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Taxonomy of Hill's Oak (*Quercus ellipsoidalis*: Fagaceae): Evidence from AFLP Data

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Abstract*—Quercus ellipsoidalis* (Hill's oak), an endemic of east-central North America, is morphologically similar to *Q. coccinea* (scarlet oak) and is subsumed into that species in several floristic treatments. This study uses data from more than 250 amplified fragment length polymorphism (AFLP) markers to investigate whether *Q. coccinea* and *Q. ellipsoidalis* are genetically distinct from one another. Whereas *Q. coccinea* and *Q. ellipsoidalis* separate from one another in all analyses, *Q. velutina* (black oak) populations collected from the geographic range of both *Q. coccinea* and *Q. ellipsoidalis* do not separate out by geographic region. This, combined with the strong differentiation between *Q. coccinea* and *Q. velutina* but weak differentiation between *Q. ellipsoidalis* and *Q. velutina*, supports the view that *Q. coccinea* and *Q. ellipsoidalis* are not simply regional variants of a single taxon. Moreover, while there is no evidence from the molecular data we collected of hybridization between *Q. coccinea* and *Q. ellipsoidalis*, the data suggest that there may be gene flow between *Q. ellipsoidalis* and *Q. velutina*. A clearer understanding of the relationships among these taxa is essential to understanding the taxonomy of *Quercus* section *Lobate* in eastern North America.

***Keywords*—**Amplified fragment length polymorphisms (AFLP), hybridization, oak taxonomy, *Quercus* section *Lobatae*.

Quercus ellipsoidalis E. J. Hill (Hill's oak) is one of the most problematic members of *Quercus* L. section *Lobatae* Loudon, the black oak section, in east-central North America. In a genus renowned as a "worst case scenario for the biological species concept" (Coyne and Orr 2004: 43), *Q. ellipsoidalis* is distinguished by the number of workers who have puzzled over its taxonomic status and proper identification (Trelease 1919; Jensen 1977, 1979; Overlease 1977; Maycock et al. 1980; Jensen et al. 1984; Hokanson et al. 1993; Shepard 1993). *Quercus ellipsoidalis*, when it was first encountered, was initially identified as *Q. coccinea* Münchh. (scarlet oak; Trelease 1919). Subsequent to the description of *Q. ellipsoidalis* based on specimens from the Chicago region in northeastern Illinois, U.S.A. (Hill 1899), many botanists accepted that this species was found in Iowa, Minnesota, Wisconsin, Michigan, and the northern counties of Illinois, Indiana, and Ohio, to the exclusion of *Q. coccinea*. *Quercus ellipsoidalis* is characterized by deeply lobed leaves with C-shaped sinuses; ellipsoid, often longitudinally striped acorns, the caps glabrous or very sparsely pubescent on the inner surface with tightly imbricated scales; and relatively small terminal buds that are glabrous or sparsely silky-pubescent (very occasionally densely pubescent) at least on the distal one-half to two-thirds (Fig. 1). Specimens housed in the major herbaria documenting the flora of northeastern Illinois and adjacent counties (WIS, MOR, and F) show geographic overlap between *Q. coccinea* and *Q. ellipsoidalis* in northwestern Indiana, with a few collections of *Q. coccinea* scattered in northeastern Illinois and southern Michigan (Fig. 2).

The region around the type locality for Hill's oak is the focus of substantial taxonomic disagreement. Some recognize *Quercus ellipsoidalis* as distinct from *Q. coccinea* and view both as present in northeastern Illinois (Trelease 1919; Jensen 1977, 1979; Jensen et al. 1984), while others hold that the two are not reliably distinguishable and best treated as a single species (Overlease 1977; Voss 1985; Shepard 1993; Swink and Wilhelm 1994). Because of difficulty in interpreting the morphological characters and apparent morphological intergradation between the two species, molecular data should be useful in evaluating whether they are best recognized as distinct at the subspecific or specific level, or whether they should be considered variants of a single, wide-ranging and variable taxon.

Characterizing the taxonomy and distribution of *Quercus ellipsoidalis* requires an investigation of its relationship with *Q. velutina* Lam. (black oak), to which it bears close similarity (Jensen 1977). Typical *Q. velutina* is characterized by large, densely canescent buds that are distinctly pentagonal in cross-section; acorn caps that are densely pubescent on the inner surface, with loose scale margins; and leaves that are frequently pubescent, even at maturity, especially on the major veins on the leaf undersides (the latter characteristic appears to be more pronounced south and east of the Chicago region; Fig. 1). While individuals at the morphological extremes are highly distinctive, intermediates between *Q. ellipsoidalis* and *Q. velutina* are not uncommon (Jensen 1977; Jensen et al. 1984). Hybridization involving some mixture of *Q. velutina*, *Q. coccinea*, *Q. rubra* L. (red oak), and *Q. palustris* Münchh. (pin oak) has been implicated in the origins of *Q. ellipsoidalis* (Hill 1899; Jensen et al. 1984). Hill (1899) believed that *Q. ellipsoidalis* was too abundant and widespread for hybridization to be a satisfying explanation of its origin, but morphological study has suggested that the type specimen for *Q. ellipsoidalis* may represent a hybrid population (Jensen et al. 1984).

The present study addresses three primary questions. (1) Can *Quercus coccinea*, *Q. ellipsoidalis*, and *Q. velutina* be distinguished from one another using molecular genetic data? If so, (2) is recognition of *Q. ellipsoidalis* as a species distinct from *Q. coccinea* warranted, and (3) which taxon is more abundant in northeastern Illinois, where the type locality of *Q. ellipsoidalis* occurs and where the nomenclature of the two is in greatest flux? These three questions are basic to understanding the taxonomy of *Q. ellipsoidalis*, the relationships among *Quercus* section *Lobatae* species of east-central North America, and the potential for gene flow between populations and between taxa. Given the frequency of individuals that have morphological characteristics of both *Q. ellipsoidalis* and *Q. velutina*, an additional question of interest is whether molecular data are compatible with the hypothesis of gene flow between these two species. We offer only a preliminary investigation of hybridization in this study.

Materials and Methods

Sampling, Plant Identification, and Labwork—142 specimens of the three species of interest, *Quercus coccinea*, *Q. ellipsoidalis*, and *Q. velutina*, were collected from localities in northeastern Illinois, Wisconsin, southern Illinois, southern Ohio, and southeastern Missouri (Fig. 2). Sampling was designed to collect unambiguous *Q. ellipsoidalis* (from Wisconsin) and unambiguous *Q. coccinea* (from southern Illinois, southern Ohio, and Missouri) for analysis in combination with populations from northeastern Illinois, where the identity of these two taxa has been questioned. Eleven specimens from two other closely related species, *Q. rubra* (red oak) and *Q. palustris* (pin oak), were collected to assess whether they are genetically distinct from the three species of interest. Identifications were based on mature material collected in summer or fall. Given the difficulty of identifying this group of oaks in northeastern Illinois, numerous precautions were taken to ensure accuracy of identification. All populations from northeastern Illinois were visited first between April and June, so that young leaves could be used in DNA extractions. This precaution turned out not to be essential—we were able to get consistently high-quality extractions from leaves collected as late as early October. Trees were individually tagged and GPS coordinates recorded on the first visit, and all northeastern Illinois specimens were revisited in the fall so that acorns and mature buds could be collected. Plants were identified by both authors using the key characters presented in *Flora of North America* (Jensen 1997). Each identified plant was placed into one of four categories: exhibiting morphological characteristics of one species only; predominantly exhibiting characteristics of one species, but with morphological evidence of hybridization with other species; presenting an admixture of morphological characters from two or more species, but unclear which is dominant (referred to as "putative hybrids" in this paper); or unable to be identified with certainty due to inadequate material or unclear morphological affinities. The fourth category of plants, 22 individuals that could not be positively identified because they lacked sufficient material for reliable morphological identification, was excluded from analyses on which figures presented in this study are based, but they are included in one set of Bayesian analyses reported (see Methods, final paragraph). These 22 individuals were excluded prior to analysis, not because they were morphologically intermediate (morphologically intermediate individuals were specifically included in analyses and are labeled as such in the figures) but because the available plant material made conclusive a priori morphological identification impossible for these 22 individuals. Including these individuals for the majority of analyses would make it difficult to assess how well morphological identifications correlate with molecular groupings. We did run a set of trial analyses including these individuals and found that their inclusion does not reduce the distinctness of the molecular genetic clusters reported in this paper and has no effects on the conclusions of our study. All individuals are vouchered at The Morton Arboretum Herbarium (MOR; Appendix 1).

