

THE EVOLUTION OF DIOECY, HETERODICHOGAMY, AND LABILE SEX EXPRESSION IN *ACER*

S. S. Renner,^{1,2} L. Beenken,¹ G. W. Grimm,³ A. Kocyan,¹ and R. E. Ricklefs⁴

¹Department of Biology, University of Munich, Menzinger Str. 67, D-80638 Munich, Germany

²E-mail: renner@lrz.uni-muenchen.de

³Institute of Geosciences, University of Tübingen, Sigwartstr. 10, D-72076 Tübingen, Germany;

⁴Department of Biology, University of Missouri-St. Louis, 8001 Natural Bridge Rd., St. Louis, MO 63121, USA

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The northern hemisphere tree genus *Acer* comprises 124 species, most of them monoecious, but 13 dioecious. The monoecious species flower dichogamously, duodichogamously (male, female, male), or in some species heterodichogamously (two morphs that each produce male and female flowers but at reciprocal times). Dioecious species cannot engage in these temporal strategies. Using a phylogeny for 66 species and subspecies obtained from 6600 nucleotides of chloroplast introns, spacers, and a protein-coding gene, we address the hypothesis (Pannell and Verdú, *Evolution* 60: 660–673. 2006) that dioecy evolved from heterodichogamy. This hypothesis was based on phylogenetic analyses (Gleiser and Verdú, *New Phytol.* 165: 633–640. 2005) that included 29–39 species of *Acer* coded for five sexual strategies (duodichogamous monoecy, heterodichogamous androdioecy, heterodichogamous trioecy, dichogamous subdioecy, and dioecy) treated as ordered states or as a single continuous variable. When reviewing the basis for these scorings, we found errors that together with the small taxon sample, cast doubt on the earlier inferences. Based on published studies, we coded 56 species of *Acer* for four sexual strategies, dioecy, monoecy with dichogamous or duodichogamous flowering, monoecy with heterodichogamous flowering, or labile sex expression, in which individuals reverse their sex allocation depending on environment–phenotype interactions. Using Bayesian character mapping, we infer an average of 15 transformations, a third of them involving changes from monoecy-cum-duodichogamy to dioecy; less frequent were changes from this strategy to heterodichogamy; dioecy rarely reverts to other sexual systems. Contra the earlier inferences, we found no switches between heterodichogamy and dioecy. Unexpectedly, most of the species with labile sex expression are grouped together, suggesting that phenotypic plasticity in *Acer* may be a heritable sexual strategy. Because of the complex flowering phenologies, however, a concern remains that monoecy in *Acer* might not always be distinguishable from labile sex expression, which needs to be addressed by long-term monitoring of monoecious trees. The 13 dioecious species occur in phylogenetically disparate clades that date back to the Late Eocene and Oligocene, judging from a fossil-calibrated relaxed molecular clock.

KEY WORDS: Dioecy, heterodichogamy, inference of character evolution, labile sex, phenotypic plasticity, sexual systems, stochastic mapping of characters.

The open architecture, modular growth, and seemingly endless modifications of the timing of anther and stigma function of flowering plants have led to a large number of sexual systems (Darwin 1877; Errera and Gevaert 1878; Yampolsky and Yam-

polsky 1922; Sakai and Weller 1999; Barrett 2002). “Sexual system” here refers to the distribution and function of gamete-producing morphological structures (Sakai and Weller 1999), not realized mating patterns. Although the terminology for realized

mating patterns (outcrossing, selfing, mixed mating) and genetic mechanisms (self-compatibility, self-incompatibility) is straightforward, that for the sexual systems can appear daunting, with names such as monoecy, andromonoecy, gynomoecy, heterostyly, enantiostyly, dioecy, androdioecy, gynodioecy, trioecy, or polygamodioecy, in addition to the overlaid phenological strategies dichogamy, duodichogamy, and heterodichogamy (Delpino 1874; Darwin 1877; Todd 1882; Stout 1928; Gleeson 1982; Ross 1982; Lloyd and Webb 1986; Barrett 1992; McArthur et al. 1992; Bertin and Newmann 1993; Renner and Ricklefs 1995; Renner 2001; Jesson and Barrett 2002; Pannell 2002; Dorken and Barrett 2003; Gross 2005; Vamosi et al. 2006). Different uses of some of these terms by different authors have repeatedly caused misunderstandings, a recent example being the different meaning given the term heterodichogamy by de Jong (1976), Gleiser and Verdú (2005), and Pannell and Verdú (2006), as will become apparent below.

Pathways between plant sexual systems can be inferred in three ways, by population-level studies, sometimes in combination with phylogeographic approaches (e.g., Pendleton et al. 2000; Dorken and Barrett 2004a; Stehlik and Barrett 2006), by species-level studies that rely on the comparative method (e.g., Weller and Sakai 1999; Renner and Won 2001; Graham and Barrett 2004; Levin and Miller 2005; Navajas-Pérez et al. 2005), and by modeling (e.g., Charlesworth and Charlesworth 1978; Pannell and Verdú 2006; Ehlers and Bataillon 2007; Gleiser et al., unpubl. ms.). These approaches address different ways in which a system can be considered a “pathway,” all of them valid. The phylogenetic approach reveals whether the sexual system of a species is similar to that of its relatives, the assumption being that all may share a common ancestral system. The population-genetic approach may reveal directional selection, and prospective modeling points to likely outcomes resulting from evolutionary stable strategies. The present study uses the phylogenetic approach to address a hypothesis about the likely evolutionary pathway to dioecy in *Acer* that was put forward by Gleiser and Verdú (2005), based on phylogenetic inference, and which subsequently led to a new model for the evolution of dioecy (Pannell and Verdú 2006).

The long-standing focus on pathways between flowering plant sexual systems (Ross 1982; Pannell and Verdú 2006), with the accompanying need to assign species to clear categories (e.g., Gleiser and Verdú 2005; this study), may have contributed to plastic sex expression receiving relatively little attention (but see Dorken and Barrett 2003, 2004; Miller and Diggle 2003; Delph and Wolfe 2005; Ehlers and Bataillon 2007). Plasticity in sex expression means that sex is determined or modulated by the environment and changes adaptively during each individual's lifetime (Charnov and Bull 1977). It is common throughout land plants (Korpelainen 1998; Taylor et al. 2005), and ranges from switches between male and female phases over the life of an individual (as

in *Arisaema* and *Catsetum*) to shorter-term changes in resource allocation to male and female function.

A clade with particularly well-documented sexual liability is *Acer* (Wittrock 1886; Haas 1933; de Jong 1976; Hibbs and Fischer 1979; Freeman et al. 1980; Barker et al. 1982; Sakai and Oden 1983; Primack and McCall 1986; Sakai 1990; Matsui 1995; Bendixen 2001; Sato 2002; Tal 2006). *Acer* comprises 124 species in the Northern Hemisphere (van Gelderen et al. 1994), most of them in China, Korea, and Japan (81%); Europe and Western Asia have 12% of the species, Eastern North America 5%, and Western North America 4%. Morphologically, maple flowers can be bisexual or unisexual. Functionally, however, they are unisexual (exceptions are exceedingly rare; de Jong 1976: 17), with most species being monoecious (flowers of both sexes on each tree), but a few dioecious (male and female trees), polygamodioecious (male trees, female trees, and monoecious trees), or androdioecious (male trees and monoecious trees). All monoecious *Acer* flower dichogamously (male and female flowering separated in time), and those with large inflorescences often put out three synchronized batches of flowers in the sequence male → female → male, a system called duodichogamy (Stout 1928; de Jong 1976; Luo et al. 2007). Protandrous trees or duodichogamous trees often are inconstant, failing to produce female flowers in some years, but not others. Lastly, some species include a certain percentage of individuals that flower in the sequence male → female (protandrous) and others with the opposite sequence (protogynous). If most trees in a population specialize in this manner, being either protogynous or protandrous, this constitutes heterodichogamy (Delpino 1874; Gleeson 1982; Renner 2001; Endress and Lorence 2004; Pannell and Verdú 2006). Unfortunately, heterodichogamy has also been used without regard to the occurrence of two genetic morphs (e.g., de Jong 1976; Gleiser and Verdú 2005).

