Pl. Syst. Evol. 266: 119–145 (2007)
DOI 10.1007/s00606-007-0545-y
Printed in The Netherlands

Plant Systematics and Evolution

Phylogeny of subtribe Pyrinae (formerly the Maloideae, Rosaceae): Limited resolution of a complex evolutionary history

C. S. Campbell[1], **R. C. Evans**[2], **D. R. Morgan**[3], **T. A. Dickinson**[4], and **M. P. Arsenault**[1]

[1]Department of Biological Sciences, University of Maine, Orono, Maine, USA
[2]Department of Biology, Acadia University, Wolfville, Nova Scotia, Canada
[3]Department of Biology, University of West Georgia, Carrollton, Georgia, USA
[4]Royal Ontario Museum, Botany Section, Toronto, Canada

Received January 16, 2006; accepted August 17, 2006
Published online: June 28, 2007

Abstract. Generic relationships in the Pyrinae (equivalent to subfamily Maloideae) were assessed with six chloroplast regions and five nuclear regions. We also plotted 12 non-molecular characters onto molecular phylogenies. Chloroplast DNA trees are incongruent with those from nuclear regions, as are most nuclear regions with one another. Some of this conflict may be the result of hybridization, which occurs between many genera of Pyrinae in the present and may have occurred in the past, and duplication of nuclear loci. Sequence divergence between genera of Pyrinae, which is significantly less than that between genera of another large clade in Rosaceae, the Rosoideae, is concentrated in terminal branches, with short internal branches. This pattern is consistent with an ancient, rapid radiation, which has also been hypothesized from the fossil record. Even with about 500,000 bp of sequence, our results resolve only several small groups of genera and leave much uncertainty about phylogenetic relationships within Pyrinae.

Key words: Rapid, ancient radiation, cpDNA, GBSSI, hybridization, gene duplication, Pyrodae, Pyreae.

Apple (*Malus*) and many of its relatives have a distinctive fruit, a pome, which led to identification of these plants as a group by Bauhin in 1623 (Kovanda 1965). Recognition of the distinctiveness of this fruit is also evident in the names of five genera in this group – *Chaenomeles*, *Chamaemeles*, *Heteromeles*, *Malacomeles*, and *Osteomeles* – wherein "meles" is derived from Greek for "little apple". This group of pome-bearing plants has long been known as subfamily Maloideae. Phylogenetic and comparative ontogenetic studies (Morgan et al. 1994, Evans et al. 2000, Evans and Campbell 2002, Evans and Dickinson 2005) suggest that the closest relatives of pome-bearing plants are three small, New World, dry-fruited genera: *Kageneckia*, *Lindleya* and *Vauquelinia*. A new classification of the Rosaceae (Potter et al., in press) includes these three genera and the traditional Maloideae in the tribe Pyreae. We refer to the pome-bearing plants as the subtribe Pyrinae.

Taxonomic studies of the approximately 950 species of Pyrinae have been motivated by their important edible fruits, such as apple (*Malus*), pear (*Pyrus*), quince (*Cydonia*), loquat (*Eriobotrya*), chokeberry (*Aronia*), and serviceberry (*Amelanchier*). Some members of these

genera as well as some cotoneasters (*Cotoneaster*), hawthorns (*Crataegus*), Japanese quinces (*Chaenomeles*), firethorns (*Pyracantha*), and mountain ashes (*Sorbus*) are valued ornamentals. Despite this interest, relationships among genera of Pyrinae remain poorly understood (Phipps et al. 1991, Robertson et al. 1991, Campbell et al. 1995, Evans 1999, Aldasoro et al. 2005, Evans and Dickinson 2005).

Pyrinae are also distinctive in their base chromosome number of 17, which is also found in *Kageneckia* and *Lindleya* ($x = 15$ in *Vauquelinia*). Isozyme studies (Chevreau et al. 1985, Weeden and Lamb 1987, Raspé et al. 1998) indicate that these $x = 17$ plants are allopolyploids. The base number of 17 led Sax (1931, 1932, 1933) to hypothesize an origin of Pyrinae through wide hybridization between ancestors of subfamily Spiraeoideae ($x = 9$) and *Prunus* ($x = 8$). Evans and Campbell (2002) falsified this wide-hybridization hypothesis with evidence suggesting that the Pyreae evolved from within a lineage that contained the ancestors of *Gillenia*, a small genus of the eastern North America with $x = 9$. The supertribe Pyrodae (Potter et al. in press) comprises *Gillenia* plus the Pyreae.