DNA was extracted from frozen leaves of single individuals using DNeasy (QIAGEN, Inc., Valencia, California). Manufacturer's protocols were followed with the following modifications: tissue mass was reduced to ca. 30 mg per individual; lysis buffer (AP1) was increased from 400 μl to 700 μl; rinse buffer (AP2) was increased from 130 μl to 200 μl; and precipitated DNA was washed four times instead of two. Extracted DNA was suspended in Qiagen elution buffer and stored at −20°C.

Total DNA was digested using two pairs of restriction enzymes: *Bfa*I / *Mse*I and *Eco*RI / *Mse*I. Sixty-four selective primer pairs were surveyed on eight individuals (representing *Quercus coccinea*, *Q. ellipsoidalis*, and *Q. velutina*) for each of the enzyme pairs. Initial inspection of the screened primers revealed a greater concentration of variable primer pairs using *Eco*RI / *Mse*I. The 20 most variable primer pairs were scored (Table 1) and the most variable primer pair (*Eco*RI-ACG / *Mse*I-CTC) was used for this study. All primer pairs reported in Table 1 exhibit sufficient variation within and among the species studied that they should provide good markers for research in other oak groups.

Amplified fragment length polymorphism (AFLP) protocols were modified from Vos et al. (1995) following Berres (2001). In selective amplifications, the *Eco*RI primer was labeled with the fluorescent dye 6FAM. Polymerase chain reaction (PCR) products were purified using the CleanSEQ dye-terminator removal system (Agencourt Bioscience Corporation, Beverly, Massachusetts) prior to analysis on an Applied Biosystems (ABI; Foster City, California) 3730 capillary sequencer with a ROX-labeled internal lane standard, fragments of known size ranging from 50–625 base pairs (bp) in 25-bp intervals.

Analysis—AFLP chromatograph files were analyzed in GeneMapper version 3.7 (ABI), eliminating bands shorter than 50 bp. Bins were generated automatically in GeneMapper and manually edited with reference to chromatograph files to correct for discrepancies in fragment size-calling and inconsistencies in the intensities of bands between different lanes. Markers that could not be positively determined to be present or absent for every individual represented were excluded from the dataset; consequently, no markers were scored as missing or ambiguous. Edited markers were scored as binary data matrices.

Ordinations were performed using nonmetric multidimensional scaling (NMS) in PC-ORD version 4.41 (McCune and Mefford 1999) on a pairwise similarity matrix calculated using Jaccard's index (Jaccard 1908; Landry and Lapointe 1996). Preliminary analyses were conducted in the "autopilot," "slow and thorough" mode of PC-ORD, which uses a maximum of 400 iterations for each of 40 runs with real data and 50 runs with randomized data to identify the number of axes, followed by 400 iterations using the optimal starting configuration from the first set of iterations. Three-dimensional solutions were favored in all preliminary analyses. To aid in interpretation and presentation, two-dimensional ordinations were obtained using stringent search criteria (50 replicate runs from random start points, each for a maximum of 100–400 iterations with a stability criterion of 0.00001). In all cases, two-dimensional solutions support the same conclusions as three-dimensional solutions and are more straightforward to visualize and interpret.

A pairwise distance matrix for all individuals was calculated in PAUP* v4b10 (Swofford 2002) using the restriction site distance of Nei and Li (1979). The neighbor-joining (NJ) method was applied to this distance matrix in PAUP*, and bootstrap branch support was estimated using 1,000 NJ bootstrap replicates. Trees were also generated using heuristic searches on the same pairwise distance matrix under a minimum evolution criterion, but they did not differ significantly from the NJ tree in topology or in bootstrap support.

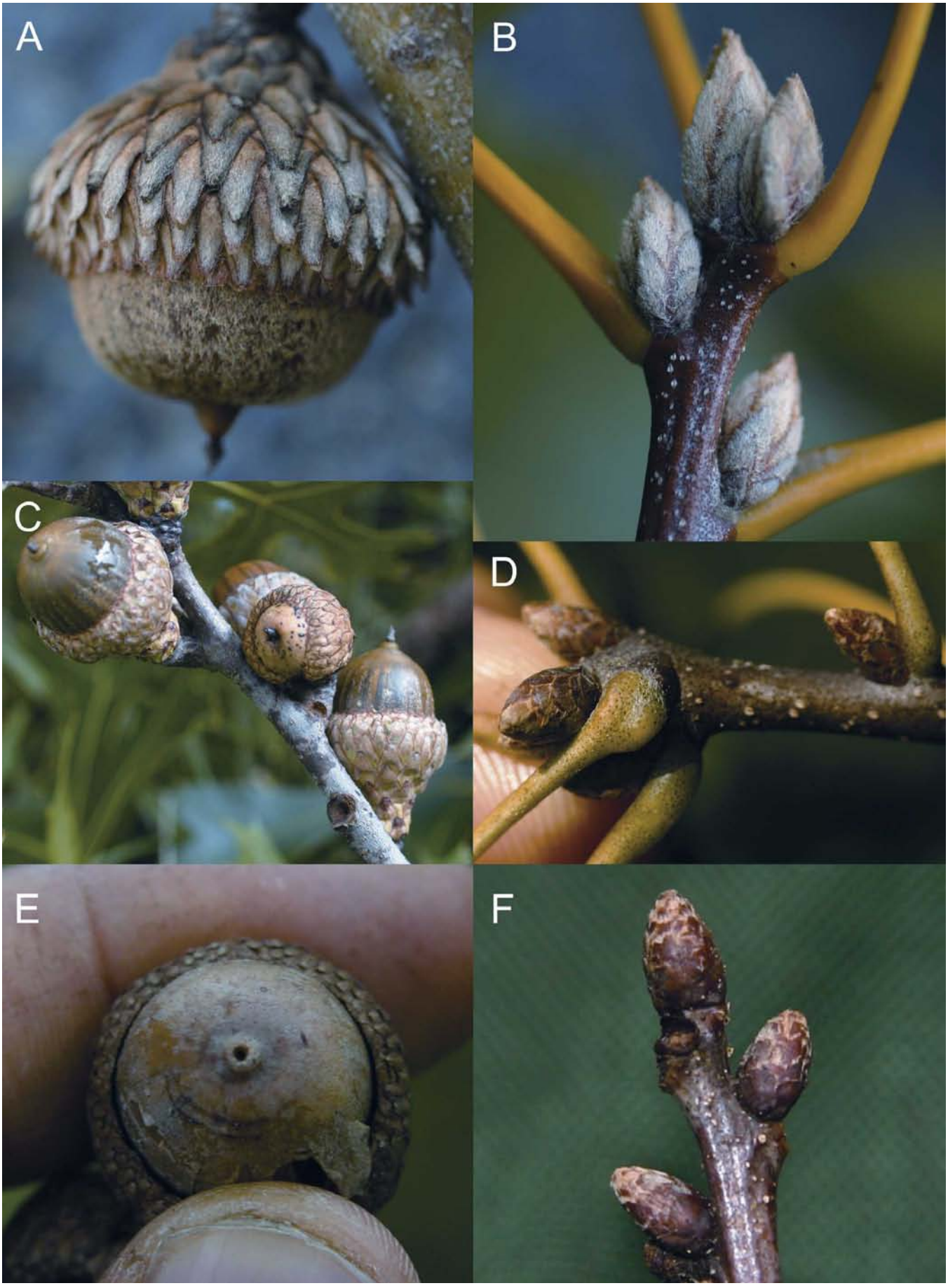
As an additional test of taxonomic hypotheses, molecular genetic structure was analyzed using the Bayesian clustering method of Pritchard et al. (2000) in STRUCTURE v 2.1 (http://pritch.bsd.uchicago.edu). The method uses Markov chain Monte Carlo (MCMC) to estimate allele frequencies and assign individuals to populations probabilistically, under the assumption that populations are at Hardy-Weinberg equilibrium and linkage equilibrium. Because AFLP loci were scored as dominant, each locus was coded as known for one copy and unknown (−9) for the other, and analyses were conducted under the "admixture" ancestral model with allele frequencies correlated among populations. The parameter for distribution of allele frequencies (λ) was estimated in five initial runs for each taxon separately with number of populations (K) set at 1, then set at the mean across runs ($\lambda = 0.6170$) for all remaining analyses. The Dirichlet parameter (α) for degree of admixture was estimated for each run. Prior geographic information was not employed.