With its rich mix of morphological and temporal sexual strategies, *Acer* presents a remarkable opportunity to study the evolution of sexual systems. The first such study was that of Gleiser and Verdú (2005) who relied on de Jong's (1976) biosystematic monograph to score the sexual system of 44 species, from which they inferred character transformations in the context of three published phylogenies that included 29, 37, or 39 species (with partial taxon overlap between studies). For trait change inference, Gleiser and Verdú created five categories loosely based on de Jong (Materials and Methods, below). Following phylogenetic inference of trait change, using either models that involved ordered states and one or two rate parameters or a model that treated the five systems as a continuous variable, they concluded (l.c., p. 633), “Three different paths to dioecy have been followed in the genus *Acer*: from heterodichogamous androdioecy; from heterodichogamous trioecy; and from dichogamous subdioecy.”

We here use a new molecular phylogeny that includes almost all dioecious species of *Acer* and a compilation of all data

on *Acer* reproductive biology to test Gleiser and Verdú's (2005) hypotheses and to infer sexual system evolution in *Acer*. Based on a molecular clock, we also place sexual system changes in a temporal framework, focusing on transitions involving dioecy.

Materials and Methods

TAXON SAMPLING AND DNA SEQUENCING

Previous phylogenies of *Acer*, including the three used by Gleiser and Verdú (2005), relied on relatively small amounts of data from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA and the chloroplast *trnL* intron and spacer. To the extent that these studies overlapped in species sampling, they yielded contradictory relationships, although not with statistical support. To remedy this problem, our phylogeny is based on a larger amount of data from five combined chloroplast loci. Online Supplementary Table S1 lists all species sequenced for this study with author names, sources, geographic provenance, GenBank accession numbers, and assignments to sections and series in the classification of van Gelderen et al. (1994). The sequenced species represent all but one of the sections accepted by de Jong (1994), whose taxon concepts we follow. The missing section is *Wardiana*, which contains only *A. wardii* W.W. Sm. A previous study (Grimm et al. 2006) found *A. wardii* embedded in the *Palmata* clade (section *Palmata* sensu de Jong 1994), which is well represented in our study. Sequences of *A. fabri* and *A. kweilinense* are from another study (Renner et al., unpubl. ms.).

Sequences of the cp *trnD-trnT* and *psbM-trnD* spacers were downloaded from GenBank (Li et al. 2006) and added to our data, with a few taxa not sequenced for these loci coded as having unknown nucleotides. Trees were rooted with both species of *Dipteronia*, two species of *Aesculus*, and *Koelreuteria bipinnata* based on a phylogeny for Sapindaceae (Harrington et al. 2005).

Total genomic DNA was isolated from silica-dried leaves or from herbarium specimens using commercial plant DNA extraction kits. The polymerase chain reactions (PCR) followed standard protocols. Reaction products were purified with a commercial clean-up kit, and cycle sequencing was performed with BigDye Terminator cycle sequencing kits (Applied Biosystems, Norwalk, CT), using 1/4- or 1/8-scale reaction mixtures. The dye terminators were removed with the help of commercial kits, according to the manufacturers' protocols. Purified sequencing reactions were run on an ABI 3130 (Applied Biosystems, Darmstadt, Germany) automated sequencer.

Primers used to amplify the *rbcl* gene were 1F of Fay et al. (1997) and 1460R of Olmstead et al. (1992). For cycle sequencing, they were supplemented by the internal primers 600F (ATT-TATGCGTTGGAGAGACCG) and 800R (CAATAACRGCATGCATYGCACGRT) (Kocyan et al. 2007). For the *trnL* intron and adjacent *trnL-F* spacer, primers c, d, e, and f of Taberlet et al.

(1991) were used for amplification and sequencing. For the *rpl16* intron, we used primers 71F of Jordan et al. (1996) and 1067F of Asmussen (1999); and for the *psbA-trnH* region, we used primers *psbA* of Sang et al. (1997) and *trnH2* of Tate and Simpson (2003). DNAs from herbarium material were amplified with low annealing temperatures and/or with internal primers. Forward and reverse reads were obtained for most samples. Sequences were edited with Sequencher (4.6; Gene Codes, Ann Arbor, MI) and aligned by eye, using MacClade 4.06 (Maddison and Maddison 2003).

PHYLOGENETIC ANALYSES

Maximum likelihood (ML) analyses were performed with RAxML version 2.2.1 (Stamatakis 2006, <http://icwww.epfl.ch/~stamatak/index-Dateien/Page443.htm>) and Bayesian analyses with MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The best-fitting evolutionary model for the concatenated data (6855 nucleotides including gaps) selected by MrModeltest version 2.2 (Nylander 2004), employing the Akaike information criterion, was the general time-reversible (GTR) model plus a gamma-shape parameter (Γ) and proportion of invariable sites (I). For Bayesian analyses we used a two-partition model, with separate estimation of substitution rates under the GTR + Γ + I model for the coding (*rbcl*) and noncoding regions. Evolutionary rates were allowed to differ across partitions by unlinking the sets of parameters. Some runs were started from an independent random starting tree, others from the last tree found in the previous run. Markov chain Monte Carlo (MCMC) runs extended for two million generations, with trees sampled every 100 generations. Each run employed Metropolis-coupled MCMC, using three heated chains in addition to the sampled (cold) chain (the default settings in MrBayes). We used a flat Dirichlet prior for the relative nucleotide frequencies and rate parameters, a discrete uniform prior for topologies, and an exponential distribution (mean 1.0) for the gamma-shape parameter and all branch lengths. Convergence was assessed by checking that final likelihoods and majority rule topologies in different runs were similar; that the standard deviations (SD) of split frequencies were <0.01 ; that the log probabilities of the data given the parameter values fluctuated within narrow limits; that the convergence diagnostic given by MrBayes approached 1; and by examining the plot provided by MrBayes of the generation number versus the log probability of the data.

For ML searches with RAxML, we used a 6664-character matrix that excluded most large gaps and employed the GTR + CAT approximation of the GTR + Γ model, using 25 rate categories to approximate the gamma-shape parameter. Model parameters were estimated in RAxML over the duration of specified runs, and searches started from complete random trees.

Statistical support was measured by ML bootstrapping and Bayesian posterior probabilities.

CODING OF SEXUAL SYSTEMS AND INFERENCE OF TRAIT CHANGE

Many species of *Acer* have been the subject of multiyear reproductive biology studies, sometimes with large sample cohorts; Table 1 is a compilation of all studies on *Acer* sexual systems that we could find. To infer sexual system evolution in the genus, we scored as many of the 66 species and subspecies in the molecular tree as possible for the following four states: dioecious, monoecious with dichogamous or duodichogamous flowering, monoecious with heterodichogamous flowering, or sexually labile. This resulted in the scoring of 52 species/subspecies (Table 1). As mentioned (Introduction), all monoecious species of *Acer* flower dichogamously and/or duodichogamously, with smaller inflorescences doing the former, larger ones the latter. Dioecious species cannot engage in dichogamous, duodichogamous, or heterodichogamous flowering because these strategies depend on each individual producing male and female flowers.

At least five species, *A. campestre*, *A. japonicum*, *A. opalus*, *A. platanoides*, and *A. pseudoplatanus*, appear to have consistently protogynous and protandrous individuals; that is, these species are at least partially heterodichogamous (references see Table 1). In addition, their populations contain inconsistent protandrous trees and/or duodichogamous trees. Unfortunately, the percentage of individuals that are protandrous or protogynous specialists or exhibit labile sex is only known for a few species. In *A. japonicum*, for example, 3.4–5.6% of 95 trees changed sex expression every year (over four years of observation; Asai 2000), although most trees were either protandrous or protogynous. We did two runs, one with *A. japonicum* as heterodichogamous (following Asai 2000 and Sato 2002), the other with *A. japonicum* coded as sexually labile. This had very little effect on the estimated transition rates, and we therefore report only results from the first run. In total, seven or eight species (Table 1) were scored as sexually labile because multiyear studies have shown that some proportion of their individuals change sex expression depending on phenotype–environment interactions.

