

## PHYLOGENETIC IMPLICATIONS OF THE MULTIPLE LOSSES OF THE MITOCHONDRIAL *coxII.i3* INTRON IN THE ANGIOSPERMS

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Previous studies have shown that the mitochondrial *coxII.i3* intron is absent in all rosids examined, in *Philadelphus* (Hydrangeaceae, Cornales), and in *Catharanthus* and *Vinca* (Apocynaceae, Gentianales). We surveyed for the presence or absence of this intron in 177 species representing all orders of angiosperms, where it is primitively present. The intron appears to have been lost independently in Gnetales, Laurales, Zingiberales, Ranunculales, Saxifragales, rosids, Santalales, Caryophyllales, Ericales, Cornales, Gentianales, Lamiales, Boraginales, Aquifoliales, Asterales, Dipsacales, as well as in the genus *Escallonia*. Depending upon the phylogenies used to interpret the losses, and due to lack of resolution in some groups, the intron could have been lost up to 27 times in the angiosperms (excluding the loss in Gnetales). The losses sometimes corroborate the monophyly of groups (rosids and Lamiales) or of subgroups within orders (in the Ranunculales, Caryophyllales, Cornales, Gentianales, and Asterales). In other groups, such as the Saxifragales, Ericales, and Dipsacales, the patterns of losses are more complex and would require further study. The presence or absence of the *coxII.i3* mitochondrial intron seems a useful phylogenetic marker in some groups, but caution in interpretation is needed as multiple parallel losses have occurred throughout the angiosperms.

**Keywords:** angiosperms, phylogenetic marker, mt DNA, *coxII.i3* intron, structural rearrangement, intron loss, eudicots, rosids.

### Introduction

Major efforts have been made in recent years to improve our knowledge of phylogenetic relationships within the angiosperms (Chase et al. 1993; Soltis et al. 1997, 1999, 2000; Angiosperms Phylogeny Group [APG] 1998; Qiu et al. 1999; Savolainen et al. 2000b). Relationships are becoming clearer, but areas of uncertainty remain, and the search for phylogenetically informative characters is ongoing. Among these, structural rearrangements of the organellar genomes have been used as phylogenetic markers in several groups of plants (Downie et al. 1991; Downie and Palmer 1994; Qiu and Palmer 1997; Qiu et al. 1998b; Graham and Olmstead 2000). For example, previous studies have suggested that the presence or absence of a group II intron in the cytochrome oxidase subunit II mitochondrial gene, the *coxII.i3* intron, could represent a suitable character in supporting the delimitation of taxa in angiosperms (DeBenedetto et al. 1992; Rabbi and Wilson 1993, and references therein; Dong et al. 1997, 1998). We chose to further investigate this structural rearrangement.

The first survey of the loss of the *coxII.i3* intron in angiosperms by DeBenedetto et al. (1992) raises the hypothesis that the loss of this intron could characterize the rosids (*sensu* APG 1998). The only exception to this pattern is the apparently independent loss of the intron in *Philadelphus*, now placed in the Hydrangeaceae (Cornales, Asterid) (Cronquist 1981; Soltis et al. 1995; APG 1998). A subsequent study supported the hypothesis that the intron loss is characteristic of the rosids but

introduced evidence for a further intron loss in two closely related genera of Apocynaceae, *Vinca* and *Catharanthus* (Rabbi and Wilson 1993). More recently, Qiu and Palmer (1997) suggested that the loss of the *coxII.i3* intron occurred in the common ancestor of rosids I and II. The *coxII.i3* intron was found in most mosses, ferns, and gymnosperms (Dong et al. 1997; Qiu et al. 1998b), suggesting that its presence is ancestral within the flowering plants. Following Qiu et al. (1998b), we use the name *coxII.i3* for this intron, rather than the *coxIIIi1* nomenclature of Dong et al. (1998), because two additional upstream introns are present in *Marchantia* (Y.-L. Qiu, personal communication).

Because the taxon sampling in the previous studies was limited within angiosperms (but see Qiu and Palmer 1997), we surveyed for the presence or absence of this intron in representative species of all orders of angiosperms, using the classification of the APG (1998) as our basis for selecting taxa and for interpreting the results. Our first objective was to determine whether the intron loss could be a good phylogenetic marker for the rosids, as defined by the APG (1998), thus helping to delimit this major angiosperm clade. Further, because independent losses were known to have occurred in at least two other angiosperm groups, we chose to survey widely across the angiosperms to assess the utility as a phylogenetic marker of the intron loss in other groups.

### Material and Methods

A total of 177 species representative of each order of basal angiosperms, monocots, and eudicots was surveyed for the presence or absence of the *coxII.i3* intron. Because our primary emphasis was on eudicots, the monocots and basal angio-

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sperms were less extensively sampled (only one representative per order). We also sampled *Gnetum* as a representative of the gymnosperms. In groups where the intron was absent, we increased our taxon sampling when material was available to better circumscribe the intron loss. Most plants were sampled from the living collections of the Montreal Botanical Garden, a few samples were obtained in the field, and others came from plants bought at markets in the Montreal area. Vouchers are deposited at the Herbarium Marie-Victorin (MT) (appendix).

To investigate the presence or absence of the intron, we amplified the intron region using two primers corresponding to the 5' and 3' exon coding sequences. The primers used were those designed by Rabbi and Wilson (1993); their sequences are given in table 1. The *coxII-A* primer is located 178 bp upstream from the intron, while the *coxII-Icr* primer is 89 bp downstream from the site of excision. In their study, a probe corresponding to the intron sequence hybridized with the larger amplified fragments (ranging from 1200 to 1635 bp) indicating the presence of the intron, but it did not hybridize with the smaller fragments (ca. 300 bp) where the intron was absent. Similarly, using the polymerase chain reaction (PCR) method and their primers, amplification of large fragments should indicate the presence of the intron, while fragments of ca. 300 bp provide evidence for absence of the intron. To verify the validity of our approach, we amplified the intron region in several previously investigated species, including *Daucus carota*, *Capsicum* sp., *Cucumis sativa*, *Beta vulgaris*, and *Zea mays*.

Total DNA was extracted from 1 g of fresh leaf material following the CTAB (hexadecylmethyl-ammonium bromide) extraction method of Doyle and Doyle (1987). The PCR reaction contained, for a final volume of 100  $\mu$ L, 1 X PCR reaction buffer (containing 1.5 mM MgCl<sub>2</sub>), 1  $\mu$ M of each primer, 200  $\mu$ M of each dNTP, 1% of Igepal, 2 U of Taq polymerase (Boehringer), and 30 ng of DNA. Samples were brought to 94°C for 1 min (denaturation), 55°C for 1 min (annealing), and finally to 72°C for 3 min (elongation) for 35 cycles. Fragments were analyzed on 1.5% agarose gel (1 X TBE) with a 100 bp ladder marker (Boehringer), and the size was estimated to the nearest 10 bp after comparison with a logarithmic curve built from the migration distances of the ladder.