The number of populations (K) was estimated using five independent runs for each value of K, with parameters estimated over 1,000,000 MCMC generations following a burn-in period of 100,000 generations. The number of populations was inferred over the interval $K = 1$ to $K = 6$ for six sets of individuals: (1) *Quercus coccinea* and *Q. ellipsoidalis* together, excluding putative hybrids; (2) *Quercus coccinea* and *Q. ellipsoidalis* together, including all putative hybrids; (3) *Q. ellipsoidalis* and *Q. velutina* together, including all putative hybrids; (4) *Q. velutina* alone; (5) *Quercus coccinea*, *Q. ellipsoidalis*, *Q. velutina*, and putative hybrids; and (6) the entire dataset of 153 individuals, which includes all individuals in the previous set plus *Q. palustris*, *Q. rubra*, and the 22 individuals that could not be positively identified and were excluded from the other analyses (see Methods, first paragraph). Although the sampling strategy was not designed to assess population genetic structure within taxa, analyses were conducted over $K = 1$ to $K = 6$ to evaluate whether there is molecular genetic structure within species.

Results

AFLP Data and Neighbor-Joining Analysis—Over the entire dataset of 153 individuals, 253 markers were scored. Markers ranged from 51–554 base pairs in length, including adapters (mean length 224.95 ± 7.17 [s.e.]). 238 markers were variable among all individuals, 146 within *Quercus coccinea*, 188 within *Q. ellipsoidalis*, and 201 within *Q. velutina*. The data matrix is available from the first author upon request.

In the neighbor-joining tree (Fig. 3), three taxa cluster cleanly: *Quercus coccinea*, *Q. palustris*, and *Q. rubra*. Of these, only the *Q. palustris* cluster has strong branch support (100%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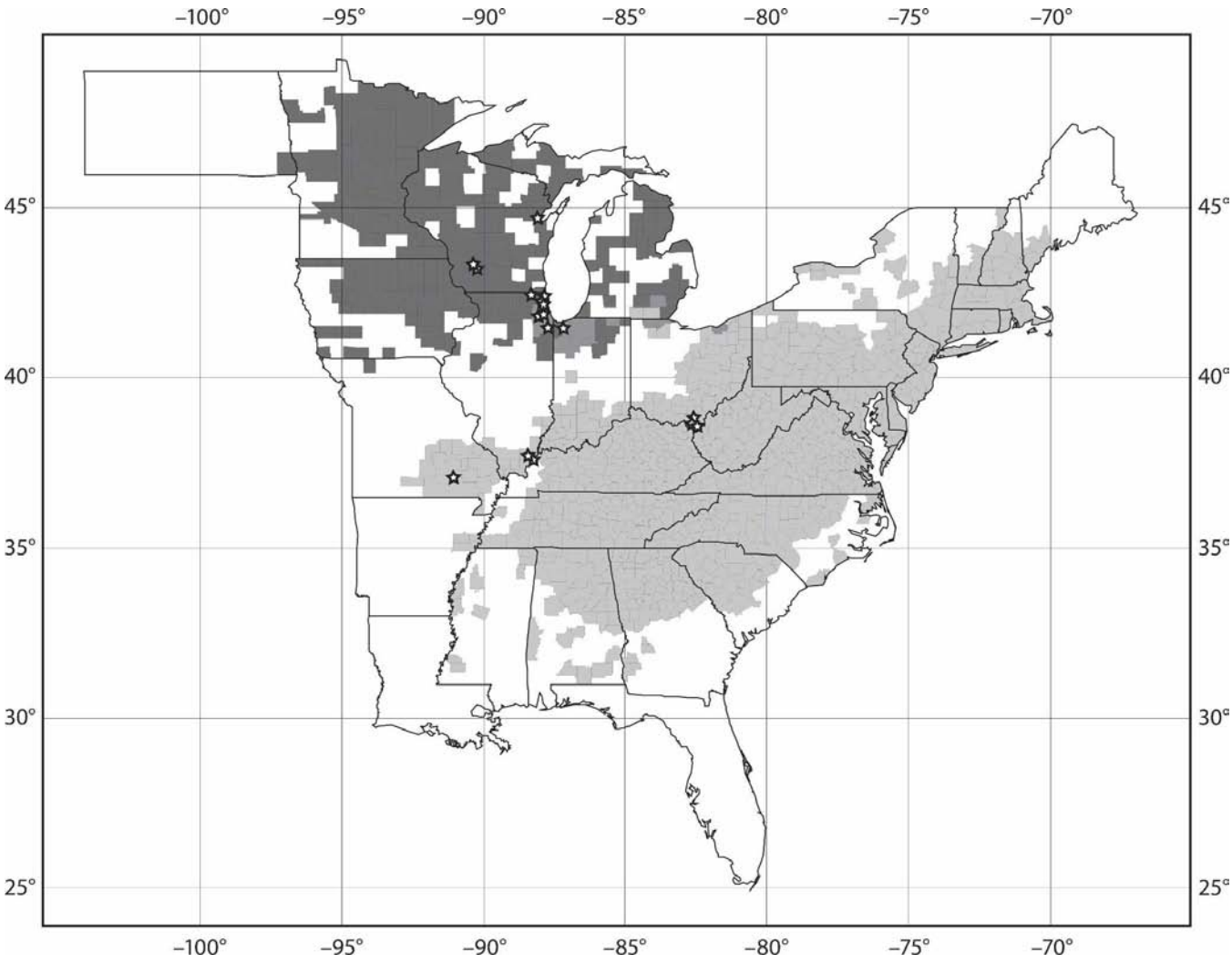

FIG. 2. Distribution map of *Quercus coccinea* and *Q. ellipsoidalis* in the United States, indicating sites at which *Q. coccinea*, *Q. ellipsoidalis*, and *Q. velutina* specimens were collected for this study. Ranges for *Q. ellipsoidalis* (dark grey) and *Q. coccinea* (light grey—counties in which both species are found are medium grey) are based on county distribution maps in Stein et al. (2003), which was based largely on printed sources. Modifications to distributions in these maps were made by reference to *Flora of North America* (Jensen 1997), which represents a relatively current understanding of the distribution of the species. Specimens at F and MOR were inspected to refine distributions for *Q. coccinea* in northeastern Illinois, and specimens at WIS and MICH were inspected to map the distribution of *Q. coccinea* in Wisconsin and Michigan respectively. As this map is based primarily on print sources rather than loans, it is not meant to imply a high degree of precision at the county level. Stars represent approximate collection localities; precise localities can be found in Appendix 1.

bootstrap support). *Quercus ellipsoidalis* and *Q. velutina* are intermixed with one another and form several clusters, making it difficult to infer interspecific relationships. We consequently do not attempt to do so in this study. One putative hybrid between *Q. ellipsoidalis* and *Q. velutina* pairs with *Q. ellipsoidalis*, three putative hybrids pair with *Q. velutina*, and one pairs with the *Q. palustris* cluster. Given the relatively clean clustering of *Q. palustris* and *Q. rubra* and our limited sampling of these species, the current study does not investigate the possible role of *Q. palustris* and *Q. rubra* in the taxonomy of *Q. ellipsoidalis*.