Of the outgroups, *Dipteronia* is monoecious and duodichogamous (de Jong 1976), whereas *Aesculus* and *Koelreuteria* are andromonoecious and dichogamous (Bertin 1982; Ronse Decraene et al. 2000; L. Beenken, pers. obs. in the Munich Botanical Garden). The distant outgroup *Koelreuteria* was excluded from trait change analyses.

Table 1 includes the sexual strategy scorings of all species in Gleiser and Verdú (2005) and de Jong (1976) whom they cite as their source. de Jong's results are based on up to 10 years of monitoring of cultivated trees, observations on wild trees of European species, and indirect evidence from mode of inflorescence branching and old fruit stalks on herbarium specimens; he listed all studied herbarium specimens in an appendix. de Jong assigned 113 species to 10 sexual expression types plus flowering modes,

designated with the letters A through G, plus three letter combinations (de Jong 1976: table 2; de Jong 1994: figure 6.3). Unfortunately, de Jong called species heterodichogamous whenever he found protandrous and protogynous inflorescences, whether on one tree, on different trees, or only on herbarium sheets. A few examples of his application of the term to species producing protandrous and protogynous inflorescences on the same tree are *A. griseum* (p. 77), *A. japonicum* (p. 59), *A. pseudoplatanus* (p. 35), and *A. platanoides* (p. 51). This use of heterodichogamy is fundamentally different from the genetic system of two temporal (genetic) morphs that other authors call heterodichogamy (Renner 2001 for a review; Pannel and Verdú 2006). Gleiser and Verdú's (2005) scorings of numerous species as heterodichogamous for which there is no information on morph frequencies suggest that they may have been misled by de Jong's use of heterodichogamy. These scoring problems affect two-thirds of the species included in their study (cf. our Table 1).

We modeled changes in sexual system by means of the stochastic character mapping technique described by Huelsenbeck et al. (2003) and implemented in the program SIMMAP (Bollback 2006; version 1.0 Beta 2.3 available at <http://www.simmap.com/simmap/simmap.html>). The Markov approach estimates the rates at which a discrete character undergoes state changes as it evolves through time (Pagel 1994; Lewis 2001; Huelsenbeck et al. 2003). We opted for Bayesian estimation of state transformations because this approach allows one to average over equally likely topologies, which is relevant because the *Acer* phylogenetic tree has a poorly resolved backbone.

The rate and number of state transformations were estimated on the 7501 post burn-in trees (with branch lengths) from a Bayesian analysis (that used the search strategies described under *Phylogenetic analyses*) of the chloroplast dataset after the exclusion of the taxa with unknown sexual systems and the distant outgroup *Koelreuteria*. This left 56 taxa and four states. For a four-state character, 12 rates must be estimated, as each sexual system can change into any other. A rule of thumb is that for each rate parameter to be estimated, there should be 10 species with known character states in the phylogenetic tree; clearly, our dataset does not approach that ratio, resulting in large confidence intervals (CIs). As recommended, branch lengths were rescaled so that the total tree length was 1, with branch length proportions maintained. We assigned no prior value on the rate parameters and instead let branch lengths define rates. The prior on the bias parameter was fixed at $1/k$, where k is the number of states, this being the recommended approach in SIMMAP for characters with more than two states.

MOLECULAR CLOCK TIME ESTIMATION

The matrix used for dating excluded the *trnD-trnT* and *psbM-trnD* spacers because of missing sequences, as well as taxa for which

Table 1. Sexual systems recorded in *Acer*, and their scoring in this study and in Gleiser and Verdú (2005). Gleiser and Verdú assigned species to five “sexual systems,” namely duodichogamous monoecy, heterodichogamous androdioecy, heterodichogamous trioecy, dichogamous subdioecy, and dioecy, listing de Jong (1976) as their basis. For comparison, we include de Jong’s original assignments. Note that de Jong’s (1976) usage of heterodichogamy differs from that of other authors in that he used the term whenever a species has protandrous and protogynous inflorescences, without considering whether these inflorescences occur on different trees or on the same tree (see text for details). Based on the studies cited in column 3, we scored species for the following sexual systems: (1) dioecious = individuals genetically male or female; (2) monoecious with duodichogamous flowering; (3) monoecious with heterodichogamous phenology; (4) labile sex expression. Monoecious *Acer* species flower dichogamously, duodichogamously, or heterodichogamously; dioecious species cannot engage in these phenological strategies. A species was scored as having labile sex expression if individuals change their sex expression during ontogeny (often drastically between years). Chromosome numbers in column 2 are from Gelderen et al. (1994). Note: Because most female flowers morphologically look bisexual, herbarium-based studies, such as Xu et al.’s treatment of Aceraceae for the Flora of China, often describe *Acer* species as andromonoecious (i.e., with male and bisexual flowers).

Species	#	Sources of information P. = page number in de Jong (1976)	Scoring in this study based on the cited sources	Scoring in Gleiser and Verdú (2005)
<i>A. acuminatum</i> Wall. ex D. Don	–	P. 94: Dioecious Xu et al., unpubl. ms.; Dioecious	Dioecious	Not included in phylogenies
<i>A. argutum</i> Maxim.	–	P. 94: Dioecious Semm 1966: Dioecious	Dioecious	Dioecious
<i>A. barbinerve</i> Maxim.	–	P. 94: Dioecious Xu et al., unpubl. ms.; Dioecious	Dioecious	Not included in phylogenies
<i>A. buergerianum</i> Miq.	26	de Jong 1976: No information on sexual system for this species	Not included because no information	Heterodichogamous androdioecious
<i>A. caesium</i> Wall. ex Brand.	26 (52?)	de Jong 1976: No information on sexual system for this species; van Gelderen et al. 1994: Monoecious but “poorly known”	Not included because too little information	Heterodichogamous androdioecious
<i>A. campbellii</i> subsp. <i>flabellatum</i> (Rehder) A.E. Murray	26	de Jong 1976: No information on sexual system for this species; van Gelderen et al. 1994: Monoecious	Monoecious; no information on phenology	Heterodichogamous androdioecious
<i>A. campbellii</i> subsp. <i>sinense</i> (Pax) P.C. de Jong	–	P. 102: Duodichogamous Xu et al., unpubl. ms.; Andromonoecious	Monoecious; insufficient information on phenology	Not included in phylogenies
<i>A. campestre</i> L.	26	P. 51: five trees over six years, two protogynous, one male or protandrous, two duodichogamous Semm 1966: Duodichogamous, protandrous and protogynous, rarely unisexual; Bendixen 2001: Duodichogamous and heterodichogamous	Monoecious with heterodichogamous flowering	Heterodichogamous androdioecious
<i>A. cappadocicum</i> Gled.	26	P. 51: two trees over six years; protandrous or duodichogamous	Not available for sequencing	Heterodichogamous androdioecious
<i>A. carpiniifolium</i> Sieb. & Zucc.	52	P. 94: Dioecious; tendency to wind pollination Ohwi 1965	Dioecious	Dioecious
<i>A. caudatum</i> subsp. ukurunduense (Trautv. & C.A. Mey.) A. E. Murray	26	van Gelderen et al. 1994: Monoecious Xu et al., unpubl. ms.: Andromonoecious	Monoecious; no information on phenology	Not included in phylogenies
<i>A. circinatum</i> Pursh	26	P. 58–61: one tree over six years, duodichogamous	Monoecious; insufficient information on phenology	Heterodichogamous androdioecious