Our results are interpreted following the consensus tree given by the APG (1998), with some modifications as suggested by the following studies. The consensus reached in several recent studies was used for the position of the primitive angiosperms (Mathews and Donoghue 1999; Qiu et al. 1999; Sanderson et al. 1999; Soltis et al. 1999, 2000); the phylogeny of the monocots was adjusted using Davis (1999); the position of the Saxifragales, Vitaceae, Caryophyllales, Dilleniaceae, and Santalales in relation to eusids and asterids is that suggested by Soltis et al. (2000). The gymnosperms, including *Gnetum*, were presumed to be monophyletic, following Winter et al. (1999), Chaw et al. (2000), and Bowe et al. (2000). The tree used for optimization of the *coxII.i3* intron thus represents a composite of consensus trees from several of the above studies. In general, character optimization should be performed on one of the most parsimonious trees rather than on a consensus (Maddison and Maddison 1992). However, in all cases where polytomies occur in the consensus, the number of losses of the *coxII.i3* intron is not affected by the lack of resolution because they occur in

Table 1

**Sequences of the Two Primers Used to Survey the Presence or Absence of the Mitochondrial *coxII.i3* Intron in Angiosperms**

Primers	Sequence
<i>coxII.i3-A</i>	AAT CCA ATC CCG CAA AGG ATT
<i>coxII.i3-Icr</i>	CCC AAT TCT GGA TCA TCT TCT

Note. The primer sequences were taken from Rabbi and Wilson (1993).

derived positions within each of the clades. Potentially problematic cases are individually discussed.

Unlike group I introns, for which recent studies have shown a propensity for multiple parallel gains through lateral transfer (intron homing; Cho et al. 1998), group II introns are not known to transfer laterally (Palmer et al. 2000). For this reason, Dollo parsimony, which prohibits parallel gains, is preferred over Camin-Sokal parsimony in optimizing the loss of the *coxII.i3* intron (Le Quesne 1975; Farris 1977a, 1977b). In our interpretation of the evolution of the *coxII.i3* intron, we make the assumption that the loss is irreversible (Downie et al. 1998). The evolutionary pattern of the multiple losses of the *coxII.i3* intron in the angiosperms was therefore optimized manually using Dollo parsimony on the consensus tree.

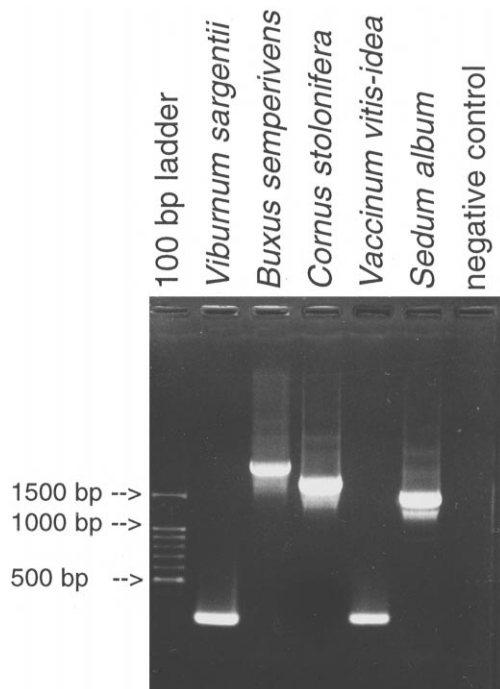
## Results

A single band was obtained in all PCR reactions. When the amplified fragment ranged in size from 710 (*Acorus calamus*) to 3400 bp (*Magnolia lilifolia*), the intron was assumed to be present (fig. 1). When the amplified fragment varied from 240 to 300 bp, the intron was considered to be absent (fig. 1). The species previously investigated (including *Daucus carota*, *Capsicum* sp., *Cucumis sativa*, *Beta vulgaris*, and *Zea mays*) gave the same results as those obtained in other studies, confirming the validity of our approach.

The presence or absence of the *coxII.i3* intron and its size for all the taxa studied are given in the appendix, and its loss is optimized on the consensus tree (fig. 2). Terminal taxa on the tree are orders, except for families that were not assigned to orders by APG (1998) or where increased resolution was needed for orders in which the intron was both present and absent (then shown as families or genera). Optimization on the phylogenetic trees suggests that independent losses occurred in Gnetales, Laurales, Zingiberales, Ranunculales, Saxifragales, rosids, Santalales, Caryophyllales, Ericales, Cornales, Gentianales, Lamiales, Boraginales, Aquifoliales, Asterales, Dipsacales, as well as in the genus *Escallonia* (figs. 2–5). Our sampling suggests that the loss in these groups may involve entire orders (or major groups as the rosids) or only a few families, genera, or even species within a particular order.

## Discussion

In many taxa, the loss of the *coxII.i3* intron corroborates recent phylogenies in supporting their monophyly or that of subgroups within them. Among the first branches of the angiosperms, the intron is present in almost all groups surveyed



**Fig. 1** PCR-amplified *coxII* gene for five species in 1.5% agarose gel treated with ethidium bromide. When the *coxII.i3* intron is absent (*Viburnum* and *Vaccinium*), the amplified fragment is constant (ca. 300 bp), but when present (*Buxus*, *Cornus*, and *Sedum*), it can vary from 790 (*Acorus calamus*; appendix) to 3400 bp (*Magnolia lilifolia*; appendix).

(i.e., basal angiosperms, most monocots; see below). For this reason and because the intron is present in most mosses, ferns, and gymnosperms (Qiu et al. 1998b), the intron is interpreted as being primitively present in the angiosperms, with its absence the result of a loss in more derived groups (fig. 2). We discuss the implications as a phylogenetic marker of the intron loss in each of the taxa where our survey suggested such a loss. We begin our discussion with taxa in which the intron loss seems to be a deep phylogenetic marker, followed by a discussion of groups in which the intron has been lost either more than once or in which the pattern of losses does not appear to corroborate recent phylogenetic studies.

#### *coxII.i3* as a Phylogenetic Marker

The absence of the intron in *Gnetum* suggests an independent loss in the gymnosperms because, besides the Gnetales, all gymnosperms have the intron, with the exception of *Metasequoia* (Qiu et al. 1998b). Although we only surveyed *Gnetum* within the order, Qiu et al. (1998b) reported a loss for *Ephedra* and *Welwitschia* also, thus providing a further phylogenetic marker for this order.

In the Ranunculales, the intron is absent in the Ranunculaceae and Berberidaceae but present in at least six of the seven other families of the order (Circaeasteraceae were not included due to lack of material). Thus, our data support recent phylogenetic analyses that suggest Ranunculaceae and Berberidaceae are sister groups (Chase et al. 1993; Hoot and Crane 1995; Kim and Jansen 1995; Savolainen et al. 2000a; Soltis et al. 2000) rather

than the more distant relationship proposed by Loconte et al. (1995).