In addition to being part of the genesis of the Pyreae, hybridization is unusually common among genera of Pyrinae. Robertson et al. (1991) summarized reports of intergeneric hybridization involving 16 genera of Pyrinae. Some of these intergeneric hybrids are highly fertile and appear repeatedly in nature (Godron 1874, Sax 1931, Oddou-Muratorio et al. 2001, Nelson-Jones et al. 2002). The occurrence of extensive intergeneric hybridization among extant Pyrinae could indicate that hybridization has been part of their evolutionary history. Hybridization among lineages can be quite disruptive to phylogenetic trees generally (McDade 1995, Linder and Rieseberg 2004) and was recognized by Phipps et al. (1991) for its potential to undermine phylogeny reconstruction using various morphological characters in Pyrinae.

Generic limits for 25 genera established by Roemer (1847) are close to those in the most recent studies of Pyrinae (Robertson et al. 1991, Kalkman 2004), which have mostly been followed in this paper. There has been disagreement about whether to delimit *Sorbus* narrowly (92 species of the North Temperate Zone) or broadly to include *Aria* (97 Eurasian species), *Chamaemespilus* (one European species), *Cormus* (one species of Europe and Asia Minor), and *Torminalis* (two species of Europe, Asia Minor, and Northern Africa). *Photinia* too has been interpreted narrowly, with about 60 southeastern Asian and Central American species, or broadly to include *Aronia* (three North American species) and *Stranvaesia* (five southeastern Asian species). Opinion has also varied about whether *Malus* (47 species of the Northern Hemisphere) should contain *Docyniopsis* (four eastern Asian species) and *Eriolobus* (one eastern Mediterranean species), and whether *Pseudocydonia* (one species from China) is congeneric with *Cydonia* (one species of southwestern and Central Asian) or *Chaenomeles* (four species of eastern Asia).

While there is certainty that Pyrinae are monophyletic (Morgan et al. 1994, Evans 1999, Evans and Campbell 2002), efforts to resolve relationships within the subtribe have not been successful (Phipps et al. 1991, Campbell et al. 1995, Evans 1999, Aldosoro et al. 2005). Campbell et al. (1995) reported that interior branch lengths within Pyrinae nuclear ribosomal DNA internal transcribed spacers (nrITS) trees are significantly shorter than terminal branches, and Evans (1999) noted a similar pattern in the chloroplast gene *ndh*F. Rapsé and Kohn (2002) observed that S-alleles in four genera of Pyrinae appeared to have coalesced much more recently than those in genera of Solanaceae. These patterns suggest a rapid, ancient radiation or cladogenesis, which is consistent with the fossil record of several genera of Pyrinae from the early middle Eocene, 48–50 million years ago (Wolfe and Wehr 1988). Rapid, ancient radiations (also called starburst speciation) are a difficult challenge to phylogenetic inference (Donoghue and Sanderson 1992, Fishbein et al. 2001,

Fishbein and Soltis 2004, Rokas et al. 2005) because short interior branches limit the historical record of early diversification. In the Pyrinae, there are also long terminal branches that are prone to the analytical artifact of long-branch attraction (Felsenstein 1978).

Our goal in this paper is to improve our understanding of the phylogeny of the Pyrinae, especially in light of potential rapid, ancient radiation and hybridizations in the history of the group. Our sample of mostly just one species per genus (Table 1) is not suitable for tests of generic limits. Our sample is instead directed toward assembly of a large molecular data set, including both coding and noncoding elements of cpDNA and nuclear regions, in order to muster sufficient phylogenetic signal to resolve deeper branches in the Pyrinae tree and to identify possible hybrid taxa. We used six cpDNA regions – the *atp*B-*rbc*L intergenic spacer plus a portion of the 3′ region of the *rbc*L gene, *trn*K region (including the *mat*K gene), 3′ region of *ndh*F, *trn*L-*trn*F region, *rpl*16 intron, and *rps*16 intron – totaling about 7,709 aligned sites. Our nuclear DNA sequences total about 8200 aligned sites and consist of the nrITS region plus four loci of the granule-bound starch synthase gene, GBSSI-1A, GBSSI-1B, GBSSI-2A, and GBSSI-2B. This gene is single copy in some angiosperms (see references in Evans et al. 2000), and it duplicated some time in the ancestry of Rosaceae and once again in the origin of the Pyreae (Evans et al. 2000, Evans and Campbell 2002). In addition to maximum parsimony, we used Bayesian analysis because it is model-based, computationally advantageous compared to maximum likelihood, and may be less prone to long-branch attraction than parsimony (Huelsenbeck 1995).