Continued

Table 1. Continued

Species	#	Sources of information P. = page number in de Jong (1976)	Scoring in this study based on the cited sources	Scoring in Gleiser and Verdú (2005)
<i>A. cissifolium</i> (Sieb. & Zucc.) K. Koch	26	P. 90: Dioecious; also L. Beenken, pers. obs. in Munich Bot. Garden	Dioecious	Dioecious
<i>A. crataegifolium</i> Sieb. & Zucc.	26	P. 61, 71, 102: Depending on the individual and/or surrounding conditions or age, some male or female trees are monoecious in other years (J. Murata, pers. comm., Botanical Gardens, The University of Tokyo, Feb. 2007)	Labile sex expression; insufficient information on phenology	Dichogamous subdioecious
<i>A. davidii</i> subsp. <i>davidii</i>	26	P. 61, 67, 71: Monoecious, but very variable	Monoecious; insufficient information on phenology	Unclear which subspecies included in phylogenies
<i>A. davidii</i> subsp. <i>grosseri</i> (Pax) P.C. de Jong	26	P. 61, 66, 71: five trees over six years, male, female, and protandrous inflorescences all on one tree; change of sex expression	Labile sex expression	Dichogamous subdioecious (unclear which subspecies included in phylogenies)
<i>A. diabolicum</i> Blume ex K. Koch	26	P. 88: Dioecious Gelderen et al. 1994: Dioecious	Dioecious	Dioecious
<i>A. distylum</i> Sieb. & Zucc.	–	P. 74: Duodichogamous, protandrous, protogynous; only studied from herbarium specimens	Monoecious; no information on phenology	Heterodichogamous trioecy
<i>A. erianthum</i> Schwer.	–	P. 61, 102: Duodichogamous Xu et al., unpubl. ms.: Andromonoecious	Monoecious; insufficient information on phenology	Not included in phylogenies
<i>A. fabri</i> Hance	–	Xu et al., unpubl. ms.: Andromonoecious	Monoecious; no information on phenology	Not included in phylogenies
<i>A. glabrum</i> Torr.	–	P. 93: Unisexual and protogynous; sex change observed in one cultivated tree studied between 1963 and 1972	Monoecious; insufficient information on phenology	Heterodichogamous trioecy
<i>A. griseum</i> (Franch.) Pax	26	P. 75, 77: six trees studied over three years, protogynous and protandrous inflorescence borne on same tree	Monoecious; insufficient information on phenology	Heterodichogamous trioecy
<i>A. heldreichii</i> Boiss.	26	P. 35: one protogynous tree observed over six years	Monoecious; insufficient information on phenology	Not included in phylogenies
<i>A. henryi</i> Pax	–	P. 90, 93: Dioecious Xu et al., unpubl. ms.: Dioecious	Dioecious	Dioecious

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Table 1. Continued

Species	#	Sources of information P. = page number in de Jong (1976)	Scoring in this study based on the cited sources	Scoring in Gleiser and Verdú (2005)
<i>A. japonicum</i> Thunb.	26	P. 59, 102: Duodichogamous, heterodichogamous Asai 2000: 95 trees, four years, eight trees changed their sex expression over the four years, 3.4–5.6% of trees changed sex expression every year; Sato 2002: 109 trees, two years, 45 protogynous, 54 protandrous (22 of the latter duodichogamous), six trees male in both years, four males in one year but m > f next year	Monoecious with heterodichogamous flowering	Heterodichogamous androdioecious
<i>A. kweilinense</i> W. P. Fang & M. Y. Fang	–	Xu et al., unpubl. ms.: Andromonoecious.	Monoecious; no information on phenology	Not included in phylogenies
<i>A. laevigatum</i> Wall.	26	P. 61, 102: Duodichogamous Xu et al., unpubl. ms.: Andromonoecious	Monoecious; insufficient information on phenology	Not included in phylogenies
<i>A. laurinum</i> Hassk.	26	P. 96: Probably dioecious Xu et al., unpubl. ms.: Androdioecious or dioecious	Dioecious	Heterodichogamous trioecy
<i>A. macrophyllum</i> Pursh	26	P. 89: Duodichogamous with occasional loss of one phase	Monoecious; insufficient information on phenology	Heterodichogamous androdioecious
<i>A. mandshuricum</i> Maxim.	26	P. 78: “Few studied specimens” only herbarium material Xu et al., unpubl. ms.: “Dioecious?”	Not included because too little information	Heterodichogamous trioecy
<i>A. maximowiczianum</i> Miq. (incl. <i>A. nikkoense</i> Maxim.)	–	P. 74–78, Table 12: 10 trees studied over up to six years. All-male trees changed to producing bisexual inflorescence and unisexual inflorescence in subsequent years	Labile sex expression	Not included in phylogenies
<i>A. miyabei</i> Maxim.	–	P. 51, 55: one tree studied over six years switched between protandrous to duodichogamous inflorescences	Monoecious; insufficient information on phenology	Not included in phylogenies
<i>A. mono</i> Maxim.	26	P. 51: one tree studied over six years, protogynous Semm 1966: Dichogamous	Monoecious; insufficient information on phenology	Heterodichogamous androdioecious

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Table 1. Continued

Species	#	Sources of information P. = page number in de Jong (1976)	Scoring in this study based on the cited sources	Scoring in Gleiser and Verdú (2005)
<i>A. monspessulanum</i> L.	26	P. 44, 102: Varying between protandrous, protogynous, and unisexual; Haas 1933: Protandrous; Semm 1966: Dichogamous, no sex change	Monoecious; insufficient information on phenology	Heterodichogamous trioecy
<i>A. negundo</i> L.	26	P. 90: Dioecious Dawson and Geber 1999: Dioecious	Dioecious	Dioecious
<i>A. nipponicum</i> H. Hara	–	P. 72: Duodichogamous, but some inflorescences just protandrous or male	Monoecious; insufficient information on phenology	Duodichogamous monoecy
<i>A. oblongum</i> DC. (<i>A. albopurpurascens</i> Hayata)	26	de Jong 1976: No information on sexual system; Khusalani 1963: Monoecious	Monoecious; no information on phenology	Heterodichogamous androdioecious
<i>A. opalus</i> Mill.	26	P. 44, 102: Individuals protogynous or unisexual Gleiser et al., unpubl.: 100 trees studied over six years; 48% protogynous; 38% pure-males; 14% protandrous	Monoecious with heterodichogamous flowering	Heterodichogamous trioecy
<i>A. palmatum</i> Thunb.	26	P. 58, 59: Duodichogamous, protogynous, occasional pure males; Xu et al., unpubl. ms.: Andromonoecious	Monoecious; insufficient information on phenology	Heterodichogamous androdioecious
<i>A. pensylvanicum</i> L.	26	P. 61–67: Male, female, and protandrous inflorescences all on one tree; change of sex expression; Hibbs and Fisher 1979: Labile sex expression	Labile sex expression	Dichogamous subdioecious
<i>A. pentaphyllum</i> Diels	–	P. 94: Only one herbarium specimen (the type collection) studied	Not included because too little information	Heterodichogamous androdioecious
<i>A. pilosum</i> Maxim.	–	de Jong 1976: No information on sexual system; Xu et al., unpubl. ms.: Andromonoecious	Monoecious	Not included in phylogenies
<i>A. platanooides</i> L.	26, 39	P. 45–55: Duodichogamous, rarely flowering purely male Wittrock 1886: 100 trees, 47–50 protogynous, 31–30 protandrous; 7–18 pure males; 3–8 duodichogamous. Haas 1933; Svobodová 1967: Of 152 trees, 110 protandrous, 36 protogynous; Tal 2006: two years, three trees protandrous, seven protogynous	Monoecious with heterodichogamous flowering	Heterodichogamous androdioecious