***Ordinations*—**Three ordinations are reported here: one involving all 120 individuals of *Quercus coccinea*, *Q. ellipsoidalis*, *Q. velutina*, and putative hybrids; one of *Q. ellipsoidalis* sepa-

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FIG. 1. Terminal bud and acorn characteristics of *Quercus velutina*, *Q. ellipsoidalis*, and *Q. coccinea*. All photos by A. Hipp of trees growing spontaneously at Taltree Arboretum in Valparaiso, Indiana, U.S.A., September 2006 (A–D) or Albany Pine Bush Preserve, Albany, New York, U.S.A., June 2006 (E, F). Vouchers (collector numbers indicated) are deposited at MOR. A, B. *Quercus velutina* (black oak; Hipp & Hitz TAL13). A. Acorn, showing loose, fringed cap scales. The pubescence on the surface of this acorn is likely to wear off, but the pubescence on the inner surface of the cap (not shown) persists. B. Terminal buds, displaying their typical dense pubescence and angular cross-section. C, D. *Quercus ellipsoidalis* (Hill's oak). C. Acorns, showing the tightly imbricated scales that distinguish the species from *Q. velutina*. Striations on the acorn body are not uncommon in *Q. ellipsoidalis*, but also not the rule. Acorn shape in *Q. ellipsoidalis* is highly variable (Hipp & Hitz TAL9). D. Terminal buds, which are much smaller than *Q. velutina*, typically glabrous to silky-pubescent on the distal half, and not angled in cross-section (Hipp & Hitz TAL1). E, F. *Quercus coccinea* (scarlet oak; population represented by Hipp 2757–2759, 2761–2772). E. Acorn nut, stylar end, showing the concentric rings of pits typical of the species. *Quercus coccinea* shows considerable variability in this characteristic, ranging from pitting absent to pits forming three or more concentric rings. F. Terminal buds, which are similar to those of *Q. ellipsoidalis* but frequently more pubescent.

TABLE 1. Variation in 20 AFLP primer pairs screened. Markers were screened on eight individuals representing *Q. coccinea*, *Q. ellipsoidalis*, and *Q. velutina*. Potentially informative markers were defined as markers that were present in at least two and at most six screened individuals.

	Mean No. Bands per Individual	Markers Scored	Markers Polymorphic	Markers Potentially Informative	Maximum Marker Length (bp)	Mean Marker Length
EcoRI-ACG / MseI-CTC	106.4	249	194	157	622	248.1
EcoRI-AGC / MseI-CTC	129.3	225	162	121	621	239.4
EcoRI-AGA / MseI-CAA	125.5	215	151	108	571	223.6
EcoRI-ACG / MseI-CGC	140.8	208	145	126	562	210.3
EcoRI-AGC / MseI-CCC	99.1	183	144	109	608	220.8
EcoRI-ACG / MseI-CTG	125.5	205	141	105	572	236.0
EcoRI-AGC / MseI-CTT	138.9	210	137	116	598	219.3
EcoRI-ATG / MseI-CAA	119.5	187	130	105	620	206.4
EcoRI-AGC / MseI-CTA	96.4	170	130	107	619	221.5
EcoRI-ACG / MseI-CCA	108.3	178	128	96	553	213.4
EcoRI-AGC / MseI-CCT	94.4	159	127	107	624	210.3
EcoRI-ACG / MseI-CTT	110.0	167	121	101	610	190.7
EcoRI-AGA / MseI-CTA	78.8	148	111	77	601	213.3
EcoRI-AGA / MseI-CGC	106.4	161	108	85	611	200.6
EcoRI-ATG / MseI-CCG	64.1	117	104	84	514	161.7
EcoRI-ATG / MseI-CAT	95.4	153	95	66	615	185.1
EcoRI-AGC / MseI-CAC	71.0	126	93	61	571	200.6
EcoRI-ATG / MseI-CTC	73.9	127	92	67	622	228.2
EcoRI-AGA / MseI-CAC	63.5	108	80	60	593	210.2
EcoRI-ACG / MseI-CAT	95.1	133	78	65	621	188.6

rately, excluding all putative hybrids; and one of *Q. velutina* separately, also excluding putative hybrids. An additional ordination of *Q. ellipsoidalis*, *Q. velutina*, and putative hybrids was performed, but this ordination does not affect conclusions based on the ordination including all three taxa and is consequently not shown. In the all-taxa ordination (Fig. 4), *Q. coccinea* separates cleanly from both other taxa with the exception of a putative *Q. coccinea* collected in northwestern Indiana (TAG-027). This individual represents the only putative *Q. coccinea* sampled from within the range of *Q. ellipsoidalis*, and it clusters with *Q. ellipsoidalis*. The other two taxa, *Q. ellipsoidalis* and *Q. velutina*, separate from one another with some overlap: two *Q. velutina* accessions (2540, TAG-028) fall within the *Q. ellipsoidalis* cluster, and one *Q. ellipsoidalis* accession (MORCOC2) falls within the *Q. velutina* cluster. One of these individuals (*Q. velutina* 2540) derives from a population that appears to represent a hybrid zone between *Q. ellipsoidalis* and *Q. velutina*.

Individuals of a given species collected at a given site do not cluster tightly with one another (Figs. 5, 6). In *Quercus ellipsoidalis*, for example, accessions from Middlefork Savanna Forest Preserve cover nearly the entire extent of the ordination (Fig. 5). However, there does appear to be some correlation between geography and molecular variation: although individuals do not fall within tight population clusters, many sites form loose clusters of individuals that intermix with individuals from a limited number of other sites. In *Q. ellipsoidalis*, the more obvious examples include Governor's State University (GSU), Greenbelt Forest Preserve, Taltree Arboretum, and Somme Prairie Nature Preserve (Fig. 5). Geographic structure appears to be weak even when regions rather than sites are the focus of analysis. The Wisconsin collections of *Q. velutina*, for example, do not all fall near one another, although they do fall along the outer edges of the ordination (Fig. 6). The southern Illinois and Ohio accessions of *Q. velutina* are particularly notable in this regard, embedded as they are among northeastern Illinois and northwestern Indiana accessions (Fig. 6). This stands in strong contrast to interspecific comparisons at the same geographic scale: whereas *Q. velutina* accessions from different regions are intermixed, upper Midwest *Q. ellipsoidalis* accessions separate cleanly from southern Illinois and Ohio *Q. coccinea* accessions (Fig. 4).

Bayesian Analysis of Population Genetic Structure—The Bayesian analyses conducted estimate the proportion of an individual's genome that can be attributed to each of K source populations (Pritchard et al. 2000). At $K = 3$, the predominant ancestry of most *Quercus coccinea*, *Q. ellipsoidalis*, and *Q. velutina* individuals corresponds to our (morphological) species identifications. The exceptions are three *Q. velutina* collections (2540, TAG-028, JW66) and one putative *Q. coccinea* (TAG-027), all of which have substantial *Q. ellipsoidalis* ancestry (Fig. 7A). More morphological *Q. velutina* than *Q. ellipsoidalis* individuals display mixed ancestry. The *Q. coccinea* individuals from southern Illinois, southern Ohio, and Missouri show negligible evidence of mixed ancestry. Surprisingly, the $K = 4$ model has the highest support (posterior probability = 1.00). Under analyses conducted at $K = 4$, eleven *Q. ellipsoidalis* (2431, 2529, 2531, Cook Co., Illinois; MORCOC2, DuPage Co., Illinois; 2482, 2550, 2567, 2573 Lake Co., Illinois; TAL1, TAL3, Porter Co., Indiana; JW49, Brown Co., Wisconsin), seven *Q. velutina* (JW26, JW37, JW38, Richland Co., Wisconsin; JW60, JW66, Brown Co., Wisconsin; MORELL3, from seed collected in Rock Co., Wisconsin; TAG-028, Porter Co., Illinois), and one putative *Q. coccinea* (TAG-027, Porter Co., Indiana) have dominant ancestry from a fourth population (Fig. 7B). Three *Q. ellipsoidalis* (2483, JS15, Lake Co., Illinois; TAG-010, Porter Co., Indiana) and one *Q. velutina* (2526, Cook Co., Illinois) are admixed approximately equally between their nominal populations and this fourth population. The fourth population comprises a high percentage of the Wisconsin and Indiana collections. Assuming $K = 4$ may have the effect of picking up geographic structure that is not evident when only three populations (representing the three taxa) are assumed. No individual having > 80% *Q. velutina* ancestry at $K = 3$ shows appreciable membership in this fourth population.