Materials and methods

Plant samples. DNAs were the same as those used in previous studies (Campbell et al. 1995, Evans et al. 2000, Evans and Campbell 2002) or were extracted using the modified CTAB procedure of Doyle and Doyle (1987) (Table 1). We were not able to amplify DNA from herbarium material of *Hesperomeles*, a genus of 11 Central and South American species, and we did not have access to material of *Docynia*, which has two Asian species. The first branches in the tree of the Pyrodae are *Gillenia* then *Kageneckia*, *Lindleya*, and *Vauquelinia* (Evans and Campbell 2002). We used *Kageneckia*, *Lindleya*, and *Vauquelinia* as outgroups of Pyrinae in analyses of cpDNA, GBSSI-1A, GBSSI-2B, and nrITS. We used *Kageneckia* and *Vauquelinia* as outgroups of Pyrinae for GBSSI-1B (no *Lindleya* was obtained for this locus). We used *Gillenia* as the outgroup for GBSSI-2A. We further explored the relationships of *Kageneckia*, *Lindleya*, and *Vauquelinia* to one another and to Pyrinae using the GBSSI-1A, GBSSI-2B, and nrITS plus GBSSI-2B data sets, with parsimony analyses using *Gillenia* as outgroup. All cpDNA sequences are newly reported, as are nrITS sequences for eight genera (see Table 2) not included in Campbell et al. (1995). We used a new sequence for *Vauquelinia californica*, because the sequence from Campbell et al. (1995; U16191) was incorrect. We include 96 GBSSI sequences in addition to those in Evans et al. (2000) and Evans and Campbell (2002).

PCR and DNA sequencing. Amplification and sequencing of nrITS1, nrITS2, and about 35 bp from the 5′ region of the 5.8S gene followed Campbell et al. (1995). PCR, cloning, and sequencing of parts of exons 1 and 9 and the intervening region of the four GBSSI loci followed Evans and Campbell (2002). PCR and sequencing of cpDNA regions was performed with primers from the literature and was supplemented, as needed, with primers developed for the Pyrodae (Table 2). PCR parameters for cpDNA were 30 cycles of 94°C for 1.5 min, 55°C for 2 min, and 72°C for 2 min, followed by 72°C for 15 min. All cpDNA regions were sequenced in both directions.

In an attempt to get all four loci for all genera, we included 188 GBSSI clones, 124 of which were the full length of the region we have used. The remaining 64 clones were sequenced using primers 3F and 8R, which yield about one-half of the GBSSI region extending from the 3′ end of exon 3 to the 5′ of exon 8. Of the 188 clones, 89 (47%) were additional sequences of a genus for a GBSSI locus. Many of these additional clones were sequenced with primers 3F and 8R. For GBSSI-1A, we had 24 additional clones for 15 genera; for GBSSI-1B, 22 additional clones for 11 genera; for

Table 1. Taxa used in this study

Taxon[a]	Range[b]	Ploidy[c]	Loci/regions	Accession[d]
Amelanchier bartramiana (Tausch) Roemer	Eastern NA	2*x*	all	1995; CSC (91-11)
Amelanchier canadensis (L.) Medik.	Eastern NA	2*x*, 4*x*	GBSSI	CSC (91-16)
Amelanchier laevis Wieg.	Eastern NA	4*x*	GBSSI	CSC (L19)
Aria alnifolia (Sieb. & Zucc.) Decne.	South Korea	2*x*	all	1995
Aronia arbutifolia (L.) Elliott	Eastern NA	2*x*	all	1995
Chaenomeles cathayensis (Hemsl.) Schneider	China	2*x*	cpDNA, nrITS	1995
Chaenomeles speciosa (Sweet) Nakai	Southeast Asia	2*x*	GBSSI	2000
Chamaemeles coriacea Lindley	Madeira	?	cpDNA, nrITS	KRR (4775)
Chamaemespilus alpina (Miller) Robertson and Phipps	Europe	2*x*, 3*x*, 4*x*	all	KRR (5276)
Cormus domestica Spach	Denmark	2*x*	all	1995
Cotoneaster lacteus W. W. Smith	China	?	all	1995
Crataegus submollis Sarg.	Eastern NA	4*x*	cpDNA, nrITS	1995
Crataegus nigra Waldst. and Kit.	Central Europe	2*x*	GBSSI	TAD (JBM 2318-50)
Crataegus rivularis Nutt.	Western NA	4*x*	GBSSI	2002
Cydonia oblonga Miller	Asia	2*x*	all	1995
Dichotomanthes tristaniaecarpa Kurz	China	2*x*	all	UCBG 41.0372
Docyniopsis tschonoskii (Maxim.) Koidzumi	E. Asia	2*x*	all	KRR (5278)
Eriobotrya japonica Lindley	China	2*x*	all	1995
Eriolobus trilobata Roemer	E. Mediterranean	2*x*	all	Hillier Bot. Gard.
Gillenia trifoliata (L.) Moench	Eastern NA	2*x*	cpDNA, GBSSI	NEWFS B76-217
Gillenia stipulata (Muhl. ex Willd.) Baill.	Eastern NA	2*x*	nrITS	KRR (s.n.)
Heteromeles arbutifolia Roemer	California	?	all	1995
Kageneckia angustifolia D. Don	South America	2*x*	cpDNA, nrITS	1994
Kageneckia oblonga Ruiz and Pav.	South America	2*x*	GBSSI	2000
Lindleya mespiloides H.B.K.	Mexico	2*x*	all	JSH (97-105)
Malacomeles denticulata (Kunth) Engler	Southwestern NA	2*x*	all	1995
Malus domestica Borkh.	Europe, Russia	2*x*	nrITS	1995
Malus sargentii Rehder	Japan	3*x*, 4*x*	cpDNA, GBSSI	2000
Mespilus canescens Phipps	Arkansas	3*x*	GBSSI	RCE s.n.
Mespilus germanica L.	eastern Europe, western Asia	2*x*	all	1995
Osteomeles schwerinae Schneider	Hawaii	2*x*	cpDNA, nrITS	1995
Osteomeles anthyllidifolia (Sm.) Lindl.	Japan, Taiwan, Hawaii	2*x*	GBSSI	RCE (004)
Peraphyllum ramosissimum Nutt.	Western NA	2*x*	all	1995
Photinia villosa (Thunb.) DC.	Eastern Asia	2*x*	all	2002

