

# Repeated evolution of dioecy from androdioecy in *Acer*

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## Summary

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- The evolution of breeding systems was studied in the genus *Acer*, with special attention to the origin of androdioecy and dioecy, using a phylogenetic approach.
- Parsimony and maximum-likelihood techniques were used to infer the ancestral character state and trends in the evolution of breeding systems. Information on breeding systems was obtained from the literature, and phylogenetic relationships were taken from three published phylogenies.
- Although a general trend from duodichogamy to dioecy through heterodichogamy has been proposed for the genus *Acer*, our results show that a general trend is not detected when phylogenetic relationships are taken into account. Dioecy appeared as a derived state that evolved at least three times and never reversed towards other states.
- Three different paths to dioecy have been followed in the genus *Acer*: from heterodichogamous androdioecy; from heterodichogamous trioecy; and from dichogamous subdioecy. Therefore, although the best documented cases of evolution of androdioecy indicate that this breeding system evolves from dioecy, in the genus *Acer* the opposite situation occurs (androdioecy leading to dioecy). Here we discuss the role of inbreeding avoidance and sexual specialization as selective forces driving the evolution of dioecy in the genus *Acer*.

**Key words:** *Acer*, androdioecy, comparative method, evolution of dioecy, evolutionary trends, heterodichogamy, sexual specialization.

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## Introduction

Although dioecy appears in only 6% of flowering plant species, it has arisen independently in over half of all plant families (Renner & Ricklefs, 1995). Ever since Darwin (1877), evolutionary biologists have tried to explain the origin of dioecy in flowering plants, but there has been increasing interest in studying the pathways to dioecy in recent decades.

Dioecy has been proposed to have evolved from monoecy (Charlesworth & Charlesworth, 1978b; Lloyd, 1980; Webb, 1999; Renner & Won, 2001; Dorken *et al.*, 2002); from heterostyly (Lloyd, 1979; Webb, 1999); from heterodichogamy (Lloyd, 1979; Dommée *et al.*, 1990; Webb, 1999; Pendleton *et al.*, 2000); and from hermaphroditism through gynodioecy (Lloyd, 1974; Charlesworth & Charlesworth, 1978a; Lloyd, 1980; Charlesworth, 1999; Webb, 1999). In this last path, the first step is the establishment of a single mutant form (male-sterile). In the same way, androdioecy could be an inter-

mediate step in the evolution of dioecy through the spread of a female-sterility mutation. The rarity of androdioecy relative to gynodioecy indicates that conditions for the invasion of a female-sterile form are more difficult to meet than conditions for the invasion of a male-sterile form, and several authors have demonstrated theoretically why this is so (Lloyd, 1975; Charlesworth, 1984, 1999; Webb, 1999; Pannell, 2002). Therefore gynodioecy has usually been considered as a more likely intermediate step to dioecy than androdioecy (Lloyd, 1975; Charlesworth & Charlesworth, 1978a; Charlesworth, 1984). Darwin (1877) discussed the role of gynodioecy in the evolution of dioecy, and speculated about the possible role androdioecy may have played, but he was aware of the lack of androdioecious species in nature and so concluded that there was no use in considering this case. Furthermore, whereas early ideas concerning androdioecy tended to focus on the invasion of males into hermaphrodite populations, the clearest examples of androdioecy appear to have evolved from dioecy

(Rieseberg *et al.*, 1992; Krahenbuhl *et al.*, 2002; Pannell, 2002); to date the evolution of dioecy from androdioecy has remained only a theoretical possibility.