As suggested from the results of previous studies (DeBenedetto et al. 1992; Rabbi and Wilson 1993), all rosids sampled lack the *coxII.i3* intron, implying a loss in the common ancestor of the clade. Qiu and Palmer (1997), who considered the intron loss to be a marker for rosids I and II, also noted this. The presence of the intron in the Vitaceae, recently proposed as the sister group of the rosids by Chase (1999), Savolainen et al. (2000a), and Soltis et al. (2000), further suggests that the loss is a synapomorphy of the rosid clade. Morphological, anatomical, and biochemical characters are needed to confirm such delimitation and to better characterize this group. The family Elatinaceae, placed in an uncertain position in eudicots by APG (1998), is now considered a member of Malpighiales by Savolainen et al. (2000b) based on a *rbcL* analysis. This hypothesis is consistent with the *coxII.i3* intron loss in *Elatine*. This position had been proposed previously by traditional studies that suggested a close affinity of this family to the Clusiaceae (e.g., Corner 1976; Cronquist 1981).

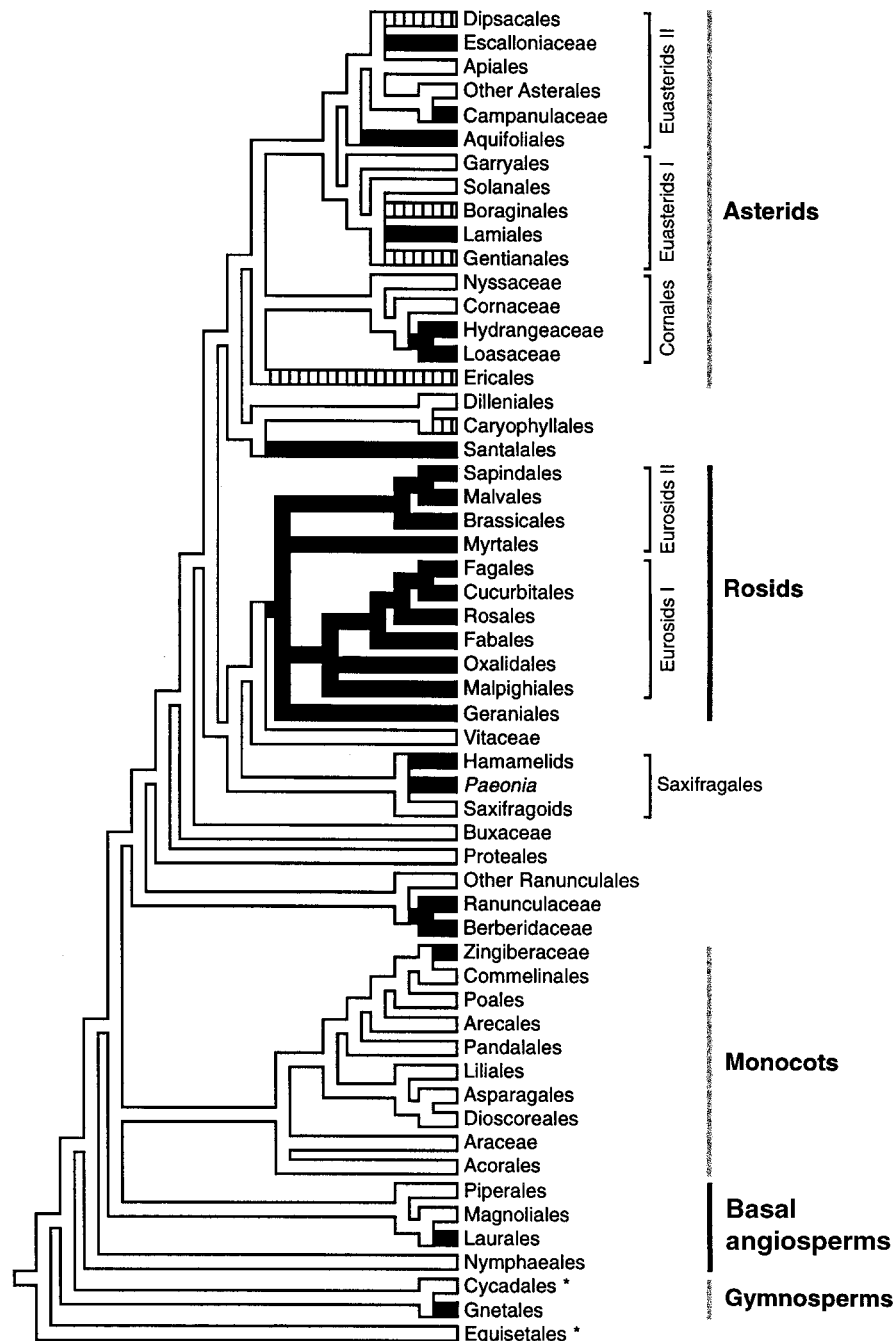
In the Cornales, the *coxII.i3* intron may have been lost only once as it is restricted to two families considered to be sister groups, the Hydrangeaceae and the Loasaceae (Chase et al. 1993; Hempel et al. 1995; Soltis et al. 1995; Xiang et al. 1998). The Cornaceae and related taxa possess the intron. The Hydrostachyaceae, which was not surveyed, should be investigated in future studies, as it has been proposed as the sister group to the Hydrangeaceae-Loasaceae clade by Hempel et al. (1995). More recently, in their phylogeny based on sequences of three genes, Soltis et al. (2000) reported that the Hydrostachyaceae was nested in the Hydrangeaceae clade. Such a topology would imply that Hydrostachyaceae lack the intron.

#### Other Independent Losses

Among the more primitive angiosperms, *Calycanthus* (Calycanthaceae), the only member of the Laurales surveyed in this study, lacks the intron. Likewise, the intron is present in all of the monocots investigated except in *Kaempferia* of the Zingiberaceae (Zingiberales), a member of the commelinoid group, but only a single species in each of the monocot orders was investigated. The intron also appears to have been lost independently in the Santalales, but only *Comandra richardsoniana* was investigated. Further sampling is needed to better delimit the extent of the loss in each of these three groups.

In the euasterids I, the *coxII.i3* intron is present in Garryales and Solanales but absent in all Lamiales surveyed and in some Boraginales and Gentianales. Recent phylogenetic analyses suggest that these represent independent losses. Within the Boraginales, the intron is present in *Hydrophyllum* but absent in *Borago* (no other genus was surveyed). In the Gentianales, the intron is present in the Gentianaceae, Loganiaceae, and most Apocynaceae. However, *Catharanthus* and *Vinca*, nested within the Apocynaceae, lack the intron (Rabbi and Wilson 1993; this study), as does *Coffea* (Rubiaceae; no other genus in the family was studied). Recent phylogenetic analyses of the Gentianales (Backlund et al. 2000; Oxelman and Bremer 2000) suggest that these would represent independent losses within the order.