Pseudocydonia sinensis (Thouin) Schneider	South Korea	?	all	1995
Pyracantha coccinea Roemer	Southern Europe to Iran	2*x*	all	KRR (5274)
Pyrus calleryana Decne.	China	2*x*	nrITS	1995
Pyrus communis L.	Europe, western Asia	2*x*	cpDNA, GBSSI	RCE (377)
Rhaphiolepis indica (L.) Lindley	China	2*x*	cpDNA, nrITS	1995
Rhaphiolepis umbellata (Thunb.) Makino	Eastern Asia	2*x*	GBSSI	RCE (357)
Sorbus aucuparia L.	Europe	2*x*	nrITS	1995
Sorbus americana Marsh.	Eastern NA	4*x*	cpDNA, GBSSI	2002
Stranvaesia davidiana Decne.	Southeast Asia	2*x*	all	UCBG 46.0702
Torminalis clusii (Roemer) Robertson and Phipps	Europe	2*x*, 4*x*	all	KRR (5275)
Vauquelinia californica (Torr.) Sarg.	Western NA	2*x*	all	2000 JSH (s.n.)

[a]generic circumscriptions follow Robertson et al. (1991), except that we recognize *Aronia* and *Stranvaesia*

[b]Country, continent, or, if in the United States, state

[c]ploidy level from the following works (or references therein): Sax (1931), Campbell et al. (1995), Evans and Campbell (2002), Kalkman (2004), Talent and Dickinson (2005), and Tropicos (http://mobot.mobot.org/W3T); a "?" indicates that ploidy is unknown

[d]a year indicates that voucher information is in Morgan et al. (1994), Campbell et al. (1995), Evans et al. (2000), or Evans and Campbell (2002); vouchers for RCE and TAD are at TRT, for JSH (James Henrickson) are at CSLA, for *Amelanchier* are at MAINE; and for KRR (Kenneth R. Robertson) are at ILLS; sample from NEWFS (New England Wildflower Society) vouchered at TRT and ACAD; samples from UCBG (University of California Botanical Garden) vouchered at TRT and JEPS (*Stranvaesia*) or UC (*Dichotomanthes*)

Table 2. Regions sequenced

Region/ gene	Number of genera[a]	Primers	Aligned length	PICs[b]	Nodes resolved[c]	Sequence divergence (exons; other)[d]
*atp*B-*rbc*L region[e]	29	atpB-F, atpB-R[f]	973	11/3	6	0.003 (0.004); 0.004 (0.002)
*trn*K intron + *mat*K gene[g]	31	TrnK-3914F, trnK-2R (Johnson and Soltis 1994) + Pyreae primers[h]	2560	38/15	6	0.007 (0.002); 0.008 (0.003)
*ndh*F 3′ region[i]	31	Graham (1997)[j]	1011	20	5	0.008 (0.004); NA
*trn*L-*trn*F region[k]	31	c, d, e, f (Taberlet et al. 1991)	1042	22	6	0; 0.007 (0.003)
*rpl*16 intron[l]	31	rpl16F71, rpl16R1516 (Small et al. 1998)	1081	34	4	NA; 0.010 (0.004)
*rps*16 intron[m]	31	rps16F, rps16R (Oxelman et al. 1997)	1042	16	5	NA; 0.007 (0.003)
cpDNA total			7709	159	8	0.006 (0.002); 0.008 (0.002)
GBSSI-1A[n]	26	Evans et al. (2000)	1903	185	12	0.030 (0.012); 0.069 (0.030)
GBSSI-1B[n]	18	Evans et al. (2000)	1913	156	6	0.037 (0.016); 0.060 (0.025)
GBSSI-2A[n]	25	Evans et al. (2000)	1982	152	9	0.030 (0.016); 0.058 (0.028)
GBSSI-2B[n]	25	Evans et al. (2000)	1899	196	15	0.038 (0.010); 0.064 (0.030)
nrITS region[o]	28	White 1990	524	145	6	0.066 (0.066); 0.132 (0.074)
Nuclear total			8232	1014	NA	0.043 (0.059); 0.087 (0.079)