Although androdioecy is an unusual breeding system, it has been identified in several species belonging to phylogenetically divergent taxa, most of which include dioecious species [Liston *et al.*, 1990, *Datisca glomerata* (Datisceae); Lepart & Dommée, 1992, *Phillyrea angustifolia* (Oleaceae); Pannell, 1997, *Mercurialis annua* (Euphorbiaceae); Valiente-Banuet *et al.*, 1997, *Neobuxmaumia mezcalaensis* (Cactaceae); Ishida & Hiura, 1998, *Fraxinus lanuginosa* (Oleaceae); Akimoto *et al.*, 1999, *Schizopepon bryoniaefolius* (Cucurbitaceae); Dommée *et al.*, 1999; Verdú, 2004, *Fraxinus ornus* (Oleaceae); Sakai, 2001, *Castilla elastica* (Moraceae)]. The presence of androdioecious species within a taxonomic group represents a challenge in studying evolutionary trends in reproductive systems. The genus *Acer* (maples) offers an interesting scenario as it contains great variability in reproductive systems, including androdioecy and dioecy. On the basis of flower morphology and physiology, de Jong (1976) defined five groups of species according to their breeding system, and postulated an evolutionary progression in breeding systems in the genus from duodichogamy (a dichogamous sequence of male–female–male flowering) through intermediate dichogamous breeding systems to dioecy. Interestingly, one of the intermediate dichogamous breeding systems corresponds to androdioecy. Later, de Jong (1994) suggested that one of these intermediate states (a subdioecious state) may depart from the main trend. Until now, this evolutionary trend has not been tested in a phylogenetic context. Furthermore, the functionality of each of these breeding systems is largely unknown for most *Acer* species. In this work we used a phylogenetic approach to test whether androdioecy in *Acer* is derived from dioecy, or whether it constitutes a previous state in the evolution towards dioecy, including breeding-systems data based on morphological descriptions. We also tested whether there is an evolutionary trend following the sequence duodichogamy–dichogamous intermediate forms–dioecy, as proposed by de Jong (1976). Finally, we discuss the trend towards dioecy in the light of the few empirical data on the functionality of the breeding system in *Acer* species.

## Methods

### Breeding-systems data

Five different breeding systems, following the evolving stages defined by de Jong (1976, 1994), could be distinguished in the genus: duodichogamous monoecy; heterodichogamous androdioecy; heterodichogamous trioecy; dichogamous subdioecy; and dioecy. The main features of these breeding systems are summarized in Table 1. Information on sex expression and morph frequencies taken from de Jong (1976) was used to classify the species included in the phylogenies into one of these breeding systems.

**Table 1** Main features of the five breeding systems in the genus *Acer*

Breeding system	Main features
Duodichogamous monoecy	Monoecious individuals with duodichogamous flowering (male–female–male sequence)
Heterodichogamous androdioecy	Males (in low frequency) and bisexual individuals; the bisexuals are heterodichogamous (two dichogamous reciprocal morphs, protandrous and protogynous)
Heterodichogamous trioecy	Males (in increasing frequencies) and females (in various frequencies), and heterodichogamous bisexuals
Dichogamous subdioecy	Mainly unisexual individuals (males and females), and dichogamous bisexuals in low proportions.
Dioecy	Unisexual individuals only (males and females)

### Phylogenetic analyses

The evolution of breeding systems in the genus *Acer* was reconstructed using parsimony methods of MACCLADE 3.08 (Maddison & Maddison, 1992). Five states of breeding system (0, duodichogamous monoecy; 1, heterodichogamous androdioecy; 2, heterodichogamous trioecy; 3, dichogamous subdioecy; 4, dioecy) were traced on the following three different published phylogenies: Ackerly & Donoghue (1998), inferred from nuclear ribosomal internal transcribed spacer (ITS) sequences and with a consistency index (CI) of 0.58; Suh *et al.* (2000), inferred from ITS of nuclear ribosomal DNA, CI = 0.45; and Tian *et al.* (2002), built with combined data from nuclear ribosomal ITS sequences and the chloroplast gene *trn* L-F, CI = 0.60. A supertree was constructed by pooling information from the three phylogenies using the SUPERTREE Server at <http://darwin.zoology.gla.ac.uk/cgi-bin/supertree.pl>, but because of the low resolution obtained (a high number of polytomies) the supertree was discarded and, instead, the analyses were run separately in each phylogeny. The breeding system was considered as an ordered character from 0 to 4 because each state represents an increasing number of unisexuals relative to dichogamous individuals. All the state changes were equally weighted because no *a priori* hypotheses exist on the transition rates between breeding systems. Both ACCTRAN and DELTRAN optimizations were explored to resolve equivocal tracings. The few polytomies in the phylogenetic trees (one in the phylogeny of Ackerly & Donoghue, 1998 and one in that of Suh *et al.*, 2000) were randomly resolved. This procedure did not change the results because the species involved in a single polytomy always had the same state character, therefore the tracing was the same irrespective of the topological solution.

