, & Stuessy. 1997. American Journal of Botany 84(9):1120-1136. *trnH*: Tate & Simpson. 2003. Systematic Botany 28(4): 723-737.
trnQ-5'rps16 *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.
trnS-trnG-trnG *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.
3'trnV-ndhC *	Shaw et al. 2007. American Journal of Botany 94(3): 275-288.

Table 1. Chloroplast regions screened for this study. Nineteen regions were screened for ease of amplification and variability on three species: *Ulmus americana*, *Ulmus laevis*, and *U. wallichiana*. Of these, fourteen (*) sequenced readily and were inspected for variability (alignments available upon request;). Phylogenetic utility was assessed by visual inspection of alignments, with an eye to nucleotide and indel variability.

Conclusions and Future Directions

Resolving phylogenetic relationships in elms will require a genuine attention to taxonomic questions in the genus, which is not particularly well understood from a systematic standpoint. Our work points to areas of difficulty (e.g., the *U. davidiana* complex) and demonstrates the utility of cpDNA sequences in identifying species complexes. Our lab is currently working on an updated taxonomic checklist for the genus (Hipp, Gog, and Weber in prep.) as well as an updated checklist of cultivar names (Hipp and Bannon in prep.). Both of these endeavors work hand-in-hand with the phylogenetic study. Given the low variability of the ITS region in *Ulmus*, understanding the effects of hybridization / reticulate evolution on the taxonomy of the group will require the use of additional nuclear loci. Such a study will be necessary for understanding how the potential for hybridization in the group-species are readily crossed in the wild and in cultivation—has impacted macroevolutionary patterns in the genus.

Acknowledgements

We could not have done this project without the extensive work by other elm researchers at The Morton Arboretum and collaborating institutions. Foremost among these are George Ware, whose influential work has shaped the development of elm cultivars for decades, and his collaborator Zhang Qinghua (Research Instituted of Forestry, Beijing), who collected from wild populations many of the Chinese species represented in the current study. Ongoing maintenance of the collection by Kunso Kim, Kris Bachtell, and others in the living collections staff has also been essential to this project. Assistance in collecting vouchers for the project was provided by volunteers and interns of the Arboretum herbarium, and the leaf photo of *Ulmus laciniata* var. *nikkoensis* was generously provided by Bruce Marlin (www.cirrusimage.com).

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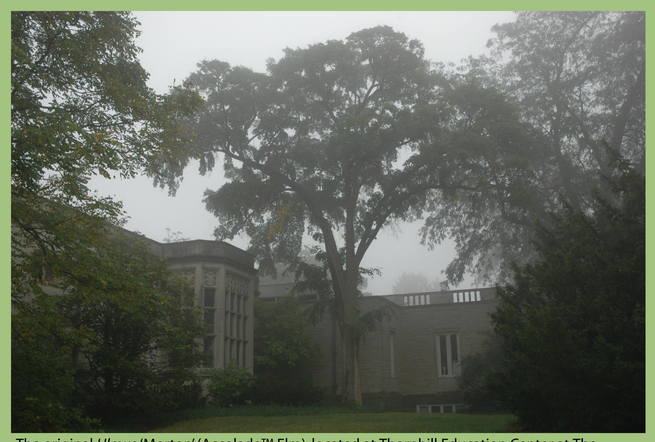

The original *Ulmus* 'Morton' (Accolade™ Elm), located at Thornhill Education Center at The Morton Arboretum. *Ulmus* 'Morton' is a putative hybrid between *U. japonica* and *U. wilsoniana* that is widely planted for its American-elm-like form and resistance to Dutch elm disease. Photo by Kris Bachtell, Director of Collection and Facilities at The Morton Arboretum.