[a]includes genera of Pyreae; *Gillenia* was also sequenced for all nuclear loci

[b]potentially informative characters; for the *atp*B-*rbc*L region[e] numbers are for the *atp*B-*rbc*L spacer and 5′ region of *rbc*L, for the *trn*K intron/ *mat*K gene, numbers are for the *mat*K gene and the 5′ and 3′ *trn*K introns; there are no PICs in the *trn*L and *trn*F genes

[c]in parsimony analyses, within Pyrinae only, with at least 50% bootstrap support

[d]mean (standard deviation), determined by the Kimura 2-parameter method in PAUP, for Pyrinae only; see text for some values in *Kageneckia*, *Lindleya*, and *Vauquelinia*

[e]includes 793 bp of aligned intergenic spacer and 180 bp of *rbcL*; sequences not obtained for *Chamaemeles* and *Pseudocydonia*; GenBank numbers (*Kageneckia*, *Lindleya*, and *Vauquelinia* and, in alphabetical order, genera of Pyrinae): DQ860478-DQ860506

[f]atpB-rbcL primers: GAAGTAGTAGGATTGATTCTC and TACAGTTGTCCATGTACCAG

[g]includes 703 aligned bp of the 5′ *trn*K intron, 1527 aligned bp of the *mat*K gene, and 331 aligned bp of the 3′ *trn*K intron; GenBank numbers (see footnote e for sequence of taxa): DQ860447-DQ860477

[h]matK primers designed for Pyreae: QR: TGACTGCAAATCCCTCCGA; 4F: CTTCGCTACTGGGTGAAAGATG; 4R: CATCTTTCACC-CAGTATCGAAG; 577R: CTCGTGAAGAAAGAGCCGT; 685F: GTATCGCACYAYGTATCATTGA; 1777F: TTCRGTGGTACG-GAGTCAAATG

[i]GenBank numbers (see footnote e for sequence of taxa): DQ851526, DQ851528, DQ851560, DQ851498-DQ851500, DQ851504, DQ851507-DQ851512, DQ851514, DQ851515, DQ851517, DQ851518, DQ851530, DQ851531, DQ851533, DQ851537-DQ851539, DQ851545, DQ851547, DQ851548, DQ851550, DQ851554, DQ851558, DQ851559

[j]ndhF2F: ACTCATGCTTATTCGAAAGC; ndhF16R: CCTACTCCATTGGTAATTCCAT; ndhF1R: GGTCGAATTTCGTCTTATTTATT

[k]includes 524 aligned bp of the *trn*L intron, 50 aligned bp of the *trn*L gene, 417 aligned bp of the *trn*L-*trn*F intergenic spacer, and 52 aligned bp of the *trn*F gene; GenBank numbers (see footnote e for sequence of taxa): DQ863219-DQ863249
[l]GenBank numbers (see footnote e for sequence of taxa): DQ860416-DQ860446
[m]GenBank numbers (see footnote e for sequence of taxa): DQ848682-DQ848712
[n]see Figs. 2-5 for genera included in Pyreae; new sequences reported here for numerous taxa; Genbank numbers: DQ874881-DQ874971
[o]excluding a 35-bp region in nrITS2 with uncertain alignment and including 69 bp at the 5′ end of the 5.8S gene; no nrITS sequences generated for *Eriolobus*, *Photinia*, and *Stranvaesia*; new sequences here reported for *Kageneckia*, *Lindleya*, *Vauquelinia californica*, *V. corymbosa*, *Chamaemeles*, *Chamaemespilus*, *Dichotomanthes*, *Docyniopsis*, *Pyracantha*, and *Torminalis*; GenBank numbers: DQ811764- DQ811773

GBSSI-2A, 36 additional clones for 15 genera; and for GBSSI-2B, seven additional clones for five genera. Many of these additional clones are the result of multiple amplifications, some undertaken at Harvard University and others at the University of Maine.

DNA sequences were readily aligned by eye using the software SeAl (Rambaut 2002). Alignment required incorporation of gaps, which were coded as missing data. Indels of at least three bases and for which there was no ambiguity about homology were assessed for their phylogenetic utility by mapping them onto maximum-parsimony (MP) trees based on DNA sequences (Simmons and Ochoterena 2000). A 35-bp region of uncertain alignment in nrITS2 was eliminated from analysis. Sequence alignments are available from the corresponding author.