Maximum-likelihood methods allow hypothesis tests that are not available to parsimony methods (Pagel, 1999a).

Branch lengths are irrelevant for parsimony but not for maximum-likelihood comparative methods (Felsenstein, 2004). Thus equal branch lengths were considered when information was not provided (Ackerly & Donoghue, 1998). Equal branch lengths are appropriate under a punctuational evolutionary model (Ackerly, 2000; Verdú, 2002). Zero branch lengths in both phylogenies (Suh *et al.*, 2000; Tian *et al.*, 2002) were led to an infinitesimal value ( $1E-10$ ) to allow the software to run without distorting the original data (see Nosil, 2002; Traveset *et al.*, 2004 for a similar procedure). The branch length of the *Dipteronia* outgroup in the Suh *et al.* (2000) phylogeny was also missing, and an arbitrary value was assigned. This value was obtained by adding 1 to the longest branch length in the tree, under the expectation that the relationship between *Dipteronia* and *Acer* ancestors is more distant than that between two nodes within the genus *Acer*. To be sure that this arbitrary assignment did not affect the results, we ran the tests with different *Dipteronia* branch lengths and checked that the conclusions did not change.

Changes in discrete characters were inferred by means of maximum-likelihood estimation techniques that use the Markov transition-rate model (Pagel, 1994; Mooers & Schluter, 1999) with the MULTISTATE (Pagel, 1994, 1997, 1999a, 1999b) and MESQUITE (Maddison & Maddison, 2003) software programs. A 'full' model requires the estimation of  $(n^2 - n)$  parameters and a minimum sample size of 10 taxa per parameter (Pagel, 1994, 1997, 1999a, 1999b). Thus our full five-state model of breeding-system evolution would contain 20 parameters, yielding an optimum sample size of 200 *Acer* species. Similarly, the full four-state model (if subdioecy is excluded) would require the estimation of 12 parameters and 120 species. These sample sizes are greater than the 111 living species in the genus (Mabberley, 1997), and for this reason it is desirable to fit simpler models without losing significant information. This simplification can be done by fitting a simpler model after the proper restriction of several parameters. One of the most typical simplifications is the nested model in which only two parameters are calculated: the forward and backward rates. In this two-parameter asymmetrical model a single forward parameter is calculated after restricting all forward parameters (changes in which state numbers increase) to be equal. Similarly, a single backward parameter is calculated once all the backward parameters (changes in which state numbers decrease) have been constrained to be equal. The asymmetry in the model comes from the fact that forward and backward rates are allowed to be different. The log-likelihoods of the full models were compared with those of the two-parameter asymmetrical models by means of the likelihood ratio (LR) test. This test subtracts the smaller from the larger log-likelihood and distributes as a  $\chi^2$  with degrees of freedom equal to the difference in the numbers of parameters between the two models. If the two models do not differ from each other at a liberal  $\alpha$  value ( $P > 0.20$ ) then the simpler model (forward/backward model) can be used to represent the data.

Such a liberal  $\alpha$  value is used to increase the power of the test, following the rationale of the MULTISTATE manual.