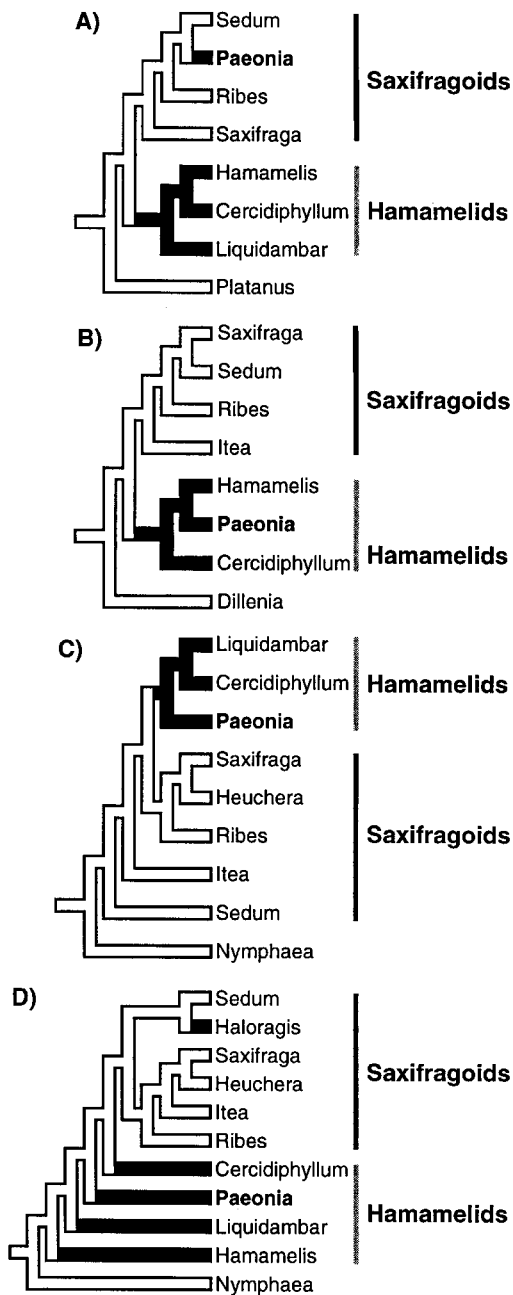
Within the euasterids II, the intron is absent in the two species of the genus *Ilex* (Aquifoliaceae) studied, but no other member



**Fig. 2** Presence (white branches) or absence (black branches) of the *coxII.i3* intron across the angiosperms (composite tree from APG 1998; modified according to Davis 1999, Mathews and Donoghue 1999, Qiu et al. 1999, Sanderson et al. 1999, Winter et al. 1999, Bowe et al. 2000, Chaw et al. 2000, and Soltis et al. 2000). Hatched branches indicate groups in which loss of the intron was partial or where phylogenies are poorly resolved (see “Discussion”). Asterisks indicate results obtained by Dong et al. (1997). Terminal taxa are orders, unless families were not assigned to orders by APG (1998) or unless increased resolution was needed in orders where the intron is both present and absent (then shown as families or genera).

of the family nor of the other two families in the order Aquifoliales, the Helwingiaceae and the Phyllonomaceae, were surveyed. In the Asterales, all Campanulaceae investigated lack the intron, while members of the other families examined possess the intron (i.e., Asteraceae, Goodeniaceae, and Menyanthaceae),

which suggests the intron loss may be a phylogenetic marker for the Campanulaceae. A loss also has occurred in *Escallonia*, previously placed in an uncertain position in euasterids II by Soltis and Soltis (1997) and APG (1998). Savolainen et al. (2000b) recently placed this taxon at the base of a clade leading



**Fig. 3** Presence (white branches) or absence (black branches) of the *coxII.i3* intron in the Saxifragales depending upon the phylogeny used to optimize the character. *A*, *rbcl*: Chase et al. (1993); *B*, *rbcl*: Qiu et al. (1998a); *C*, 18S: Soltis and Soltis (1997); *D*, *rbcl*, 18S, and *atpB*: Soltis et al. (2000). The genus *Paeonia* is in bold to represent the different interpretations possible when it is placed in a saxifragoid clade or in a hamamelid clade.

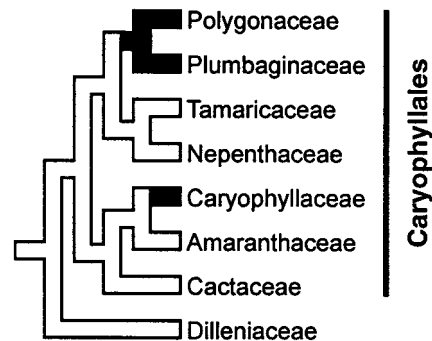
to Apiales and Asterales, which suggests that this represents an independent loss.

*More Complex Cases*

In most recent studies of the Saxifragales, the clade consisting of *Cercidiphyllum*, *Hamamelis*, and *Liquidambar* (hamamelid

clade) is considered to be a monophyletic group sister to the Saxifragales s. str. (saxifragoid clade; Chase et al. 1993; Soltis and Soltis 1997; Soltis et al. 1997; Qiu et al. 1998a). In those studies, the genus *Paeonia* is considered to be either a member of a hamamelid clade or of a saxifragoid clade (shown as unresolved in fig. 2). When *Paeonia* is placed within a saxifragoid clade (fig. 3A: Chase et al. 1993; Soltis et al. 1997), two independent losses are inferred, one in *Paeonia* and one in the hamamelid clade. When placed within a hamamelid clade (fig. 3B: Soltis and Soltis 1997; fig. 3C: Qiu et al. 1998a), the intron loss is interpreted as having occurred only once within the order. Thus, depending upon the position of *Paeonia* within the Saxifragales, one or two intron losses may be inferred within the order. The phylogenetic study of Soltis et al. (2000) shows the hamamelids as paraphyletic instead of monophyletic, a pattern of relationships that would suggest five independent losses of the intron in the Saxifragales (fig. 3D). Despite the combined use of three genes in the Soltis et al. (2000) study, the relationships within the Saxifragales remain poorly supported, which indicates that the topologies presented need further corroboration (fig. 3).

The topologies obtained in the numerous molecular phylogenies published on the Caryophyllales would suggest that the *coxII.i3* intron has been lost twice in this order (fig. 4; Giannasi et al. 1992; Rettig et al. 1992; Chase et al. 1993; Downie and Palmer 1994; Williams et al. 1994; Fay et al. 1997; Lledo 1998; Nandi et al. 1998). One of these losses occurred in the ancestor of the Caryophyllaceae (all Caryophyllaceae sampled lack the intron) and is confined to this family within the Caryophyllales s. str. (e.g., Cronquist 1981), an additional feature that suggests the family is unique within the group. A second loss has occurred in the common ancestor of Polygonaceae and Plumbaginaceae, two families considered to be sister groups in recent molecular phylogenetic analyses (Fay et al. 1997; Lledo et al. 1998) and in some traditional classification systems (e.g., Cronquist 1981). However, we cannot be sure that this loss is limited to this clade because the Simmondsiaceae, which occurs as sister to these two families in the Fay et al. (1997) and the Savolainen et al. (2000b) studies, was not surveyed here. The loss would be limited to



**Fig. 4** The two losses of the *coxII.i3* intron in the Caryophyllales optimized on a consensus tree from three recent studies (Fay et al. 1997; Lledo et al. 1998; Nandi et al. 1998). The black branches indicate the loss of the intron. Terminal taxa are families, and they may represent one or more species surveyed in this study (appendix). Dilleniaceae is the outgroup for the order.