Continued

Table 1. Continued

Species	#	Sources of information P. = page number in de Jong (1976)	Scoring in this study based on the cited sources	Scoring in Gleiser and Verdú (2005)
<i>A. pseudoplatanus</i> L.	52	P. 35, 40: Duodichogamous, protandrous, protogynous, all three types of inflorescences on the same tree; Scholz 1960: Some trees consistently protandrous or protogynous, whereas others switched their sequence of flowering; Semm 1966: Duodichogamous, protandrous, protogynous, rarely unisexual; Binggeli 1992: "In most cases, all inflorescences of a tree always start flowering with a male or female sequence and switch to the other sex one or more times." (i.e., were duodichogamous). Proportion of protandrous and protogynous individuals varying widely between studies. Tal 2006: Over two years, 55 trees duodichogamous (seven of them lacking second male phase), 18 protogynous, one pure male; no tree found to switch sex expression	Monoecious with heterodichogamous flowering	Heterodichogamous androdioecious
<i>A. pseudosieboldianum</i> (Pax) Kom.	26	de Jong 1976: No information on sexual system	Not included because too little information	Heterodichogamous androdioecious
<i>A. pycnanthum</i> Koch	78	Saeki 2005: Dioecious; sex ratio for several natural populations 35% male, 32% female, and 33% nonflowering	Dioecious	Not included in phylogenies
<i>A. rubrum</i> L.	78, 91, or 108	P. 78–86: Purely female trees, purely male trees, bisexual trees, with changing sex expression over time: "Inconstant females", "inconstant males", "variable plants" Haas 1933; Primack and McCall 1986: 79 trees, seven years, Sakai 1990: Polygamodioecious	Labile sex expression	Heterodichogamous trioecy
<i>A. rufinerve</i> Sieb. & Zucc.	26	P. 61–71: Male, female, and protandrous inflorescences all on one tree; change of sex expression; Matsui 1995: Labile sex expression	Labile sex expression	Dichogamous subdioecious

Continued

Table 1. Continued

Species	#	Sources of information P. = page number in de Jong (1976)	Scoring in this study based on the cited sources	Scoring in Gleiser and Verdú (2005)
<i>A. saccharinum</i> L.	52	P. 78–86: Functionally male trees, functionally female trees, and bisexual trees; Semm 1966: Bisexual trees, rarely unisexual trees; Sakai 1978: 700 trees, five years, Sakai and Oden 1983: Inconstant females, inconstant males, variable sex expression	Labile sex expression	Heterodichogamous trioecy
<i>A. saccharum</i> subsp. grandidentatum (Torr. & A. Gray) Desm.	–	Baker et al. 1982: Labile sex expression	Labile sex expression	Not included in phylogenies
<i>A. saccharum</i> Marsh. subsp. saccharum	26, 52, 53, 78	P. 44: two cultivated trees studied over three years, both protogynous; Gabriel 1968: six trees over three years, protandrous or protogynous inflorescence, sometimes on same tree	Monoecious; insufficient information on phenology	Heterodichogamous androdioecious
<i>A. sieboldianum</i> Miq.	26	de Jong 1976: No information on sexual system	Not included because too little information	Heterodichogamous androdioecious
<i>A. spicatum</i> Lam.	26	P. 72, 74: Duodichogamous, once protogynous	Monoecious; insufficient information on phenology	Duodichogamous monoecy
<i>A. stachyophyllum</i> Hiem.	26	P. 94: Dioecious Xu et al., unpubl. ms.: Dioecious	Dioecious	Not included in phylogenies
<i>A. sterculiaceum</i> subsp. franchetii (Pax) A.E. Murray	26	P. 88: Dioecious Xu et al., unpubl. ms.: Dioecious	Dioecious	Not included in phylogenies
<i>A. takesimense</i> Nakai = <i>A. pseudosieboldianum</i> subsp. takemiense (Nakai) de Jong	–	de Jong 1976: No information on sexual system	Not included because no information	Heterodichogamous androdioecious
<i>A. tataricum</i> subsp. ginnala (Maxim.) Wesm.	26	P. 86, under <i>A. ginnala</i> : four trees studied, one protogynous, three duodichogamous mixed with protandrous inflorescence Semm 1966: Duodichogamous	Monoecious; insufficient information on phenology	Heterodichogamous androdioecious

Continued

Table 1. Continued

Species	#	Sources of information P. = page number in de Jong (1976)	Scoring in this study based on the cited sources	Scoring in Gleiser and Verdú (2005)
<i>A. tataricum</i> subsp. <i>semenovii</i> (Regel & Herder) A. E. Murray	–	P. 86, under <i>A. tataricum</i> : two trees studied, one protogynous, one duodichogamous mixed with protandrous inflorescence Semm 1966: Duodichogamous	Monoecious; insufficient information on phenology	Heterodichogamous androdioecious
<i>A. tegmentosum</i> Maxim.	–	de Jong 1976: No information on sexual system	Not included because no information	Dichogamous subdioecious
<i>A. trautvetteri</i> Medw.	26	P. 35: Duodichogamous, protogynous, two trees over three years, one tree protogynous, one duodichogamous	Monoecious; insufficient information on phenology	Heterodichogamous androdioecious
<i>A. triflorum</i> Kom.	–	de Jong 1976: No information on sexual system for this species; Xu et al., unpubl. ms.: Androdioecious	Not included because too little information	Heterodichogamous trioecious
<i>A. truncatum</i> Bunge	26	P. 45: “some living specimens” studied but no further information for this species given Xu et al., unpubl. ms.: Andromonoecious	Monoecious; insufficient information on phenology	Heterodichogamous androdioecious
<i>A. tschonoskii</i> Maxim.	26	P. 61, 66: Monoecious (citing other authors); observation on one cultivated tree; Xu et al., unpubl. ms.: Andromonoecious	Monoecious; insufficient information on phenology	Dichogamous subdioecious
<i>A. wardii</i> W.W. Smith	–	P. 72: “limited number of herbarium specimens ... flowers of one sex only”; Xu et al., unpubl. ms.: Andromonoecious	Not available for sequencing	Dichogamous subdioecious
<i>Aesculus flava</i> Sol. (<i>A. octandra</i> Marshall)		P. 102: Duodichogamous, protandrous	Monoecious	Not included in trait inference
<i>Aesculus parviflora</i> Walter		P. 102: Duodichogamous and protandrous	Monoecious	Not included in trait inference
<i>Dipteronia dyeriana</i> Henry		P. 102: Duodichogamous, but data scarce	Monoecious	Duodichogamous monoecy
<i>Dipteronia sinensis</i> Oliver		P. 102: Herbarium and living tree	Monoecious	Duodichogamous monoecy
<i>Koelreuteria bipinnata</i> Franch.		P. 102: Duodichogamous (but only one cultivated tree)	Only included to root phylogenies	Not included

we did not sequence *rbcl* (Online Supplementary Table S1). It consisted of 52 taxa and 4521 nucleotides. The relaxed-clock approach used was that of Thorne and Kishino (2002; freely available at <http://statgen.ncsu.edu/thorne/>). Parameter values under the F84 + G model with five rate categories were estimated with PAML's *baseml* (Yang 1997; freely available at <http://abacus.gene.ucl.ac.uk/software/paml.html>), this being the only model implemented in Thorne's software. We then used Thorne's *estbranches* to calculate branch lengths and their variance, given the sequence data, PAML's model parameter file, and a specified rooted topology. The topology used as input was the highest likelihood tree obtained for the same data with RAxML. The *estbranches* output became the prior for MCMC searches in *multidivtime* that sought to find the most likely model of rate change (with rate change assumed to be log-normally distributed), given the tree topology, fossil constraints, and a Brownian motion parameter (n) that controls the magnitude of autocorrelation per million years (MY) along the descending branches of the tree. Prior gamma distributions on parameters of the relaxed-clock model were as follows: the mean of the prior distribution for the root age was set to 63 MY, based on the earliest known fruits of *Dipteronia brownii* from the Late Paleocene of Wyoming (McClain and Manchester 2001); the SD of this prior was also set to 63. The mean and SD of the prior distribution for the ingroup root rate were set to 0.0001 substitutions/site/MY by dividing the median of the distances between the ingroup root and the tips by 63 MY. The prior and SD for n were set to 0.02, following the manual's recommendation that the time between root and tips multiplied by n be about 1. Markov chains in *multidivtime* were run for one million generations, sampling every 100th generation for a total of 10,000 trees, with a burn-in of 1000 trees before the first sampling of the Markov chain. Like *Dipteronia*, *Acer* is known from Paleocene fruits from North America and Eurasia (Wolfe and Tanai 1987; Crane et al. 1990; Kittle et al. 2005), but because they cannot be associated with particular nodes in the phylogeny, we decided not to employ them as age constraints.