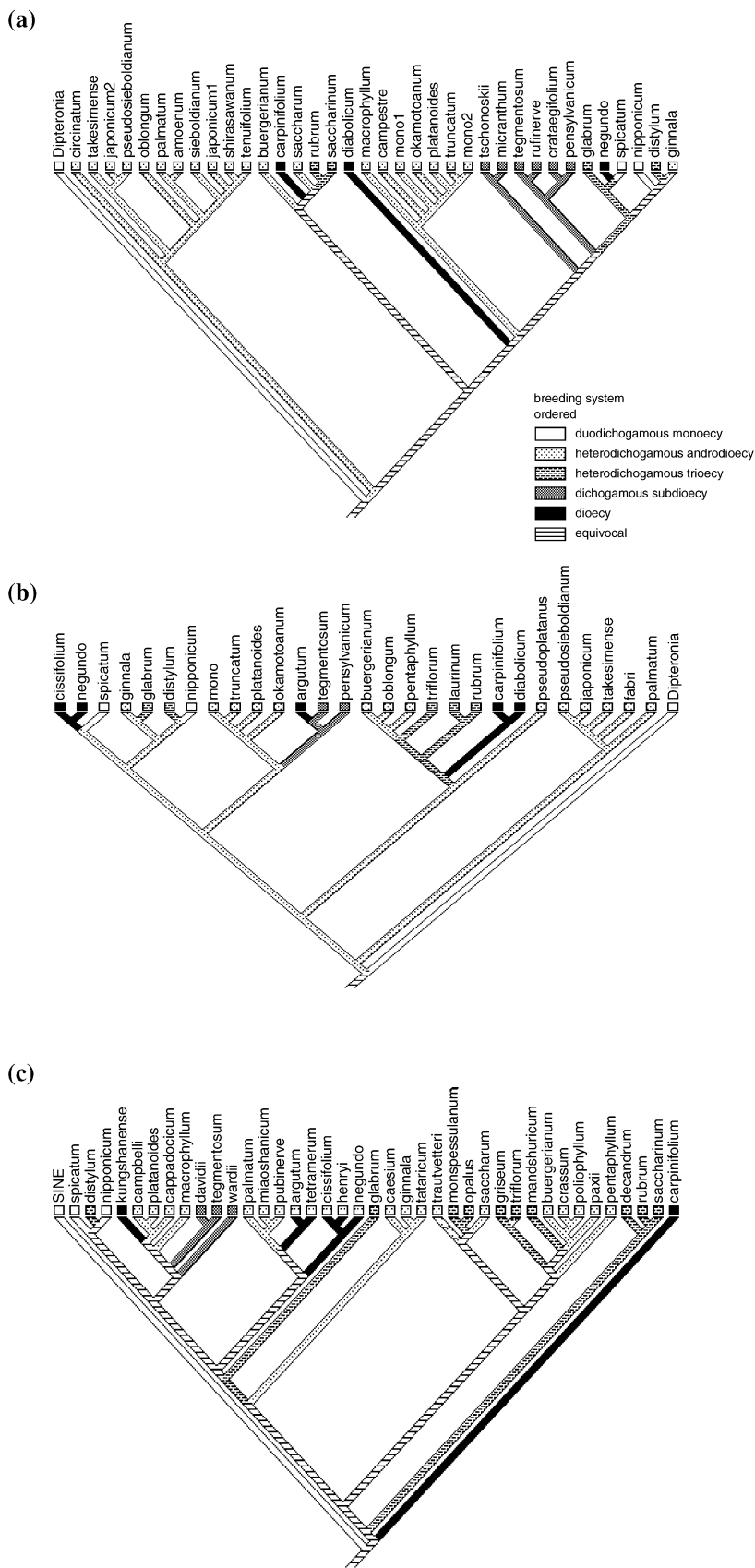
The two-parameter asymmetrical model provides not only a statistical method to simplify the full model, but also a theoretical hypothesis in which a bias in gains vs losses (or *vice versa*) can be tested (Nosil, 2002). Thus if an evolutionary trend towards dioecy from duodichogamy exists on *Acer* through intermediate breeding systems (heterodichogamy, androdioecy, subdioecy), then we would expect a bias on gains in state numbers and therefore a forward rate greater than a backward rate. To test if forward > backward rates, we compared the two-parameter asymmetrical model with another simpler, nested model in which both rates were constrained to be equal. Again, in the absence of significant differences, the simpler model (the one-parameter model in which forward = backward) can be used to represent the data.

Although the presence of a bias (gains > losses or forward > backward rates) almost inevitably suggests a trend, the absence of the bias does not necessarily suggest its nonexistence (McShea & Venit, 2002). For this reason we tested specifically with another algorithm for the existence of an evolutionary trend towards dioecy in the CONTINUOUS software (Pagel, 1997, 1999). This software allows the detection of directional trends by comparing an unbiased random-walk model of evolution (Brownian motion) against a directional random-walk model (Pagel, 1999). The directional model has an additional directional change parameter that measures the regression of trait values across taxa against total path length from root to tips of the phylogeny (Pagel, 1997, 1999a). Both models are compared by means of a generalized least-squares approach in which the log-likelihoods of the directional and standard models are compared statistically (Pagel, 1994, 1999). We could treat the breeding system as a continuous variable, as requested by the CONTINUOUS software, because its different states represent a continuous increase in the percentage of unisexuals relative to dichogamous individuals (a dioecious species contains 100% unisexuals and 0% dichogamous individuals).