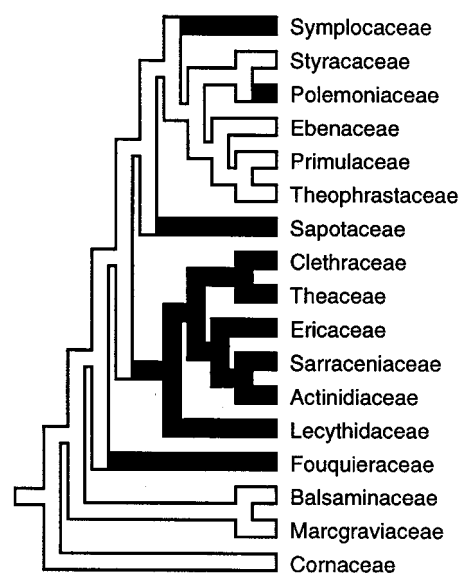
these two families as all other potential sister groups possess the intron (Lledo et al. 1998; Soltis et al. 2000).

Among the families of the Ericales studied, the intron is absent in the Actinidiaceae, Clethraceae, Ericaceae, Fouquieriaceae, Lecythidaceae, Polemoniaceae, Sarraceniaceae, Sapotaceae, Symlocaceae, and Theaceae but is present in Balsaminaceae, Ebenaceae, Macgraviaceae, Primulaceae, Styracaceae, and Theophrastaceae (appendix). Recent phylogenetic analyses of the Ericales differ from each other in numerous respects and often do not include all of the above families (Anderberg 1993; Chase et al. 1993; Kron 1996; Morton et al. 1996; Nandi et al. 1998). Thus, it is difficult to assess with confidence the possible number of losses of the intron within the order based on these studies. However, Savolainen et al. (2000b) have recently published a *rbcL* phylogeny that includes more families, particularly in the Ericales, and the relationship among them is better resolved. Following their topology, the *coxII.i3* intron appears to have been lost at least five times in the Ericales; this is a minimum since we did not survey all the families (fig. 5). One of the groups clearly defined by the loss is the Ericaceae and the families surrounding them: Clethraceae, Theaceae, Sarraceniaceae, Actinidiaceae, and Lecythidaceae. This would suggest that families not surveyed here but considered as nested within this group according to Savolainen et al. (2000b), i.e., Cyrillaceae and Roridulaceae, should also lack the intron. The close affinity among these families, particularly Clethraceae, Actinidiaceae, Sarraceniaceae, and Ericaceae, had also been suggested by Kron (1996) and Nandi et al. (1998).

In the Dipsacales, the *coxII.i3* intron has been lost in three of the four families following APG (1998): Adoxaceae, Caprifoliaceae, and Valerianaceae. Recent phylogenetic analyses of the Dipsacales would suggest these represent three independent losses of the intron within the order (Donoghue et al. 1992; Backlund and Bremer 1997; Savolainen et al. 2000b; Soltis et al. 2000). However, studies of the Dipsacales still have insufficient taxon sampling for a rigorous interpretation of our results.

#### Variation in Intron Length

In some groups, variation in the size of the intron may be of phylogenetic use. For example, within the Ranunculales, Papaveraceae have a larger intron than the other families of the order. They bear an intron of more than 1740 bp, while other families in the order all have an intron less than 1430 bp. Albertazzi et al. (1998) found that the intron of *Acorus* is the smallest observed in the angiosperms, a result supported by our analysis of a wider and complementary sampling of angiosperms. Such variations in intron length may be due to the presence of different insertion elements in different taxa (Rabbi and Wilson 1993; Dong et al. 1997) rather than to the presence of nonhomologous introns at the same position. However, when the intron is lost, the amplified fragment size is constant (ca. 300 bp), which Ouyang et al. (1997) attribute to the loss having occurred via an RNA/cDNA intermediate (see Downie et al. 1991 for the chloroplast genome). The transfer of the *coxII* gene to the nucleus is reported by Nugent and Palmer (1991) and Adams et al. (1999) in the Fabaceae, which they suggested also is effected through an RNA intermediate. Such a transfer could result in the loss of the intron for some



**Fig. 5** Presence (white branches) or absence (black branches) of the *coxII.i3* intron in the Ericales. The intron is optimized on a *rbcL* gene tree taken from the Savolainen et al. (2000b) study. Terminal taxa are families and may represent one or more species surveyed in this study (appendix). The Cornaceae are the outgroup for the order.

taxa but not in the Fabaceae (a member of the rosids) or in other groups where the intron was lost prior to the transfer.

#### Conclusion

This survey of the presence or absence of the *coxII.i3* intron in all orders of angiosperms indicates the potential as a phylogenetic marker of such structural mutations of the mitochondrial genome. However, the pattern of losses is often complex and only can be interpreted in conjunction with robust phylogenetic studies for the groups in which the intron is partially or completely absent. The *coxII.i3* intron could have been lost as many as 27 times in the angiosperms or 29 times in seed plants with the losses in Gnetales and *Metasequoia*. However, more robust phylogenies are needed in some groups to determine the exact circumscription and number of losses (e.g., Saxifragales, Ericales, and Dipsacales). Despite the numerous parallel losses of the *coxII.i3* intron in the angiosperms, we conclude that the absence of this intron could be a useful marker that corroborates recent molecular phylogenies and sometimes traditional classifications showing the monophyly of the Gnetales, Ranunculales (in part), rosids, Caryophyllales (in part), Cornales (in part), Lamiales, and Campanulaceae. Nevertheless, as noted by Graham and Olmstead (2000), structural rearrangements should not be looked upon as infallible markers of common ancestry because they can arise in parallel.