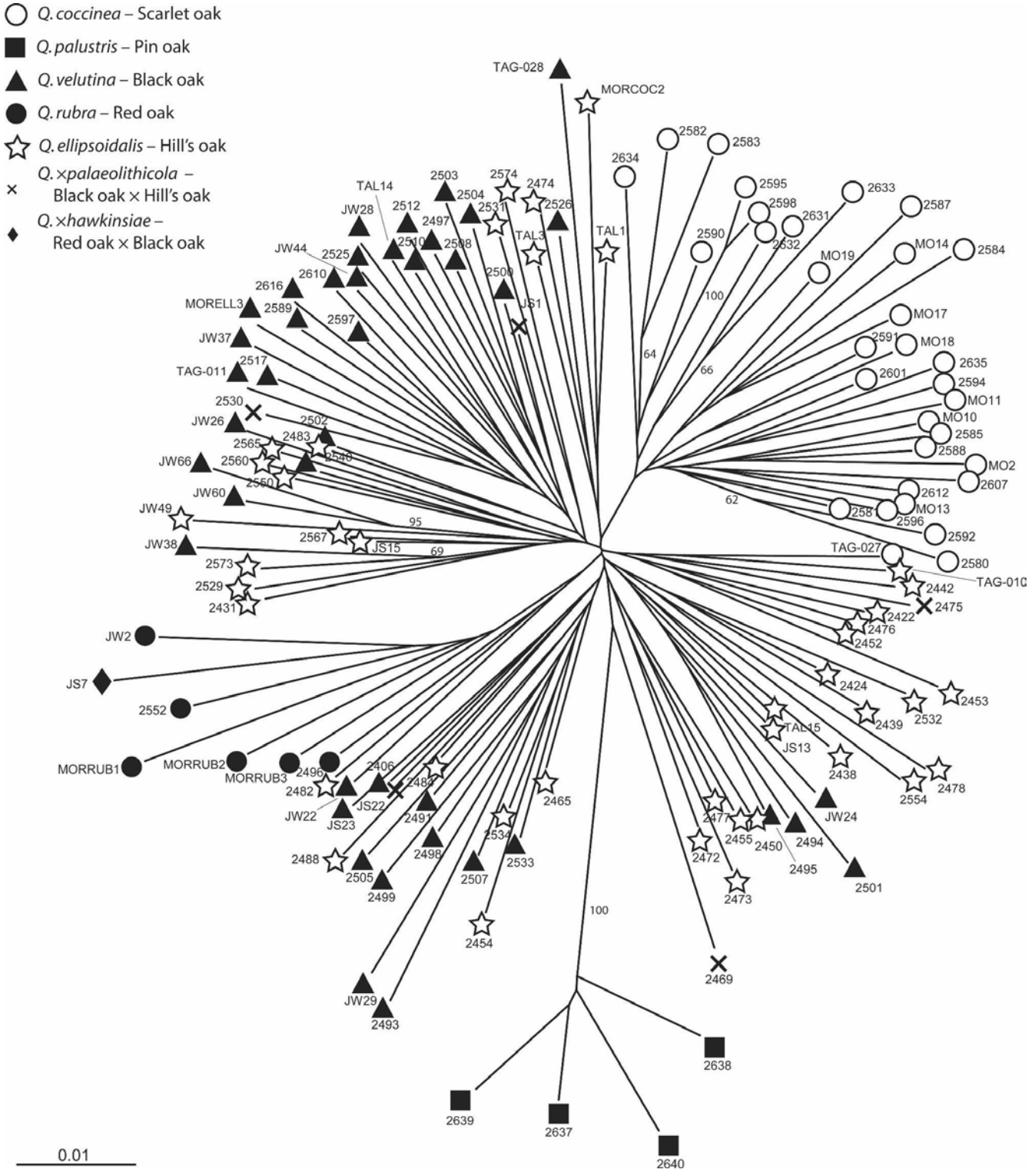

FIG. 3. Neighbor joining tree of 131 individuals representing *Quercus coccinea*, *Q. ellipsoidalis*, *Q. velutina*, *Q. ellipsoidalis* × *Q. velutina* [*Q.* ×*palaeolithicola*], *Q. palustris*, *Q. rubra, and Q. rubra* × *Q. velutina* [*Q.* ×*hawkinsiae*]. Neighbor joining was conducted on a pairwise distance matrix calculated using Nei and Li's (1979) restriction site method. Branch support was estimated using 1,000 neighbor joining nonparametric bootstrap replicates; only bootstrap support values > 50% are shown. Individual labels are voucher numbers reported in Appendix 1.

Subsets of the individuals above were also analyzed (no figures). Analysis of *Quercus coccinea* and *Q. ellipsoidalis* together supports recognition of only two populations (posterior probability of $K = 2$ is 0.9986) with all individuals except the putative *Q. coccinea* from northwestern Indiana (TAG-027) clustering according to a priori identifications. Analyzed alone, *Q. velutina* gives no indication of geographic differentiation (posterior probability of $K = 1$ is 0.7109). Assuming additional populations ($K > 1$) results in relatively even admixture of all individuals, with no separation by populations or by geographic region. *Quercus ellipsoidalis*, *Q. velutina*, and putative hybrids analyzed together support $K = 5$ popula-

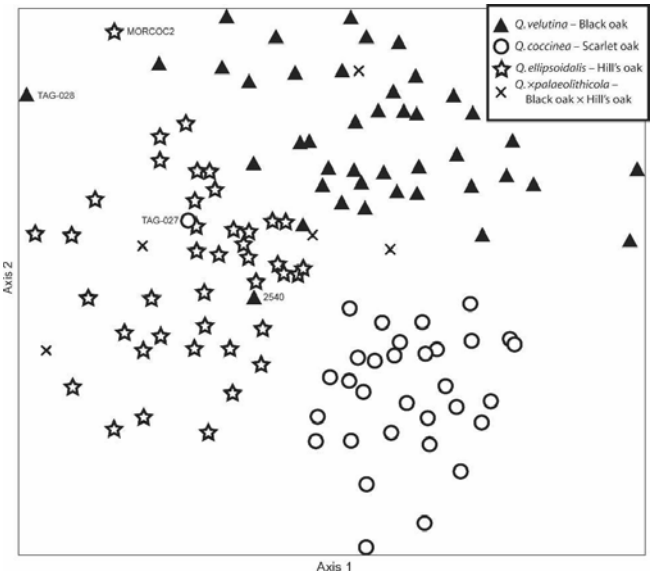

FIG. 4. Two-dimensional ordination of 120 individuals representing *Quercus coccinea*, *Q. ellipsoidalis*, *Q. velutina*, and *Q. ellipsoidalis* × *Q. velutina* [*Q.* ×*palaeolithicola*]. Ordination was conducted using nonmetric dimensional scaling on a pairwise distance matrix calculated using Jaccard's (1908) similarity index. Final stress was reached after approximately 65 iterations. Final stress = 30.40305, final instability = 0.01349, number of iterations = 100. R^2 = 0.216 (axis 1) + 0.287 (axis 2) = 0.503.

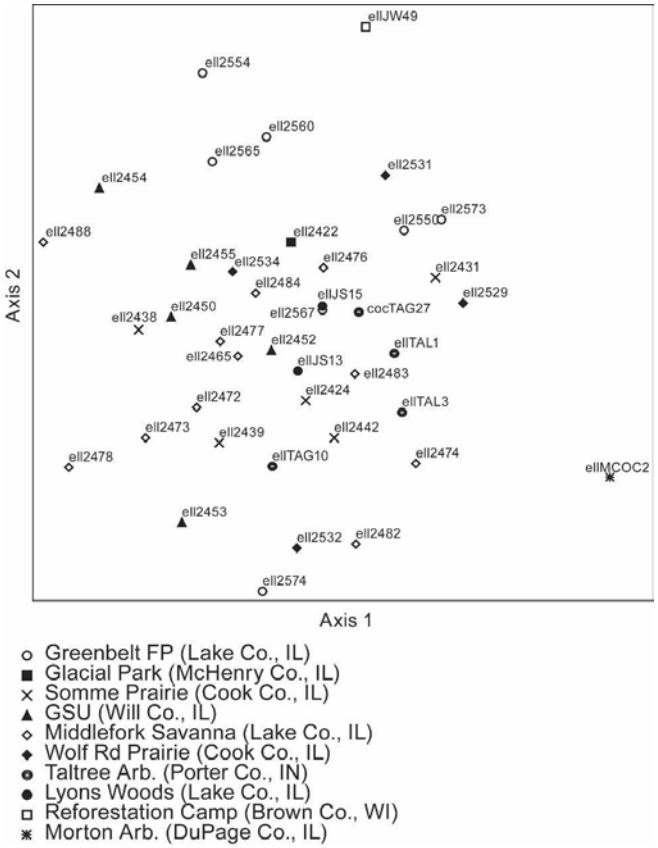

FIG. 5. Two-dimensional ordination of 34 *Quercus ellipsoidalis* individuals, with collecting sites overlaid. Ordination was conducted using nonmetric dimensional scaling. Final stress was reached after approximately 70 iterations. One putative *Q. coccinea* accession from northwestern Indiana (TAG-027) is included in this analysis because it clusters with *Q. ellipsoidalis* in all analyses. Final stress = 29.23082, final instability = 0.00323, number of iterations = 400. R^2 = 0.306 (axis 1) + 0.233 (axis 2) = 0.539.