In addition, all the analyses were run again after excluding the subdioecy state to account for the later suggestion of de Jong (1994), in which this state is thought to depart from the main path leading to dioecy.

## Results

The ancestral breeding system in the genus *Acer* was unambiguously reconstructed as heterodichogamous androdioecy in two of the three phylogenies (Fig. 1a,b), whereas the third phylogeny (Fig. 1c) traced an equivocal ancestor state between heterodichogamous androdioecy and trioecy (DELTRAN optimization did not resolve this ambiguity and ACCTAN resolved towards trioecy). Furthermore, duodichogamy always appeared as a derived and rare breeding system. In contrast, heterodichogamous androdioecy was a common ancestral



**Fig. 1** Breeding system evolution within the genus *Acer* and outgroup species traced in different phylogenetic trees (Ackerly & Donoghue, 1998; Suh *et al.*, 2000; Tian *et al.*, 2002) in which the most parsimonious states are shown.

**Table 2** Numbers of stasis and changes between states from one node to the next

From	To				
	Duodichogamous monoecy	Heterodichogamous androdioecy	Heterodichogamous trioecy	Dichogamous subdioecy	Dioecy
(a)					
Duodichogamous monoecy	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0
Heterodichogamous androdioecy	0/0/1	33/42/35	0/2/0	0/0/0	0/2/0
Heterodichogamous trioecy	1/2/1	0/1/3	5/10/9	0/2/1	1/1/2
Dichogamous subdioecy	0/0/0	0/0/1	0/0/1	8/8/13	0/0/1
Dioecy	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0
(b)					
Duodichogamous monoecy	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0
Heterodichogamous androdioecy	2/2/2	31/31/31	3/3/3	1/1/1	1/1/1
Heterodichogamous trioecy	0/0/0	1/1/1	6/6/6	0/0/0	1/1/1
Dichogamous subdioecy	0/0/0	0/0/0	0/0/0	3/3/3	1/1/1
Dioecy	0/0/0	0/0/0	0/0/0	0/0/0	4/4/4
(c)					
Duodichogamous monoecy	0/0/3	0/0/0	0/0/1	0/0/0	0/0/0
Heterodichogamous androdioecy	0/0/0	18/18/30	0/0/3	0/0/0	0/0/1
Heterodichogamous trioecy	0/2/0	0/3/2	9/20/14	0/2/1	0/3/1
Dichogamous subdioecy	0/0/1	0/0/1	0/0/0	2/2/7	0/0/1
Dioecy	0/0/0	0/0/1	0/0/0	0/0/0	6/6/9

Stasis numbers are shown on the diagonal, forward changes above and backward changes below the diagonal. The three numbers in each cell represent the unambiguous, unresolved; DELTRAN-resolved; and ACCTTRAN-resolved changes, respectively. Tables from top to bottom correspond to phylogenies published by (a) Ackerly & Donoghue (1998); (b) Suh *et al.* (2000); (c) Tian *et al.* (2002).

state, presenting a lot of stasis but also evolutionary (mainly forward) changes (Table 2).

In all three phylogenies in which the breeding systems were traced by parsimony methods, dioecy was a derived state that evolved at least three times independently (Fig. 1). This independent origin could be less evident in the phylogeny of Tian *et al.* (2002) because most of the branches leading to dioecy are equivocal; nevertheless these ambiguities never involved a dioecious state, meaning that dioecy must have arisen at least three times. Three different paths towards dioecy have been followed in the genus: from heterodichogamous androdioecy; from heterodichogamous trioecy; and from dichogamous subdioecy. Dioecy was the only state that never reversed towards other states. All possible reversions between the rest of the states were detected before or after the ACCTTRAN/DELTRAN resolution of equivocal tracings (Table 2). In general, the numbers of forward and backward changes were not statistically different from each other (none of the  $\chi^2$  tests departed significantly from 1 : 1).