Phylogenetic analyses. We used a single sequence to represent each genus for all regions except GBSSI, for which we obtained multiple clones for many of the genera (see above). Phylogenies were reconstructed using MP in PAUP 4.0b10 (Swofford 2001) and Bayesian analysis in MrBayes 3.1 (Ronquist and Huelsenbeck 2003). MP heuristic searches included 1000 replications of RANDOM addition and tree bisection-reconnection (TBR) branch swapping. Sets of equally parsimonious trees were summarized using strict consensus. Bootstrapping (Felsenstein 1985) was implemented in PAUP using 1000 replicates of heuristic searches with SIMPLE addition sequence and TBR branch swapping. All regions were analyzed separately and combined if they were not incongruent. We defined incongruence topologically as the occurrence in strict consensus trees of any clades that share one or more but not all taxa and that are supported by at least 70% bootstrap by one data set. All cpDNA regions are congruent with one another using this definition, so we combined them for analysis. The only nuclear regions that are topologically congruent with one another are GBSSI-2B and nrITS.

Prior to Bayesian analysis, we used Modeltest 3.7 (Posada and Crandall 1998) and the Akaike Information Criterion to select an evolutionary model. The evolutionary model selected for Bayesian analysis of cpDNA was GTR + I + Γ. The "GTR" or general time reversible model (Yang 1994b) specifies unequal base frequencies and six substitution rates. "I" indicates a fixed proportion of invariable sites, and "Γ" indicates all rates are free to vary with rates fit to a gamma distribution

(Yang 1994a). Models for Bayesian analysis were HKY + I + Γ for GBSSI-1A, HKY + Γ for GBSSI-1B, TVM + Γ for GBSSI-2A as well as GBSSI-2B, and GTR + I + Γ for the combination of GBSSI-2B plus nrITS. The "HKY" (Hasegawa et al. 1985) model calls for different rates of transitions and transversions. The "TVM" or transversional model calls for unequal base frequencies and five substitution rates (see documentation with Modeltest). We used this model and the specified parameters in MrBayes. All Bayesian analyses were run with four chains for 1,000,000 generations by which time the average standard deviation of split frequencies was close to or less than 0.01. The first 25% of sampled trees were discarded as "burnin", and the remaining trees were summarized as 50% majority-rule consensus trees in PAUP.

We computed sequence divergence (fractional dissimilarity) with the Kimura 2-parameter method in PAUP. We compared sequence divergence for nrITS 1 plus nrITS 2 in the 25 genera of Pyrinae for which we have nrITS data with 25 genera of Rosoideae – *Acaena*, *Aremonia*, *Agrimonia*, *Alchemilla*, *Chamaerhodos*, *Cliffortia*, *Comarum*, *Dasiphora*, *Drymocallis*, *Fallugia*, *Filipendula*, *Fragaria*, *Geum*, *Hagenia*, *Leucosidea*, *Margyricarpus*, *Polylepis*, *Potaninia*, *Potentilla*, *Poteridium*, *Rosa*, *Rubus*, *Sanguisorba*, *Sibbaldianthe*, and *Sieversia* (see Potter et al. in press for sources of sequences). We compared sequence divergence for *trn*L-*trn*F in the same 25 genera from Pyrinae and Rosoideae. We assessed homogeneity of variances of sequence divergences for the two taxonomic groups, and tested for significant differences in mean sequence divergence with an appropriate T-test in Microsoft Excel.

Non-molecular character mapping. Twelve non-molecular characters were chosen from various sources in the literature (Table 3) and include four characters from floral morphology, two fungal host associations, one phytochemical character, two alternate codings of fruit type, and three wood anatomy characters. These characters were mapped onto one of the most parsimonious nrITS plus GBSSI-2B trees to investigate whether traditional morphological, chemical, and anatomical characters support relationships obtained from analyses of molecular data. Characters were unordered, and polymorphisms were optimized with DELTRAN.

Results

Sequence divergence. Among genera of Pyrinae, sequence divergence for cpDNA introns and intergenic spacers ranges from 0.004 (*atp*B-*rbc*L spacer) to 0.010 (*rpl*16 intron) and averages only 0.008 (0.002; Table 2). Divergence in exons ranges from 0 in *trn*L-*trn*F to 0.008 in *ndh*F and averages 0.006 (0.002). Overall cpDNA divergence, coding plus non-coding regions, in Pyreae is 0.008 (0.003) and drops to 0.007 (0.002) in Pyrinae alone. Divergence among *Kageneckia*, *Lindleya*, and *Vauquelinia* is 0.010 (0.002).

Sequence divergence across all nuclear regions is about seven times greater than in cpDNA for exons and almost eleven times greater for non-coding regions (Table 2). Overall divergence across the Pyreae is 0.069 (0.077), and, as in cpDNA, lower within Pyrinae (0.062 + 0.007) than among *Kageneckia*, *Lindleya*, and *Vauquelinia* (0.081 ± 0.004).

Pyrinae have significantly less sequence divergence than Rosoideae. For nrITS, divergence is 0.101 (0.060) among 25 genera of Pyrinae, which is significantly ($p < 0.001$) less than the mean (0.19 ± 0.064) among 25 genera of Rosoideae. For *trn*L-*trn*F, divergence averages 0.006 (0.003) among 25 genera of Pyrinae, which is significantly ($p < 0.001$) less than the mean (0.073 + 0.028) among 25 genera of Rosoideae.