Results

PHYLOGENETIC RELATIONSHIPS

The final dataset used for phylogenetic inference included 6664 characters, following the elimination of 191 positions because of ambiguous alignment. The data comprised 1142 aligned positions from the *trnL* region, 1287 from the *rpl16* intron, 783 from the *psbA-trnH* spacer, 1524 from the *trnD-trnT* spacer, 679 from the *psbM-trnD* spacer, and 1440 from the *rbcl* gene. Topologies resulting from individual analyses of these six loci contained no well-supported conflicting nodes, and we therefore concatenated the data. The mean gamma-shape parameter estimated by MrBayes for the *rbcl* gene was $\alpha = 0.10$, indicating little rate het-

erogeneity, that for the noncoding regions was $\alpha = 1.09$, indicating moderate rate heterogeneity. Figure 1 shows a ML tree obtained from the combined data. Although the backbone of the phylogeny is poorly resolved, many of the traditionally recognized series and sections (marked in Fig. 1) are recovered, sometimes with high statistical support. Section *Acer*, however, is polyphyletic, because *A. caesium* and *A. pseudoplatanus* do not group with the remaining species of that section, nor do *A. caudatum* and *A. spicatum* of series *Caudata* group together.

EVOLUTION OF DIOECY IN ACER

Dioecy evolved in three clades and two isolated species (circled in Fig. 1). With the divergence between *Dipteronia* and *Acer* constrained to minimally 63 MY based on the fossil *D. brownii*, the initial radiation of the largest dioecious clade (consisting of *A. acuminatum*, *A. argutum*, *A. barbinerve*, *A. cissifolium*, *A. henryi*, and *A. stachyophyllum*; Figs. 1, 2) took place about 37 MY ago, with a 95% CI of 22 to 56 MY. The split between the dioecious *A. diabolicum* and *A. sterculiaceum* is estimated as having occurred 26 MY ago (CI: 12–44 MY), and that of *A. laurinum* from *A. pycnanthum* at 29 (16–46) MY. Divergence times of *A. negundo*, *A. carpinifolium*, and *A. craetaegifolium* cannot be estimated because of their statistically unsupported placements (Fig. 1). The estimated age of the clade comprising four of the nine species with sexual plasticity is 28 (16–46) MY (Fig. 2).

The rate and frequency distribution of the 12 possible sexual system transformations including those leading to dioecy are shown in Table 2, and Figure 2 shows a randomly chosen character history of the 75,010 replicates (10 replicates of character transformations on each of 7501 post burn-in Bayesian trees). The mean total number of transformations (sexual system changes) was 15.2, with the most frequent transitions those from monoecy with (duo)dichogamous flowering to dioecy (4.5) and from monoecy with (duo)dichogamous flower to monoecy with heterodichogamous flowering (3.7). The relative transformation rates (Table 2) suggest that returns from dioecy to most of the other systems are unlikely, with the exception of a switch to labile sex expression. The switch from heterodichogamous flowering to dioecy was among the lowest of all observed transition rates (Table 2). However, given the small overall number of transitions, the 95% CIs around all estimates are large (or cannot be calculated), and partly overlap (Table 2).

Discussion

THE DISTRIBUTION OF DIOECY AND HETERODICHOGAMY IN ACER

Of the 124 species of *Acer*, 13 are dioecious (van Gelderen et al. 1994; our Table 1). This may be a slight underestimate because there are three Chinese entities that are closely related

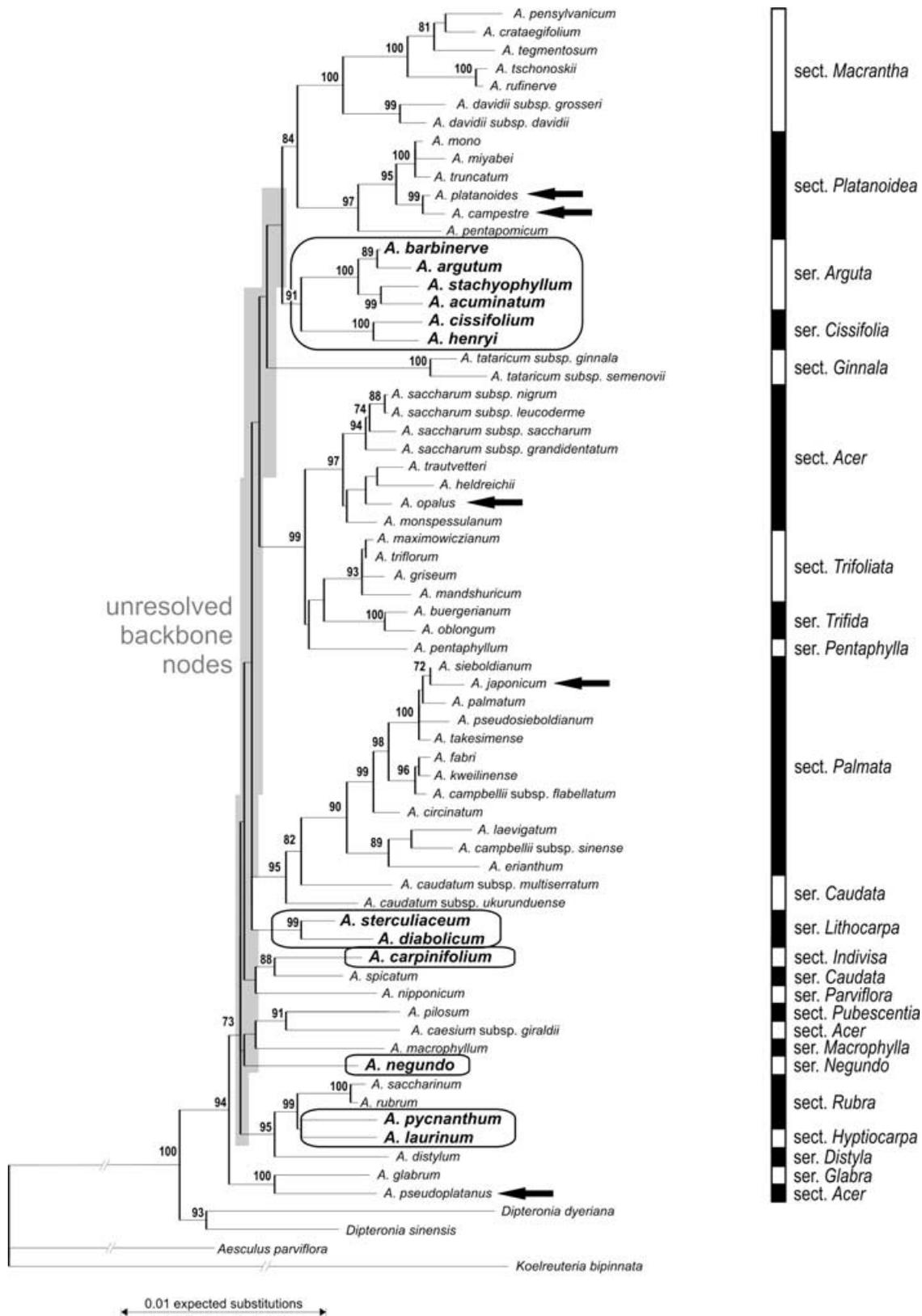


Figure 1. Maximum-likelihood phylogram for 66 species/subspecies of *Acer* plus four outgroups, based on 6664 nucleotides from four chloroplast DNA loci. Values above nodes indicate ML bootstrap support $\geq 70\%$. Dioecious species are shown in bold. The remaining species are monoecious with duodichogamous or heterodichogamous flowering (marked by arrows) or sexually labile (compare Fig. 2 for sexual systems).