Under maximum likelihood, all the full models could be simplified to simpler forward/backward models because both nested models never differed significantly from each other even at a high liberal  $\alpha$  value (see LR test values for model simplification, Table 3). Forward rates did not differ from backward rates in any of the phylogenies studied, as the log-likelihoods of the forward/backward model and the one-parameter model, in which forward = backward, were not

significantly different (see LR test values for gain/losses bias, Table 3). A general evolutionary trend towards dioecy was not detected in any of the three phylogenies when the breeding system was treated as a continuous variable, as long as the log-likelihoods of the directional random-walk models never differed significantly from those of the standard (nondirectional) constant-variance random walk models (see LR test values for evolutionary trend, Table 3).

Exclusion of the subdioecy state from the analyses did not alter the results described above, and for simplicity, they are not shown here. The single difference is that one reversion from dioecy to androdioecy was detected in the Tian *et al.* (2002) phylogeny after ACCTTRAN optimization.

## Discussion

The current phylogenetic analysis shows that dioecy has evolved repeatedly from androdioecy in the genus *Acer*. Nevertheless, there is no general trend in the genus because dioecy has also arisen in some clades from other paths, as dichogamous subdioecy and heterodichogamous trioecy, indicating that there is no common mechanism for the appearance of unisexuals in the genus. The ancestral breeding system in *Acer* was reconstructed as heterodichogamous androdioecy in two of the three phylogenies, and was ambiguous in the third. Nevertheless, the possibility of a duodichogamous ancestor in the genus could not be ruled out



**Table 3** Maximum-likelihood tests comparing two nested models following different hypotheses in different phylogenetic trees (Ackerly & Donoghue, 1998; Suh *et al.*, 2000; Tian *et al.*, 2002)

Hypothesis	Phylogeny	Model					Statistical test	
		Full (20)	Forward-backward (2)	Forward = backward (1)	Non-directional (1)	Directional (2)	LR test	P
Model simplification	Ackerly	-29.074	-38.758				19.36	0.36
	Suh	-28.263	-35.835				15.14	0.65
	Tian	-41.944	-47.775				11.66	0.86
Gains/losses bias	Ackerly		-38.758	-38.838			0.16	0.69
	Suh		-35.835	-35.918			0.17	0.68
	Tian		-47.775	-47.880			0.21	0.64
Evolutionary trend	Ackerly				-58.259	-58.255	0.01	0.92
	Suh				-7.635	-6.868	1.53	0.21
	Tian				-46.152	-46.164	0.02	1.00

Each hypothesis compares two models by means of a likelihood ratio (LR) test shown in the right-hand columns. Number of parameters estimated by each model is shown in parentheses.

entirely. The breeding system of the common ancestor of *Acer* and the outgroup was ambiguous in the three phylogenies, and this ambiguity was always between duodichogamy and other breeding systems. Thus the ancestor of *Acer* could have been duodichogamous; the earliest members of *Acer* (those just after the split with the outgroup) could have retained this breeding system, and the transition to the other states could have occurred before the radiation of the genus.

The conclusions drawn in this paper depend on the accuracy of the underlying phylogenetic hypotheses (Ackerly, 2000). It can be noted that the *Acer* species represented in each of the three published phylogenies are not a random sample of the genus, but a geographically biased sample depending on the author's country (the Chinese authors include Chinese species that are not included in the other phylogenies). However, the consistency of the results across the three different phylogenies makes the conclusions very robust.

Most of the phylogenetically documented cases of the evolution of androdioecy indicate the opposite path (that androdioecy evolves from dioecy) (Fitch *et al.*, 1995; Weller & Sakai, 1999; Haag & Kimble, 2000; Krahenbuhl *et al.*, 2002; but see Wallander, 2001). Liston *et al.* (1990) proposed that androdioecy can evolve from dioecy under a situation of pollen limitation. If seed production by females is limited by a lack of pollen, a mutation allowing pollen production in females will be strongly selected, assuming these females are self-compatible. Recently, Wolf & Takebayashi (2004) have demonstrated theoretically that self-fertilization is a requirement for androdioecy to evolve from dioecy, while high outcrossing rates are necessary for androdioecy to evolve from hermaphroditism. All the species in the genus *Acer* are 'obligate outcrossers', as self-fertilization is avoided by a temporal segregation of the sexual phases. This situation satisfies the premise for androdioecy to have evolved not from dioecy

but from hermaphroditism. In support of this theoretical argument, we provide here phylogenetic evidence confirming that androdioecy has not evolved from dioecy in the genus *Acer*. The alternative hermaphroditic origin of androdioecy could not be tested in this work, as androdioecy appeared to be the most probable ancestor state. In that case it would be necessary to trace back the evolution of androdioecy at a higher taxonomic level.