We obtained sequences for all 31 genera in our sample of Pyreae for all cpDNA regions except the *atp*B-*rbc*L spacer, for which we did not get *Chamaemeles* and *Pseudocydonia*. Only about 2 % of the total of 7709 aligned cpDNA sites are parsimony informative, and fewer than seven nodes are resolved by each of the six regions (Table 2).

Chloroplast DNA. In the cpDNA tree (Fig. 1), eight clades are supported by at least 70% BS, and 12 clades have at least 96% Bayesian posterior probability values. *Amelanchier* and *Peraphyllum* are sister taxa, and these two genera are closely related to *Malacomeles*. *Crataegus* and *Mespilus* form a

Table 3. Non-molecular characters of particular phylogenetic interest in the Pyreae

Character	States	Description	Source
1. Stamen number	0	5	Hutchinson 1964, Robertson et al. 1991
	1	10	
	2	15	
	3	20	
	4	>20	
2. Style connation	0	absent	Robertson et al. 1991
	1	present	
3. Ovary adnation to hypanthium	0	free	Robertson et al. 1991, Rohrer et al. 1994
	1	base	
	2	>0.5	
4. Ovule number/locule and position with respect to each other	0	one	Hutchinson 1964; Sterling 1964, 1965a, 1965b, 1965c, 1966; Robertson et al. 1991, Evans 1999, Evans and Dickinson 1999, Evans and Dickinson 2005
	1	two/collateral	
	2	two/superposed	
	3	>2/files	
5. Gymnosporangium host	0	no	Savile 1979, Farr et al. 2005
	1	yes	
6 Cercospora host	0	no	Farr 1989, Farr et al. 2005
	1	yes	
7. Flavone C-glycoside synthesis	0	absent	Challice 1974
	1	present	
8. Fruit type	0	follicetum	Spjut 1994
	1	coccetum	
	2	pome	
	3	polypyrenus drupe	
9. Generic groupings following phenetic analysis of 18 fruit characters	0	group 1	Rohrer et al. 1991
	1	group 2	
	2	group 3	
	3	group 4	
	4	group 5	
	5	group 6	
	6	group 7	
	7	group 8	
	8	group 9	
10. Ray composition in wood	0	square cells	Zhang 1992
	1	Krib's III-I heterogeneous	
	2	Krib's III homogeneous	
11. Crystals in ray cells	0	Absent	Zhang 1992
	1	Present	
12. Prismatic crystals in axial parenchyma	0	Absent	Zhang 1992
	1	Present	

clade, which is sister to the *Amelanchier-Peraphyllum-Malacomeles* clade (only 62% BS and 94% Bayesian posterior probability). Small clades with strong support are also formed by *Eriobotrya* plus *Rhaphiolepis*, *Chamaemespilus* plus *Torminalis*, and *Docyniopsis* plus *Malus*. There is less support for several clades consisting of other pairs of genera. Two larger clades within Pyrinae are supported by at least 96% posterior proba-