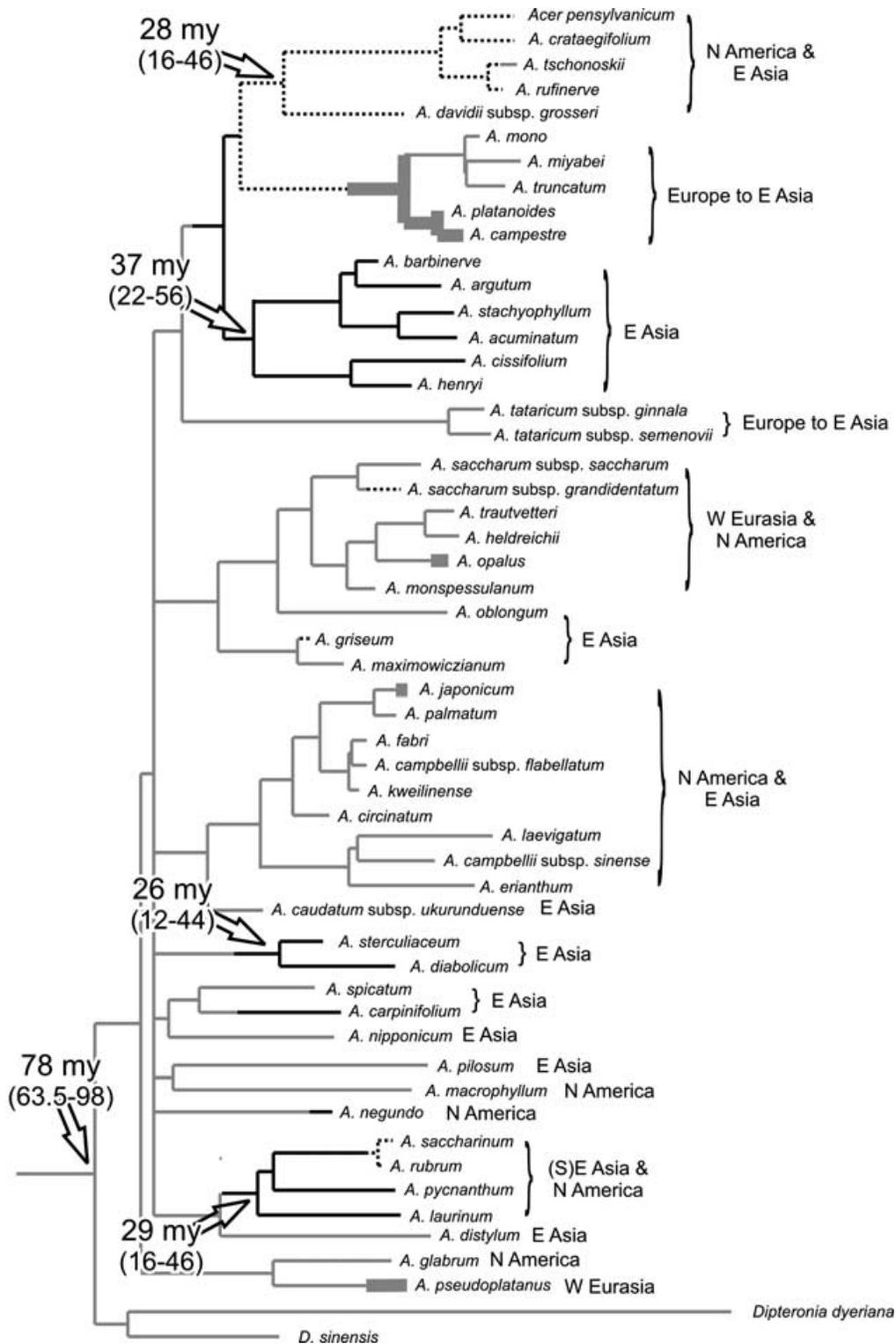


Figure 2. One of 75,010 character histories inferred on a sample of Bayesian trees obtained with the same chloroplast data as used for Figure 1 except that taxa with insufficient information on sexual system were excluded (see Materials and Methods). Black lines designate dioecy, gray lines monoecy with duodichogamous flowering, thick gray lines monoecy with heterodichogamous flowering, and hatched lines a plastic sexual system. Table 1 lists the references for each species' sexual system. Character histories were mapped under a four-state continuous time Markov process, with trees and branch lengths sampled by the Markov chain. Compare Table 2 for mean total numbers of transformations and transformation frequency distributions among the 75,010 replicates. The ages in millions of years (with confidence intervals) are from a Bayesian relaxed molecular clock (see Materials and Methods).

Table 2. Estimated character transformations and their confidence limits for the sexual strategies monoecy with dichogamous flowering, monoecy with heterodichogamous flowering, dioecy, and labile sex expression scored for the species of *Acer* listed in Table 1. Compare Fig. 2 for a randomly selected character history out of the 75,010 replicates simulated on a post burn-in sample of Bayesian phylograms obtained from 6674 nucleotides of combined chloroplast loci. Dwell time is the proportion of the total branch length in a particular character state, averaged over all sampled histories. By dividing the estimated total transformations by the dwell time, the transition rate can be determined (i.e., the likelihood of a branch having a particular state changing to another state).

Transformations	Total 15.21	Dwell time	Rate	Confidence limits
Dioecy>monoecy-dichogamy	0.38	0.19	2.00	0–9.10
Dioecy>monoecy-heterodichogamy	0.05	0.19	0.26	0–5.22
Dioecy>labile sex expression	1.56	0.19	8.21	4.45–12.52
Monoecy-dichogamy>monoecy-heterodichogamy	3.65	0.66	5.53	4.42–6.53
Monoecy-dichogamy>dioecy	4.53	0.66	6.86	4.74–8.07
Monoecy-dichogamy>labile sex expression	2.41	0.66	3.65	2.87–4.92
Monoecy-heterodichogamy>monoecy-heterodichogamy	0.34	0.04	8.50	0–33.90
Monoecy-heterodichogamy>dioecy	0.02	0.04	0.50	0–0
Monoecy-heterodichogamy>labile sex expression	0.05	0.04	1.25	0–16
Labile sex expression>monoecy-dichogamy	1.57	0.12	13.08	7.30–21.41
Labile sex expression>monoecy-heterodichogamy	0.31	0.12	2.58	0–9.87
Labile sex expression>dioecy	0.31	0.12	2.58	0–9.72
Monoecy-dichogamy		0.66		
Monoecy-heterodichogamy		0.04		
Dioecy		0.19		
Labile sex expression		0.12		

or synonymous with the dioecious species *A. diabolicum* and *A. sterculiaceum* and that are also dioecious (*Acer leipoense* W. P. Fang & T. P. Soong from Southwest Sichuan, *A. yangbiense* Y. S. Chen & Q. E. Yang from Yunnan, and *A. sinopurpurascens* Cheng from Central China). If these three are biological species and were to group with *A. diabolicum* and *A. sterculiaceum* as expected from their morphology, dioecy in *Acer* would have evolved five times (Fig. 2). None of the dioecious species are phylogenetically close to the five (partly) heterodichogamous species, *A. campestre*, *A. japonicum*, *A. opalus*, *A. platanoides*, and *A. pseudoplatanus* (Fig. 1), and switches from heterodichogamous flowering to dioecy were among the lowest of all transition rates (Table 2). This does not fit with the hypothesis that dioecy in *Acer* evolved from heterodichogamous ancestors or relatives (Gleiser and Verdú 2005), although see below.