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## Appendix

**Table A1**

**Vascular Plants Surveyed for the Presence or Absence of the *coxII.i3* Intron**

Species	Collection number	Accession number or locality	Intron size (bp)	Intron
Fern allies:				
Equisetales:				
Equisetaceae:				
<i>Equisetum arvense</i> L.	...	Dong et al. 1997	...	+
Gymnosperms:				
Cycadales:				
Cycadaceae:				
<i>Cycas revoluta</i> Thunb.	...	Dong et al. 1997	...	+
Gnetales:				
Gnetaceae:				
<i>Gnetum gnemon</i> L.	July 233	2240-50-77	—	—
Basal angiosperms:				
Nymphaeaceae:				
<i>Nymphaea odorata</i> Ait.	July 247	MBG	1910	+
Laurales:				
Calycanthaceae:				
<i>Calycanthus fertilis</i> Walt.	July 213	MBG	—	—
Magnoliales:				
Magnoliaceae:				
<i>Liriodendron tulipifera</i> L.	July 110	2314-95	2020	+
<i>Magnolia liliflora</i> Desr.	July 80	1471-89	3130	+
Piperales:				
Piperaceae:				
<i>Piper betle</i> L.	July 161	6383-39	1190	+
Monocots:				
Alismatales:				
Araceae:				
<i>Syngonium triphyllum</i> Birdsey	Barabé s.n.	3404-88	1210	+
Acorales:				
Acoraceae:				
<i>Acorus calamus</i> L.	July 226	MBG	440	+
Dioscoreales:				
Dioscoreaceae:				
<i>Dioscorea alata</i> L. cv. Florido	July 222	1879-88	1260	+
Liliales:				
Liliaceae:				
<i>Lilium</i> cv. Kenora	July 205	3004-81	1550	+
Pandanales:				
Pandanaaceae:				
<i>Pandanus utilis</i> Bory	July 240	2080-74	1210	+
Commelinoids:				
Bromeliaceae:				
<i>Guzmania patula</i> Mez & Wercklé	July 232	4127-84	1210	+
Arecales:				
Arecaceae:				
<i>Sabal etonia</i> Swingle ex Nash	July 214	3243-76	1260	+
Commelinales:				
Pontederiaceae:				
<i>Eichhornia crassipes</i> (Martius) Solms	July 201	MBG	1150	+

Table A1

(Continued)

Species	Collection number	Accession number or locality	Intron size (bp)	Intron
Poales:				
Poaceae:				
<i>Zea mays</i> L.	July 252	Market	800	+
Zingiberales:				
Zingiberaceae:				
<i>Kaempferia pulchra</i> Ridl.	July 231	3086-84	–	–
Eudicots:				
Buxaceae:				
<i>Buxus sempervirens</i> L. cv. Alyce	July 105	1257-98	2083	+
Proteales:				
Platanaceae:				
<i>Platanus occidentalis</i> L.	July 85	1724-73	1483	+
Ranunculales:				
Berberidaceae:				
<i>Caulophyllum thalictroides</i> (L.) Michx.	July 133	2716-94	–	–
<i>Mahonia aquilefolium</i> cv. Compacta	July 184	838-96	–	–
<i>Nandina domestica</i> Thunb.	July 189	965-91	–	–
<i>Podophyllum emodi</i> Wall.	July 191	979-73	–	–
Eupteleaceae:				
<i>Euptelea polyandra</i> Siebold & Zucc.	July 224	1242-61-71	1210	+
Lardizabalaceae:				
<i>Akebia trifoliata</i> Koidz.	July 173	1843-96	1400	+
Menispermaceae:				
<i>Menispermum canadense</i> L.	July 130	1177-74	1370	+
Ranunculaceae:				
<i>Actea rubra</i> (Ait.) Willd.	July 128	2711-94	–	–
<i>Aquilegia coerulea</i> E. James	July 163	811-90	–	–
<i>Caltha palustris</i> L.	July 74	MBG	–	–
<i>Clematis</i> cv. Rovalty	July 108	1990-95	–	–
<i>Coptis trifolia</i> L.	Brouillet 99-4	Newfoundland	–	–
<i>Delphinium</i> cv. Aphrodite	July 171	2723-90	–	–
<i>Helleborus niger</i> L.	July 131	2662-94	–	–
<i>Thalictrum aquilegifolium</i> L.	July 116	499-30	–	–
Papaveraceae:				
<i>Dicentra eximia</i> (Ker Gawl.) Torr.	July 107	351-84	1810	+
<i>Macleaya microcarpa</i> (Maxim.) Fedde	July 208	2085-76	1740	+
Core eudicots:				
Dilleniaceae:				
<i>Dillenia indica</i> L.	July 156	1603-65	1230	+
Vitaceae:				
<i>Leea coccinea</i> Bojer	July 230	1245-89	1780	+
<i>Vitis riparia</i> Michx.	July 122	MBG	1710	+
Caryophyllales:				
Amaranthaceae:				
<i>Beta vulgaris</i> L.	July 251	Market	1550	+
Cacatceae:				
<i>Pereskia aculeata</i> Mill.	July 217	2115-41	1260	+
Caryophyllaceae:				
<i>Dianthus sylvestris</i> Wulfen	July 212	882-40	–	–
<i>Cerastium tomentosum</i> L. cv. Columnare	July 237	1472-86	–	–
<i>Gypsophila repens</i> L. cv. Rosea	July 194	1557-95	–	–
<i>Petrorhagia saxifraga</i> (L.) Link	July 238	1491-86	–	–
Nepenthaceae:				
<i>Nepenthes alata</i> Blanco	July 94	2567-83	1480	+
Plumbaginaceae:				
<i>Armeria alpina</i> Willd.	July 243	1025-46	–	–
<i>Plumbago zeylanica</i> L.	July 241	473-53	–	–
Polygonaceae:				
<i>Coccoloba wifera</i> (L.) L.	July 239	1425-89	–	–
<i>Rheum X culturum</i> cv. Canada red	July 242	2534-94	–	–



Table A1

(Continued)

Species	Collection number	Accession number or locality	Intron size (bp)	Intron
<i>Rumex patientia</i> L.	July 215	2561-94	–	–
Tamaricaceae:				
<i>Tamarix ramosissima</i> Ledeb. cv. Summer glow	July 147	1840-54-93	890	+
Santalales:				
Santalaceae:				
<i>Comandra richardsiana</i> Fernald	Brouillet 99-19	Quebec	–	–
Saxifragales:				
Altingiaceae:				
<i>Liquidambar styraciflua</i> L.	July 97	394-68	–	–
Cercidiphyllaceae:				
<i>Cercidiphyllum japonicum</i> Siebold & Zucc.	July 86	2015-38-68	–	–
Crassulaceae:				
<i>Sedum album</i> L.	July 95	3317-87	1300	+
Grossulariaceae:				
<i>Ribes odoratum</i> H.L. Wendl.	July 73	1605-73	1380	+
Haloragaceae:				
<i>Haloragis erecta</i> (Murr.) Schindler cv. Melton Bronze	July 216	253-99	–	–
Hamamelidaceae:				
<i>Fothergilla major</i> Lodd.	July 88	688-49-71	–	–
<i>Hamamelis vernalis</i> Sarg.	July 98	1017-36	–	–
Iteaceae:				
<i>Itea virginica</i> L. cv. Little Henry	July 206	MBG	1390	+
Paeoniaceae:				
<i>Paeonia broteri</i> Boiss & Reut.	July 106	1558-51	–	–
<i>Paeonia kavachensis</i> Aznav.	July 244	MBG	–	–
Saxifragaceae:				
<i>Mukdenia rossii</i> (Oliv.) Koidz.	July 91	837-49	1480	+
<i>Heuchera cylindrica</i> Douglas ex Hook.	July 111	1128-82	1290	+
Rosids:				
Staphyleaceae:				
<i>Staphylea trifoliata</i> L.	July 174	3888-84	–	–
Zygophyllaceae:				
<i>Guaiacum officinale</i> L.	July 157	1484-50	–	–
Geraniales:				
Geraniaceae:				
<i>Geranium maculatum</i> L.	July 92	172-79	–	–
Eurosids I:				
Celastrales:				
Celastraceae:				
<i>Euonymus Hamiltonianus</i> Wall.	July 102	1115-38-74	–	–
Parnassiaceae:				
<i>Parnassia glauca</i> Raf.	July 190	1977-47	–	–
Cucurbitales:				
Cucurbitaceae:				
<i>Cucumis sativus</i> L.	Bruneau s.n.	Market	–	–
Begoniaceae:				
<i>Hillebrandia sandwicensis</i> D. Oliver	July 82	2960-57	–	–
Datisceae:				
<i>Datisca cannabina</i> L.	July 127	MBG	–	–
Fabales:				
Fabaceae:				
<i>Gleditsia amorphoides</i> (Griseb.) Taub.	Lewis 2171	Ecuador	–	–
Polygalaceae:				
<i>Polygala senega</i> L.	Brouillet 99-11	Quebec	–	–
Fagales:				
Betulaceae:				
<i>Corylus cornuta</i> Marshall	Forest s.n.	Quebec	–	–
Fagaceae:				
<i>Quercus rubra</i> L.	Forest 97100810	Quebec	–	–
Juglandaceae:				