tions (posterior probability = 1.00). Two of the inferred population types are found predominantly in *Q. ellipsoidalis*, two predominantly in *Q. velutina*, and one is found across both taxa.

In analysis of all 153 individuals (including all five taxa and the 22 individuals that had inadequate material for a priori identification), the six-population model was most strongly supported (posterior probability = 1.00; figure not shown). Beginning at $K = 1$, assuming additional populations has the effect of successively distinguishing additional species: *Quercus coccinea* forms a cluster separate from all other taxa at $K = 2$, *Q. ellipsoidalis* separates out at $K = 3$, then *Q. palustris* forms a cluster at $K = 4$ and *Q. rubra* forms a cluster at $K = 6$. At $K = 5$ and $K = 6$, a population is inferred that includes several *Q. ellipsoidalis* and *Q. velutina*. This population is essentially the same as the putative fourth population of the *Q. coccinea*-*Q. ellipsoidalis*-*Q. velutina* analysis with the addition of eight of the individuals not included in previous analyses.

DISCUSSION

Interpreting Genetic Divergence Between Quercus coccinea and Q. ellipsoidalis—All analyses demonstrate a strong separation of *Quercus coccinea* from the other taxa investigated, much stronger than the separation between *Q. ellipsoidalis* and *Q. velutina* (Figs. 3, 4, 7). The divergence between *Q. coccinea* and *Q. ellipsoidalis* must be explained either as divergence between two taxa or as genetic divergence within a single, wide-ranging species. Although geographic distance may play some role in the strong separation between these two species—the *Q. coccinea* and *Q. ellipsoidalis* populations closest to one another in our molecular study are separated by more than 400 km—the fact that region and population explain so little of the genetic clustering in *Q. velutina* across the same geographic range (Fig. 6) suggests that long-distance gene flow is common in this species group (cf. Dow and Ashley 1998) and that geographic distance alone does not account for the genetic divergence observed. However, the apparent lack of genetic differentiation between *Q. velutina* accessions of northern Illinois, Indiana, and Wisconsin on one hand and southern Illinois and Ohio on the other may be due in part to the fact that *Q. velutina* ranges nearly continuously across the extent of our study area: geographically distant populations may be genetically similar because alleles migrate between them via geographically proximate populations. In contrast, *Q. coccinea* and *Q. ellipsoidalis* are both absent from central Illinois, central Indiana, and western Ohio (Fig. 2). It might be argued, then, that if in fact *Q. coccinea* and *Q. ellipsoidalis* represent a single, wide-ranging species, and if populations exchange pollen and seed primarily with close neighbors (Sork et al. 1993, 2002; Dutech et al. 2005), the populations sampled are genetically divergent from one another simply because they lie near the endpoints of an approximately arc-shaped species distribution. We find this explanation dissatisfying, because if it were the case, we would expect to see some clinal variation between the two in our data. For example, the northeastern Illinois and northwestern Indiana populations of *Q. ellipsoidalis* should show more evidence of introgression from *Q. coccinea* than the Wisconsin populations do. As we have shown, our data demonstrate no such variation. Moreover, it would be surprising to find a

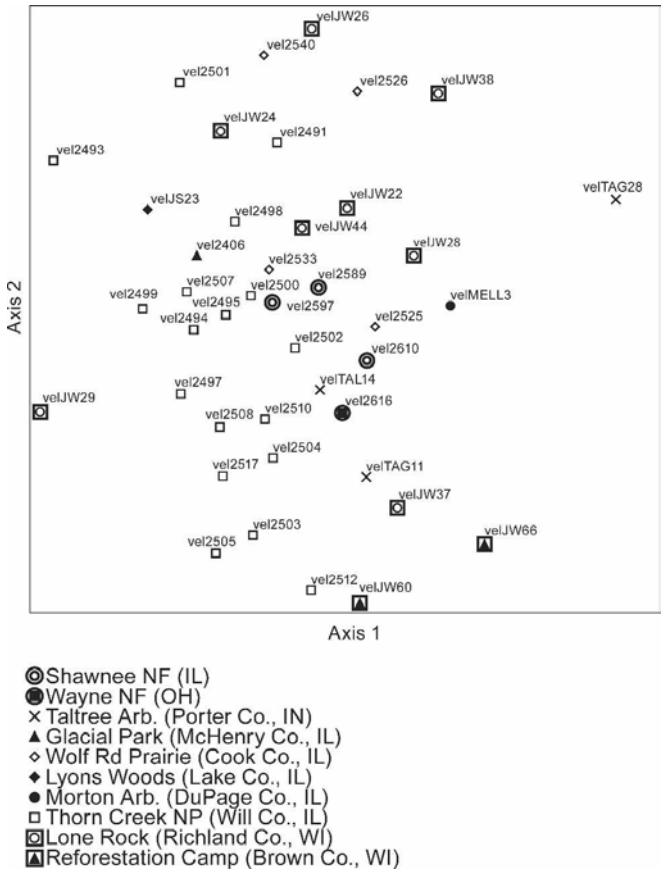

FIG. 6. Two-dimensional ordination of 44 *Quercus velutina* individuals, with collecting sites overlaid. Ordination was conducted using nonmetric dimensional scaling. Final stress was reached after approximately 60 iterations. Final stress = 28.02297, final instability = 0.00026, number of iterations = 400. R^2 = 0.403 (axis 1) + 0.165 (axis 2) = 0.568.

very close relationship between *Q. ellipsoidalis* and *Q. velutina*, but an equally distant relationship between both of these species and *Q. coccinea*, if in fact *Q. coccinea* and *Q. ellipsoidalis* were genetically cohesive (i.e. a single species). We consequently conclude that *Q. coccinea* and *Q. ellipsoidalis* are taxonomically distinct from one another. To what degree the similarity between *Q. ellipsoidalis* and *Q. velutina* is a consequence of recent taxonomic divergence versus introgression remains to be seen.

Identity of the Northeastern Illinois Oaks—Our study includes specimens from the three northeastern Illinois counties from which scarlet oak has been reported (Fig. 2). We have inspected all specimens at F and MOR on which these reports are based, and several have one ring or, less commonly, two concentric rings of pits at the stylar end of the acorn. These rings are mostly solitary, 2.75–3.5 (–5 mm) in diameter. In studying specimens from Wisconsin and northwestern Illinois, we find that this is not outside the morphological range for *Q. ellipsoidalis*. Moreover, these specimens for the most part do not have the strongly convex, glossy acorn cap scales typical of scarlet oak. We sampled two of the northeastern Illinois populations from which *Q. coccinea* has been reported (The Morton Arboretum and Governors State University) as well as a third population on which we found individuals with rings of pits at the stylar end of the acorn (Somme Prairie), and while *Q. ellipsoidalis* and *Q. velutina* were found at all three sites, we could not locate any *Q. coccinea*. Three specimens inspected at F from the Calumet area of southern Cook County, Illinois bear similarity to *Q. coccinea*, and we will be sampling the population they represent as part of a study of the distribution of *Q. coccinea* in the Chicago region. We conclude that *Q. coccinea* is at best quite rare in northeastern Illinois and leave open the possibility that it may be present in restricted areas of southern Cook County.