From inspection of the tree (Fig. 1) it appears that dioecy in maples is not associated with species proliferation and that the dioecious clades all originated relatively early, with no additional transitions to separate sexes later on. However, current methods cannot distinguish between infrequent trait evolution and a trait's infrequency due to its occurrence in species-poor clades (Maddison 2006). Morphological traits known to be associated with dioecy, such as wind pollination and fleshy fruits (Renner and Ricklefs 1995), do not appear to play a large role in *Acer*, with only one of the dioecious species, *A. negundo*, being wind-pollinated (Freeman et al. 1976) and some nondioecious species, for example *A. saccharinum*, also being wind-pollinated (Sakai

and Oden 1983). All *Acer* have wind-dispersed fruits. There is also no obvious association of dioecy and ploidy level (Table 1, column 2).

Gleiser and Verdú (2005, p. 635) inferred that heterodichogamy was the most likely ancestral condition in the genus. However, the scoring problems (cf. our Table 1), small taxon sample (only six dioecious species were included), and weak phylogenies available at the time cast doubt on these inferences. de Jong (1976) hypothesized that the ancestral sexual system of *Acer* was monoecy with dichogamous or duodichogamous flowering and that heterodichogamous flowering evolved later. His arguments for this were that duodichogamy is the sexual strategy of *Dipteronia*, the closest relative of *Acer*; that andromonoecy is the sexual system in *Aesculus* and other Sapindaceae close to *Acer/Dipteronia*; and that monoecious duodichogamous flowering is the most common phenological strategy in *Acer*. Although our data fit with de Jong's hypothesis, more solid inference of the ancestral phenological strategy in *Acer* will require more data on the flowering patterns in the basal-most species (compare Fig. 1 and Table 1).

Based on the results of Gleiser and Verdú (2005), *Acer* became the prime example of the path from heterodichogamy via androdioecy to dioecy modeled by Pannell and Verdú (2006), because "dioecy appears to have evolved repeatedly via androdioecy from a heterodichogamous ancestral state in the maple genus, *Acer*" (i.e., p. 670). Although our data do not address the theoretical model, *Acer* should probably no longer be considered a

strong example of the proposed pathway. This is because heterodichogamy as modeled (Pannell and Verdú 2006) involves reciprocal mating types that occur at a 1:1 ratio as in walnut and pecan (Gleeson 1982; Thompson and Romberg 1985). In *Acer*, there is little evidence of such classic heterodichogamy. Instead, heterodichogamous trees coexist with males and inconstant protandrous and duodichogamous individuals (e.g., Wittrock 1886; Svobodová 1967; Asai 2000; Sato 2002; Tal 2006; additional studies are cited in Table 1). Fluctuations in flowering apparently can stabilize monoecy with heterodichogamous flowering, rather than leading to dioecy (Gleiser et al., unpubl. ms.).

INCONSTANT MALES, GENETIC MALES (ANDRODIOECY), AND LABILE SEX

That inconstant individuals are very common in *Acer* has long been noted (de Jong 1976; Gleiser et al., unpubl. ms.; Table 1 for additional references). de Jong suggested that occasional all-male flowering, rarely all-female flowering, in normally dichogamous or duodichogamous trees is stress-induced, because the same trees usually are duodichogamous or protandrous in other years. In some species, however, the phenotypic sex of all individuals appears to be ontogenetically labile (Wittrock 1886; Haas 1933; de Jong 1976; Hibbs and Fischer 1979; Freeman et al. 1980; Barker et al. 1982; Sakai and Oden 1983; Primack and McCall 1986; Sakai 1990; Matsui 1995; Bendixen 2001; Asai 2000; Sato 2002). As is true of phenotypic plasticity in general, labile sex is likely to evolve when stress periods occur at appropriate frequency (Gabriel 2005), such that individuals fare better by constantly adjusting their sex allocation than by adopting a fixed sexual strategy. From the phylogeny (Figs. 1, 2), it appears that species with labile sex determination are concentrated in section *Macrantha* (the *A. davidii*-*A. pensylvanicum* clade), a North American/Asian clade that may be 28 MY (16–46) old (Figs. 1, 2). This clustered occurrence of sexual phenotypic plasticity indicates that it is a heritable sexual strategy.

In well-studied sexually plastic species, such as *A. saccharum* subsp. *grandidentatum* and *A. rufinerve*, links between precipitation, water access, and sex expression have been demonstrated (Barker et al. 1982; Matusi 1995; Ushimaru and Matsui 2001; Nanami et al. 2004; also for *A. pensylvanicum*: Hibbs and Fischer 1979, *A. pseudoplatanus*: Jones 1945a; Scholz 1960; Semm 1966; Binggeli 1992, *A. rubrum*: Primack and McCall 1986; Sakai 1990, and *A. saccharinum*: Sakai 1978; Sakai and Oden 1983). These and other studies (Jones 1945b; Wittrock 1886; Haas 1933; Asai 2000; Bendixen 2001) suggest that at least in some species of *Acer*, male and female reproductive outputs are differentially affected by habitat quality as envisioned by theories of plant sex choice (Charnov and Bull 1977; Freeman et al. 1980; Korpelainen 1998; Guillon et al. 2006).

The commonest and perhaps ancestral (see above) sexual system in *Acer* is monoecy with dichogamous and duodichogamous flowering. Duodichogamy is only known from four or five unrelated groups of flowering plants (Luo et al. 2007) and may be favored by intrasexual competition among pollen parents for access to ovules (Lloyd and Webb 1986: p. 147). Such competition is expected to be strongest if few ovules are offered or matured into fruits. Indeed, all known duodichogamous taxa (*Castanea*, *Cladium*, *Dipteronia*) have few ovules, and *Acer*, with two ovules per ovary of which only one develops into a seed, is no exception. Spring-blooming trees with vast numbers of flowers, such as maples, may also experience pollinator scarcity, at least in some years. Both factors may favor duodichogamy, which in turn may facilitate the establishment of inconstant males or male mutants (i.e., androdioecy) if inconstant males physiologically are able to “commit” to fruiting late under particularly good conditions (other pollen donors still be available because of the second male phase of duodichogamous trees).

Together, the macroevolutionary perspective on sexual system evolution in *Acer* provided here and results from recent modeling efforts (Ehlers and Bataillon 2007; Gleiser et al., unpubl. ms.) suggest that monoecy with variable flowering strategies and the ability of some fraction of trees to change sex expression set up conditions that slowed down or prevented the evolution of sex specialization (dioecy). Even if some of the species included here turn out erroneously scored in terms of their flowering pattern (duodichogamy vs. heterodichogamy), this would not change the distribution of dioecy relative to monoecy nor the conclusion that dioecy in *Acer* evolved very few times, and mostly a long time ago. The phylogenetic clustering of the sexually most plastic species (Fig. 2) was unexpected. There are several other clades of flowering plants that appear to be characterized by labile sex expression (also referred to as environmental sex determination, gender diphasy, or sex choice [Freeman et al. 1980; Schlessman 1988; Korpelainen 1998; Guillon et al. 2006]), for instance, species in the genera *Gurania* and *Psiguria* in the Cucurbitaceae, several oil palms (*Elaeis*), *Arisaema* (Araceae), and Catesetinae orchids (Schlessman 1988), implying that sexual plasticity can be an inherited trait.

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Supplementary Material

The following supplementary material is available for this article:

Table S1. Species with their placement in the classification of van Gelderen et al. (1994) and status as nomenclatural types, DNA sources, geographic origin, chloroplast regions sequenced and GenBank accession numbers. BG stands for botanical garden, s.n. for sine numero (without collection number), and TROPICOS for the specimen database of the Missouri Botanical Garden at <http://mobot.mobot.org/W3T/Search/vast.html>.

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