Table A1

(Continued)

Species	Collection number	Accession number or locality	Intron size (bp)	Intron
<i>Pterocarya stenoptera</i> C. DC.	July 136	2398-78	–	–
Myricaceae:				
<i>Myrica gale</i> L.	July 146	80-54	–	–
Malpighiales:				
Clusiaceae:				
<i>Hypericum perforatum</i> L.	July 141	2611-94	–	–
Elatinaceae:				
<i>Elatine californica</i> A. Gray	July 245	MBG	–	–
Euphorbiaceae:				
<i>Andrachne colchica</i> Fisch. & C.A. Mey ex Boiss	July 175	710-71	–	–
Linaceae:				
<i>Linum perenne</i> L.	July 119	413-84	–	–
Salicaceae:				
<i>Salix matsudana</i> Koidz.	July 77	3368-37-89	–	–
Violaceae:				
<i>Viola canadensis</i> L.	July 134	580-36	–	–
Oxalidales:				
Cephalotaceae:				
<i>Cephalotus follicularis</i> Labill.	July 151	2689-96	–	–
Oxalidaceae:				
<i>Oxalis regnellii</i> Miq.	July 155	2615-95	–	–
Rosales:				
Elaeagnaceae:				
<i>Elaeagnus angustifolia</i> L.	July 126	MBG	–	–
Moraceae:				
<i>Morus alba</i> L.	July 135	2329-50-70	–	–
Rhamnaceae:				
<i>Rhamnus cathartica</i> L.	July 123	MBG	–	–
Rosaceae:				
<i>Rosa roousseauiorum</i> B. Boivin	Drouin 1202	Quebec	–	–
Ulmaceae:				
<i>Ulmus americana</i> L.	July 78	1446-79	–	–
Eurosids II:				
Brassicales:				
Caricaceae:				
<i>Carica papaya</i> L.	July 162	913-71	–	–
Brassicaceae:				
<i>Alyssum saxatile</i> L.	July 75	4910-38	–	–
Resedaceae:				
<i>Reseda odorata</i> L.	July 210	MBG	–	–
Tropaeolaceae:				
<i>Tropaeolum majus</i> L.	July 181	MBG	–	–
Malvales:				
Cistaceae:				
<i>Helianthemum</i> cv. Henfield Brillant	July 121	MBG	–	–
Malvaceae:				
<i>Tilia platyphyllos</i> Scop.	July 137	1000-49	–	–
Thymelaceae:				
<i>Daphne cneorum</i> L.	July 89	2011-47	–	–
Myrtales:				
Combretaceae:				
<i>Combretum microphyllum</i> Klotzsch	July 218	3151-40	–	–
Lythraceae:				
<i>Punica granatum</i> L. cv. Wonderful	July 153	1482-92	–	–
Myrtaceae:				
<i>Syzygium jambos</i> (L.) Alston	July 159	6061-39	–	–
Onagraceae:				
<i>Fuchsia magellanica</i> Lam. cv. Aurea	July 124	MBG	–	–
Sapindales:				
Anacardiaceae:				

Table A1

(Continued)

Species	Collection number	Accession number or locality	Intron size (bp)	Intron
<i>Cotinus coggygria</i> Scop.	July 99	1723-56-56	–	–
Sapindaceae:				
<i>Acer saccharinum</i> L.	July 81	2483-77	–	–
Simaroubaceae:				
<i>Ailanthus altissima</i> (Mill.) Swingle	July 138	1422-96	–	–
Asterids:				
Cornales:				
Cornaceae:				
<i>Cornus stolonifera</i> Michx.	July 103	3288-37-55	1600	+
Hydrangeaceae:				
<i>Deutzia glabrata</i> Kom.	July 87	1927-41-56	–	–
<i>Hydrangea heteromalla</i> D. Don	July 143	6487-37	–	–
<i>Kirengeshoma palmata</i> Yatabe	July 202	820-87	–	–
<i>Philadelphus purpuracens</i> (Kochne) Render	July 132	112-55	–	–
Loasaceae:				
<i>Cevallia sinuata</i> Lag.	Spellenberg 12942	New Mexico	–	–
<i>Mentzelia multiflora</i> (Nutt.) A. Gray	Spellenberg 12937	New Mexico	–	–
Nyssaceae:				
<i>Nyssa salvatica</i> Marshall	July 192	320-93	–	+
Ericales:				
Actinidiaceae:				
<i>Actinidia arguta</i> (Siebold & Zucc.) Planch. Ex Miq.	July 140	2039	–	–
Balsaminaceae:				
<i>Impatiens</i> cv. Hawaii	July 180	3281-85	1060	+
Clethraceae:				
<i>Clethra alnifolia</i> L.	July 203	4204-37-65	–	–
Ebenaceae:				
<i>Diospyros virginiana</i> L.	July 178	2731-77	1360	+
Ericaceae:				
<i>Arctostaphylos uva-ursi</i> (L.) Spreng.	July 120	456-63-84	–	–
<i>Enkianthus campanulatus</i> (Miq.) Nichols. cv. Red Bells	July 177	1706-95	–	–
<i>Moneses uniflora</i> (L.) A. Gray	Brouillet s.n.	Newfoundland	–	–
<i>Monotropa uniflora</i> L.	Brouillet 99-102	Newfoundland	–	–
<i>Rhododendron</i> cv. PJM	July 79	1546-74	–	–
<i>Vaccinium Vitis-Idaea</i> L.	July 101	427-75	–	–
Fouquieriaceae:				
<i>Fouquieria splendens</i> Engelm.	Spellenberg 12939	New Mexico	–	–
Lecythidaceae:				
<i>Bertholletia axcelsa</i> Bonpl.	July 234	1878-59	–	–
Marcgraviaceae:				
<i>Marcgravia picta</i> Willd.	July 172	1304-68	970	+
Polemoniaceae:				
<i>Phlox paniculata</i> L. cv. Tenor	July 179	2850-84	–	–
<i>Polemonium reptans</i> L.	July 125	MBG	–	–
Primulaceae:				
<i>Primula elatior</i> (L.) J. Hill	July 104	1653-82	1400	+
<i>Steironema hybridum</i> (Michx.) Raf.	July 183	MBG	1360	+
Sarraceniaceae:				
<i>Sarracenia leucophylla</i> Raf.	July 150	1748-91	–	–
Sapotaceae:				
<i>Chrysophyllum cainito</i> L.	July 158	MBG	–	–
Styracaceae:				
<i>Halesia carolina</i> L.	July 145	35-36	1250	+
<i>Pterostyrax itispida</i> Siebold & Zucc.	July 200	MBG	1310	+
Symplocaceae:				
<i>Symplocos paniculata</i> (Thunb. Ex Murray) Miq.	July 211	2266-96	–	–
Theaceae:				
<i>Stewartia pseudocamellia</i> Maxim.	July 204	1670-70	–	–
Theophrastaceae:				
<i>Jacquinia pungens</i> A. Gray	July 236	1315-72	1210	+