The one definitive scarlet oak population in northeastern Illinois is located at Tinley Creek Forest Preserve in southern Cook County, growing in a stand with *Liquidambar styraciflua* L. (sweetgum), *Tilia americana* L. var. *heterophylla* (Vent.) Loud. (white basswood), *Quercus shumardii* Buckley (Shumard's oak), *Q. lyrata* Walter (overcup oak), and *Q. montana* Willd. (chestnut oak), all species of more southern hardwood forests that are well outside their natural range in northeastern Illinois (Shepard 2005). There is substantial reason to believe that this stand is planted rather than indigenous (M. Bowles, G. Ware, pers. comm. 2006; W. Vanderploeg, pers. comm. 2007), and we did not sample from this *Q. coccinea* population for the current study.

Discrepancy Between Morphological and Molecular Estimates of Admixture—Many oaks we have inspected from herbarium specimens collected in northwestern Indiana show aspects of the morphology of *Quercus coccinea*, but often not the pitting at the stylar end of the acorn. *Quercus coccinea* and *Q. ellipsoidalis* are particularly difficult to distinguish from one another in northwestern Indiana, where they are believed to cooccur (Overlease 1977; Jensen 1986). Moreover, there is morphological discontinuity between *Q. ellipsoidalis* of northern Indiana and adjacent northeastern Illinois, which has been explained as a possible consequence of introgression between *Q. coccinea* and *Q. ellipsoidalis* in northern Indiana only (Jensen 1986). For this study, we sampled one oak population from northwestern Indiana (Taltree Arboretum), including a single individual that bears the morphological key characteristics of *Q. coccinea* (TAG-027). The acorns from this specimen have the characteristic pitting at the stylar end and glossy, convex / tuberculate cap scales that appear to be typical of scarlet oak (Fig. 1). These facts not withstanding, specimen TAG-027 clusters with *Q. ellipsoidalis* in all analyses (Figs. 3, 4, 7).

We have ruled out laboratory and field error as explanations for this result by replicating the AFLP reactions from specimens collected twice in two different years from this same individual. There are at least three alternative explanations. First, the northwestern Indiana population we sampled might be intermediate between typical *Quercus coccinea* and typical *Q. ellipsoidalis*, and these two might represent endpoints of a genetic (and morphological) continuum. As we have argued in the previous section, our data do not support this explanation. The second possible explanation is that hybridization between *Q. coccinea* and *Q. ellipsoidalis* with subsequent backcrossing to *Q. ellipsoidalis* has produced plants that have the morphology of *Q. coccinea* but AFLP genotypes more similar to *Q. ellipsoidalis*. This result might be expected if the *Q. coccinea* phenotype is under positive selection, but *Q. ellipsoidalis* is more abundant on the landscape or otherwise has more opportunity to pollinate. Such a phenomenon of asymmetric gene flow has been demonstrated, for example, in *Populus* L. (Lexer et al. 2005). The third possible explanation is that *Q. coccinea* is absent from the upper Midwest, but that the morphological variation in *Q. ellipsoidalis* in north-

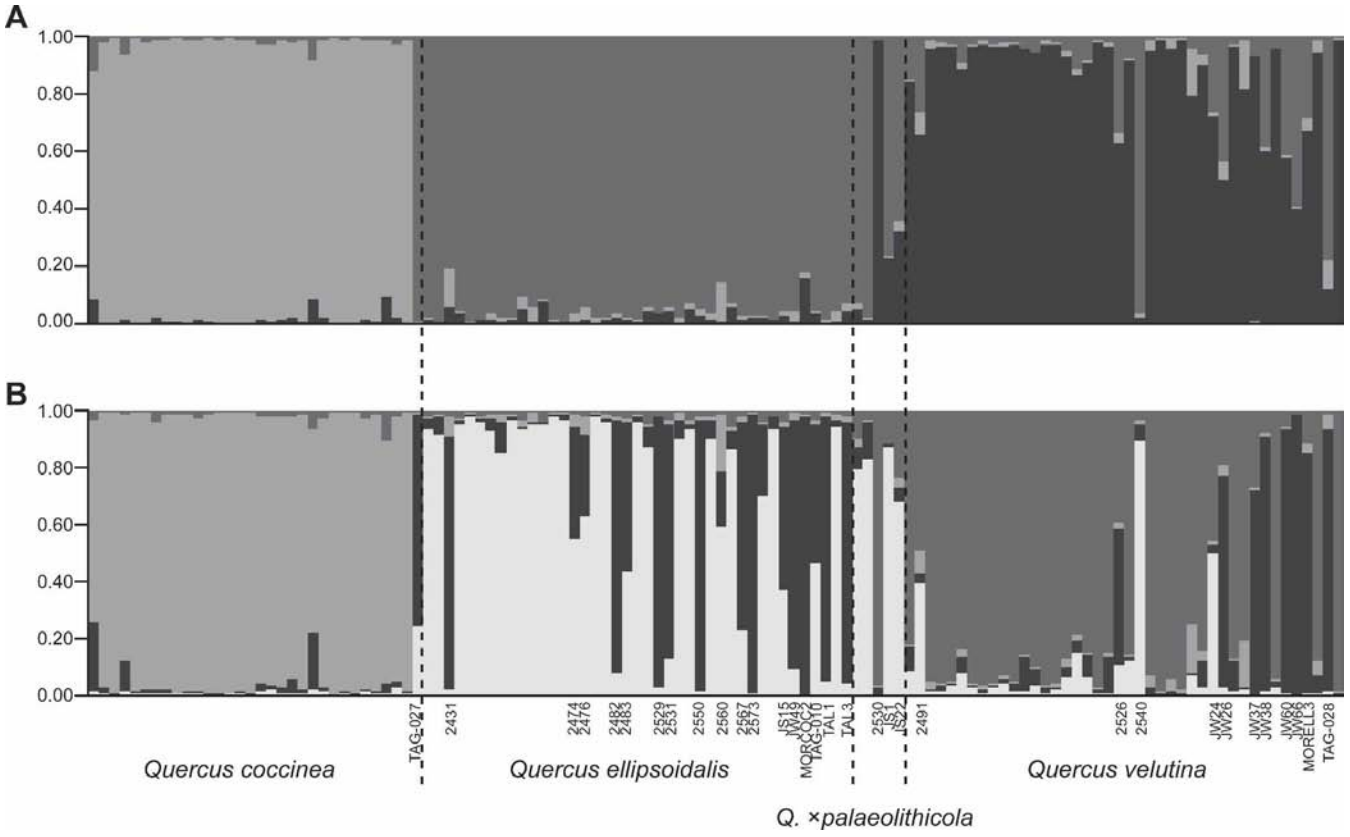

FIG. 7. Bayesian clustering of 120 individuals representing *Quercus coccinea*, *Q. ellipsoidalis*, *Q. velutina*, and *Q. ellipsoidalis* × *Q. velutina* [*Q.* ×*palaeolithicola*]. Clustering was performed using the method of Pritchard et al. (2000). Run parameters utilized the "admixture" model with allele frequencies correlated between populations. A single admixture parameter (α) was inferred for all populations and the parameter for allele frequency correlation (λ) was set at 0.6170 for all runs based on initial trials. Panel A: Population genetic structure assuming $K = 3$ (posterior probability = 1.29×10^{-29}). Panel B: Population genetic structure assuming $K = 4$ (posterior probability = 1.00).

western Indiana encompasses the typical morphology of scarlet oak. In this case, correctly identifying TAG-027 and specimens like it will require clarifying the morphological characters used to distinguish *Q. coccinea* from *Q. ellipsoidalis*. More intensive sampling in northern Indiana and southern Michigan populations where *Q. ellipsoidalis* and *Q. coccinea* are thought to cooccur is needed to test these alternative hypotheses.