The evolution of dioecy has generated much debate, and two main hypothesis have been put forward: the predominant view, that separation of the sexes arose because it reduces inbreeding; and a less common view, that selection for sexual specialization has driven the evolution of dioecy (Freeman *et al.*, 1997). As Darwin (1877) pointed out, there are effective alternatives for avoiding inbreeding apart from dioecy, such as herkogamy and dichogamy. Dichogamy is present in all nondioecious *Acer* species, so an inbreeding-avoidance mechanism was acquired early within the genus. Thus inbreeding avoidance does not seem to be a likely explanation for the origin of dioecy in this genus. Sexual specialization is the most plausible force driving the evolution of separate sexes in the genus *Acer*. It has been argued that sexual specialization is the selective agent responsible for the evolution of dioecy via monoecy (Freeman *et al.*, 1997). The following traits often co-occur with sexual specialization: an outbreeding mechanism such as dichogamy; spatial segregation of the sexes; and sexual lability (Freeman *et al.*, 1997). Many of these features have already been reported in maple species. For example, spatial segregation of the sexes has been detected in *Acer grandidentatum* (Baker *et al.*, 1982) and *Acer negundo* (Dawson & Ehleringer, 1993). Sexual changes have been reported in *Acer pensylvanicum* (Hibbs & Fischer, 1979), *Acer rufinerve* (Matsui, 1995) and *Acer japonicum* (Sato, 2002). In *A. japonicum*, an androdioecious species with populations composed of

males, protandrous and protogynous trees, sexual changes between male and protandrous morphs were detected. In addition, the flowering period of the male trees coincided with the male stage of protandrous individuals, suggesting that stable males may have been derived from the protandrous morph. In another morphologically androdioecious species, *Acer opalus*, the same morphs are present in the populations and sexual changes between males and protandrous trees also occur (Gleiser *et al.*, 2004; Verdú *et al.*, 2004a). Nevertheless, an important difference exists between these two species: while males in *A. japonicum* are present at very low frequency (< 10%), in *A. opalus* the frequency of males is  $\approx$  50% (Verdú *et al.*, 2004a). According to theoretical models (Lloyd, 1975; Charlesworth & Charlesworth, 1978a; Charlesworth, 1984) the frequency of males in a functionally androdioecious population is always < 50%, and reaches 50% only when the male function of hermaphrodites approaches 0, that is, when the hermaphrodites specialize as females and then the population is functionally dioecious (Pannell, 2002). Both situations are present in *Acer*, supporting the evolution of dioecy from androdioecy through sexual specialization. It must be noted that the functionality of androdioecy in genus *Acer* has not still been demonstrated, and our results are based on morphological data. This fact deserves careful attention, because morphological androdioecy has often turned out to be cryptic dioecy (Pannell, 2002; Verdú *et al.*, 2004b). In the case of *A. japonicum*, however, the sex ratios are consistent with a functionally androdioecious breeding system. Future studies on the functionality of breeding systems are necessary to strengthen (or weaken) our conclusions.

In conclusion, dioecy is a derived state in the genus *Acer*, but the pathway proposed by de Jong (1976), from duodichogamy through heterodichogamy to dioecy, has no phylogenetic support. Instead, the ancestral state was found to be heterodichogamous androdioecy, although the possibility of a duodichogamous ancestor could not be entirely discarded. Furthermore, dioecy evolved from three different pathways: from heterodichogamous androdioecy; from heterodichogamous trioecy; and from dichogamous subdioecy. None of the current theoretical models describes these pathways towards dioecy; we believe that such models will help us to understand the evolution of dioecy.

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