Table A1

(Continued)

Species	Collection number	Accession number or locality	Intron size (bp)	Intron
Euasterids I:				
Boraginaceae:				
<i>Borago officinalis</i> L.	July 182	MBG	–	–
<i>Hydrophyllum virginianum</i> L.	July 235	1373-95	1210	+
Garryales:				
Aucubaceae:				
<i>Aucuba japonica</i> Thunb. cv. Variegata	July 220	6973-38-68	1260	+
Eucommiaceae:				
<i>Eucommia ulmoides</i> Oliv.	July 198	706-99	1310	+
Gentianales:				
Apocynaceae:				
<i>Amsonia tabernaemontana</i> Walter	July 115	2664-40	1330	+
<i>Cathartus roseus</i> (L.) G. Don	July 166	MBG	–	–
Gentianaceae:				
<i>Gentiana gracilipes</i> Turrill	July 188	587-96	1470	+
Loganiaceae:				
<i>Strychnos nux-vomica</i> L.	July 221	2243-51	1260	+
Rubiaceae:				
<i>Coffea</i> sp.	July 160	MBG	–	–
Lamiales:				
Acanthaceae:				
<i>Pachystachys lutea</i> Ness	July 154	2125-78-97	–	–
Bignoniaceae:				
<i>Catalpa bignonioides</i> Walter	July 169	MBG	–	–
Buddlejaceae:				
<i>Buddleja davidii</i> Franch. cv. Harlequin	July 176	MBG	–	–
Gesneriaceae:				
<i>Saintpaulia nitida</i> B.L. Burtt.	July 83	2763-98	–	–
Globulariaceae:				
<i>Globularia cordifolia</i> L.	July 76	1420-83	–	–
Lamiaceae:				
<i>Salvia pratensis</i> L.	July 117	2633-96	–	–
Lentibulariaceae:				
<i>Pinguicula moranensis</i> Kunth	July 152	3051-84	–	–
Oleaceae:				
<i>Syringa oblata</i> Lindl.	July 96	4196-38-50	–	–
Pedaliaceae:				
<i>Proboscidea altheaefolia</i> (Benth.) Decne.	Spellenberg 12938	New Mexico	–	–
Plantaginaceae:				
<i>Veronica latifolia</i> L.	July 114	35-45	–	–
Orobanchaceae:				
<i>Rhinantus minor</i> L.	Brouillet s.n.	Newfoundland	–	–
Scrophulariaceae:				
<i>Verbascum phoenicum</i> L. cv. Flush of white	July 112	428-97	–	–
Verbenaceae:				
<i>Verbena boraginensis</i> L.	July 209	MBG	–	–
Solanales:				
Convolvulaceae:				
<i>Convolvulus arvensis</i> L.	July 207	MBG	1260	+
Solanaceae:				
<i>Capsicum</i> sp.	Bruneau s.n.	Market	880	+
Euasterids II:				
Escalloniaceae:				
<i>Escallonia bifida</i> Link & Otto	July 229	3069-84	–	–
Apiales:				
Apiaceae:				
<i>Daucus carota</i> L.	Bruneau s.n.	Market	1380	+
Pittosporaceae:				
<i>Pittosporum tobira</i> (Thunb.) W.T. Aiton cv. Variegatum	July 219	39-43-71	1260	+
Aquifoliales:				

**Table A1**  
(Continued)

Species	Collection number	Accession number or locality	Intron size (bp)	Intron
Aquifoliaceae:				
<i>Ilex verticillata</i> (L.) A. Gray	July 139	4228-37-72	—	—
<i>Ilex glabra</i> (L.) A. Gray cv. Densa	July 168	2649-82	—	—
Asterales:				
Asteraceae:				
<i>Chrysanthemum caucasicum</i> (DC.) Pers.	July 186	1233-66	1360	+
Campanulaceae:				
<i>Campanula ineretina</i> Rupr.	July 170	958-38	—	—
<i>Lobelia siphilitica</i> L.	July 225	MBG	—	—
<i>Platycodon grandiflora</i> (Jacquin) A. DC.	July 193	2220-76	—	—
Goodeniaceae:				
<i>Scaevola</i> sp.	Brouillet s.n.	MBG	1300	+
Menyanthaceae:				
<i>Menyanthes trifoliata</i> L.	July 109	MBG	1190	+
Dipsacales:				
Adoxaceae:				
<i>Viburnum sargentii</i> Kochne	July 100	1023-36-55	—	—
Caprifoliaceae:				
<i>Sambucus canadensis</i> L.	July 142	MBG	1250	+
<i>Lonicera</i> cv. Mandarin	July 144	1252-98	—	—
<i>Symphoricarpos albus</i> (L.) S.F. Blake	July 149	2362-76-76	—	—
<i>Weigela</i> cv. Feerie	July 148	1479-57-69	—	—
Dipsacaceae:				
<i>Scabiosa colombaria</i> L. cv. Pinkmist	July 187	1296-97	1360	+
Valerianaceae:				
<i>Valeriana pyrenaria</i> L.	July 118	1111-85	—	—

Note. Plants categorized according to the classification of APG (1998). The collection number, the accession number of the plant when from the Montreal Botanical Garden (MBG), or the locality, the intron size (in bp), and the presence or absence of the *coxII.i3* intron is indicated for every species. All vouchers are deposited at MT, except for those of G. P. Lewis (K). Intron size was deduced from fragment size, which was estimated to the nearest 10 bp after comparison with a logarithmic curve built from the migration distances of the ladder. For locations, MBG indicates that the specimen was collected in the Montreal Botanical Garden, but that no accession number was available.

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