While *Quercus ellipsoidalis* as identified based on morphological characters separates cleanly in all analyses, several *Q. velutina* sampled exhibit substantial genetic admixture or cluster with *Q. ellipsoidalis*. These individuals were all collected from sites at which *Q. velutina* and *Q. ellipsoidalis* grow together. Putative hybrids based on morphological data for the most part do not exhibit admixture. Our analyses did not use reference samples of assumed pure *Q. ellipsoidalis* and pure *Q. velutina*, and consequently our admixture estimates may be biased (Pritchard et al. 2000; Dodd and Afzal-Rafii 2004). However, none of the southern Illinois or southern Ohio collections of *Q. coccinea* shows significant admixture with *Q. ellipsoidalis*, and the Thorn Creek Nature Preserve population, which is composed almost exclusively of *Q. velutina*, also shows no evidence of admixture. This suggests that our finding of admixture in many populations at which both *Q. ellipsoidalis* and *Q. velutina* are found is not spurious. Other researchers have found similar discrepancy between morphological and molecular estimates of admixture. Using Random Amplified Polymorphic DNA (RAPD) markers, González-Rodríguez et al. (2004) found more individuals genetically intermediate than morphologically intermediate in a hybrid zone of *Quercus affinis* Scheidw. and *Q. laurina* Bonpl. In some populations, individuals that were genetically either intermediate or more similar to *Q. affinis* resembled *Q. laurina* morphologically. Craft et al. (2002) found that morphological intermediates between *Q. douglasii* Hook. & Arn. and *Q. lobata* Née were unlikely to be of mixed ancestry based on microsatellite data. Conversely, only one of four individuals with the highest likelihood of hybrid ancestry was morphologically intermediate.

Although F_1 hybrids can often be identified by character intermediacy (Estabrook et al. 1996, though see Kleinschmit et al. 1995), hybrids in the second generation and later may fall anywhere along a phenotypic continuum between the two parents and often resemble one parent only (Stebbins 1950; Rieseberg and Ellstrand 1993). Our findings suggest that many putative hybrids are late-generation backcrosses toward *Quercus ellipsoidalis*, and that *Q. ellipsoidalis* readily introgresses into *Q. velutina*. This would be expected if (1) *Q. ellipsoidalis* has a larger effective population size or is otherwise more effective at dispersing pollen or acorns or both, and (2) *Q. velutina* phenotypic characters are more strongly selected than Hill's oak characters. Additional study will be needed to evaluate these hypotheses.

Correlations With Prior Studies—Our conclusions regarding the taxonomic distinctness of *Quercus coccinea* and *Q. ellipsoidalis* stand at odds with the conclusions of two previous morphological studies, which suggest that these names represent variants of a single, wide-ranging species (Over-

lease 1977; Shepard 1991, 1993). Jensen (1986) argued that Overlease's conclusion that northern Indiana populations are intermediate between *Q. ellipsoidalis* to the north and *Q. coccinea* to the south may rest on analysis of mixed populations in northern Indiana. Shepard's studies have not been published in whole (Shepard 1993 is a thesis; Shepard 1991 is an abstract), and consequently his work is difficult to review in detail. However, the principal components analyses (PCA) of morphological data presented in the thesis show a very close relationship between *Q. coccinea* of Shawnee National Forest (southern Illinois) and the *Q. coccinea* population of Tinley Creek Forest Preserve (southern Cook County, northeastern Illinois), and a more distant relationship with the *Q. ellipsoidalis* populations of northeastern Illinois. This finding is compatible with the findings of our study, although our study shows stronger divergence between the two species than Shepard's does.

We interpret the strong difference in population genetic structure within *Quercus velutina* on one hand and between *Q. coccinea* and *Q. ellipsoidalis* on the other as evidence that *Q. coccinea* and *Q. ellipsoidalis* are not simply variants of a single, wide-ranging species. Further study is needed to rule out the possibility that the difference in species ranges may be the cause of this difference. Based on our findings, we support recognizing *Q. coccinea* and *Q. ellipsoidalis* as distinct species.

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APPENDIX 1. Species names, collector numbers, and population locations for specimens used in molecular analyses. All vouchers are deposited at MOR. Second specimens were collected in autumn for most Northeastern Illinois vouchers, and collector numbers for these latter collections are referenced on specimen labels.

***Quercus coccinea* Münchh.** *Hipp & Hitz TAG-027,* Taltree Arboretum, Porter Co., IN. *Hipp & Kirschbaum 2631,* Wayne National Forest, Lawrence Co., OH. *Hipp & Kirschbaum 2632–2635,* Lake Vesuvius Recreation Area, Lawrence Co., OH. *Hipp & Schlismann 2580–2585, 2587, 2588, 2590–2592, 2594–2596, 2598, 2601, 2607, 2612,* Shawnee National Forest, Gallatin Co., IL. *Wibbenmeyer 192 & 193 (representing MO2, MO10, MO11, MO13, MO14, MO17–MO19)* Chilton Creek Preserve area, Carter Co., MO. ***Quercus ellipsoidalis* E.J. Hill.** *Hipp 2424, 2431, 2438, 2439, 2442,* Somme Prairie Nature Preserve, Cook Co., IL. *Hipp & Hitz TAL1, TAL3, TAL15, TAG-010,* Taltree Arboretum, Porter Co., IN. *Hipp & Schlismann 2465, 2472–2474, 2476–2478, 2482–2484, 2488,* Middlefork Savanna Forest Preserve, Lake Co., IL. *Hipp & Weber 2422,* Glacial Park Nature Preserve, McHenry Co., IL. *Hipp & Weber 2450, 2452–2455,* Governors State University, Will Co., IL. *Hipp & Weber 2550, 2554, 2560, 2565, 2567, 2573, 2574,* Greenbelt Forest Preserve, Lake Co., IL. *Hipp & Weber MORCOC2,* The Morton Arboretum, DuPage Co., IL. *Hipp & Weber 2529, 2531, 2532, 2534,* Wolf Road Prairie Nature Preserve, Cook Co., IL. *Schlismann et al. JS13, JS15,* Lyons Woods Forest Preserve, Lake Co., IL. *Weber & Weber JW49,* Reforestation Camp, Brown Co., WI. ***Quercus ellipsoidalis* × *Q. velutina* (= *Q.* ×*palaeolithicola* Trelease).** *Hipp & Schlismann 2469, 2475,* Middlefork Savanna Forest Preserve, Lake Co., IL. *Hipp & Weber 2530,* Wolf Road Prairie Nature Preserve, Cook Co., IL. *Schlismann et al. JS1, JS22,* Lyons Woods Forest Preserve, Lake Co., IL. ***Quercus palustris* Münchh.** *Hipp 2637–2640,* Avon Lake, Lorain Co., OH. ***Quercus rubra* L.** *Hipp et al. 2496,* Thorn Creek Woods Nature Preserve, Will Co., IL. *Hipp & Weber 2552,* Greenbelt Forest Preserve, Lake Co., IL. *Hipp & Weber MORRUB1–MORRUB3,* The Morton Arboretum, DuPage Co., IL. *Weber & Weber JW2,* SE of Richland Center, Richland Co., WI. ***Quercus rubra* × *Quercus velutina* (= *Q.* ×*hawkinsiae* Sudw.).** *Schlismann et al. JS7,* Lyons Woods Forest Preserve, Lake Co., IL. ***Quercus velutina* Lam.** *Hipp & Hitz TAL14, TAG-011, TAG-028,* Taltree Arboretum, Porter Co., IN. *Hipp et al. 2491, 2493–2495, 2497–2505, 2507, 2508, 2510, 2512, 2517,* Thorn Creek Woods Nature Preserve, Will Co., IL. *Hipp & Kirschbaum 2616,* Wayne National Forest, Lawrence Co., OH. *Hipp & Schlismann 2589, 2597, 2610,* Shawnee National Forest, Gallatin Co., IL. *Hipp & Weber 2406,* Glacial Park Nature Preserve, McHenry Co., IL. *Hipp & Weber 2525, 2526, 2533, 2540,* Wolf Road Prairie Nature Preserve, Cook Co., IL. *Schlismann et al. JS23,* Lyons Woods Forest Preserve, Lake Co., IL. *Weber & Weber JW22, JW24, JW26, JW28, JW29, JW37, JW38, JW44,* Lone Rock Unit – Lower Wisconsin State Riverway, Richland Co., WI. *Weber & Weber JW60, JW66,* Reforestation Camp, Brown Co., WI. *Hipp & Weber MORELL3,* The Morton Arboretum, DuPage Co., IL.