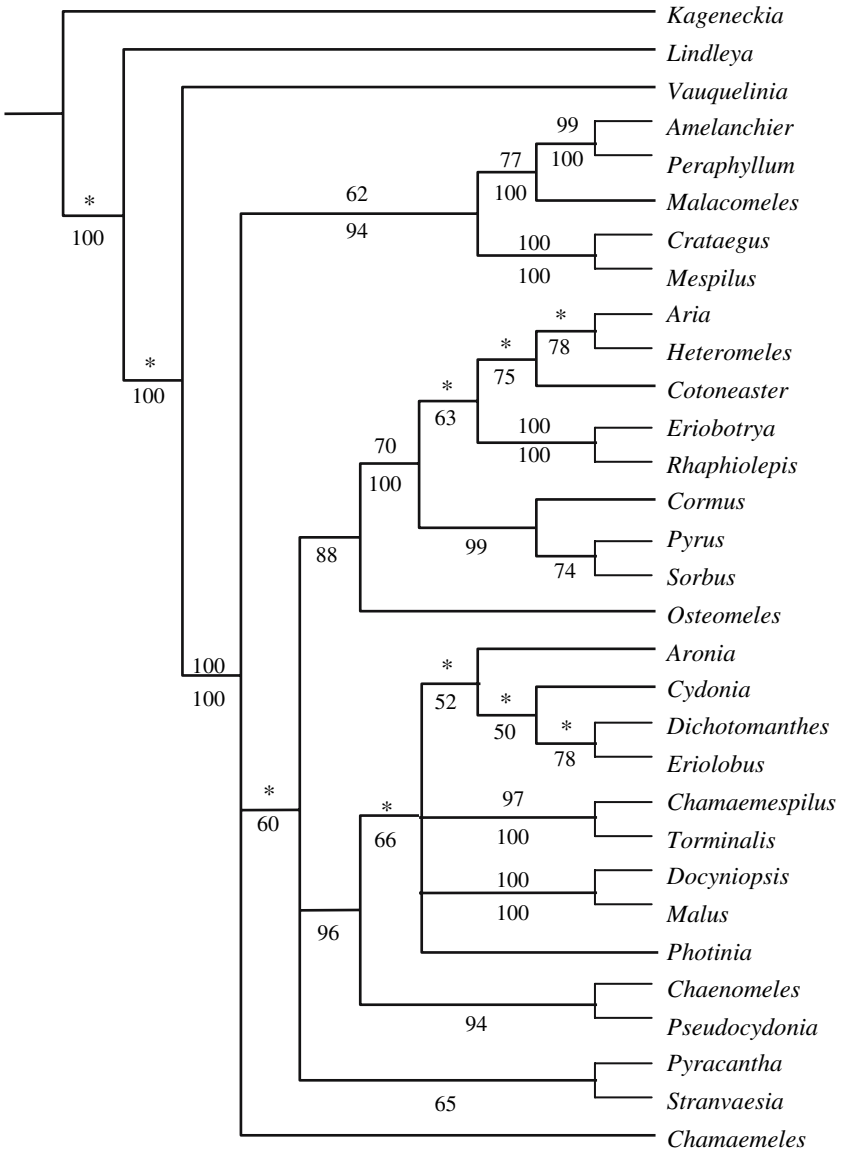

Fig. 1. The Bayesian 50% majority-rule consensus tree for all cpDNA sequences from 31 genera of Pyreae. The parsimony strict consensus of 2674 trees (769 steps, ci = 0.559, ri = 0.612) is less resolved than the Bayesian tree but does not differ otherwise in topology. Numbers above branches are parsimony bootstrap values, and numbers below branches are Bayesian posterior probabilities. Nodes indicated by an asterisk were not resolved in the parsimony strict consensus tree

bility. One of these has eight genera: *Aria*, *Cotoneaster*, and *Heteromeles*, along with the *Eriobotrya-Rhaphiolepis* and *Cormus-Pyrus-Sorbus* clades. The other large clade contains 11 genera: *Aronia*, *Cydonia*, *Dichotomanthes*, *Eriolobus*, *Photinia*, plus the *Chamaemespilus-Torminalis* and *Docyniopsis-Malus* clades. The maximum parsimony strict consensus cpDNA tree is less resolved than but otherwise topologically identical to the Bayesian tree.

We observed 15 potentially informative cpDNA indels that involve at least three base pairs and that appear to be clearly homologous. Two indels, a six-base insert in *mat*K and a five-base insert in *trn*L-*trn*F, support the *Kageneckia-Lindleya* clade. One indel, a

22-base insertion in *trn*L-*trn*F, occurred in *Aronia*, *Cydonia*, *Dichotomanthes*, *Chamaemespilus*, *Torminalis*, and *Pseudocydonia*, all of which belong to the clade of 11 genera that is supported by 96% Bayesian posterior probability (Fig. 1). Other members of this clade lack this insertion, but it occurs in *Osteomeles*. Similarly, *Crataegus* and *Mespilus* both have an indel, a seven-base insertion, that is also found in *Photinia*. There is considerable homoplasy in the remaining 11 indels.

Nuclear DNA. We are missing considerably more data for nuclear markers than for cpDNA regions. We did not obtain nrITS sequences for *Eriolobus*, *Photinia*, and *Stranvaesia*, and GBSSI sequences for between five (16%) and 13 (37%) genera for the four copies of this gene (Table 2). Preliminary analyses of the four GBSSI loci using all 188 available clones show that, in all but a few exceptions, clones from a genus coalesce with bootstrap values greater than 95%, and we tentatively consider them to be allelic. The only exceptions in GBSSI-1A and GBSSI-1B involved an additional clone of *Chamaemespilus* for each locus that is a partial sequence, the one for GBSSI-1A with only about 500 bp. Exceptions to coalescence of clones from a genus for GBSSI-2A are discussed below, and there are no exceptions for GBSSI-2B. Because coalescence of clones from a genus is the case in the great majority of genera, we used one clone to represent each genus in analyses of GBSSI-1A, −1B, and −2B. We used all available clones for locus GBSSI-2A to explore a perplexing pattern of relationships (see below).

About 12% of the 8232 nuclear sites are parsimony informative, and between six (GBSSI-1B) and 15 (GBSSI-2B) nodes are resolved by individual regions (Table 2). For all four GBSSI loci, the maximum parsimony strict consensus trees were less resolved than but otherwise topologically identical to the Bayesian tree.

GBSSI-1A trees (Fig. 2) resolve 12 nodes, seven of which have at least 70% bootstrap and 95% posterior probability values: *Aria* plus *Chamaemespilus*, *Cormus* plus *Sorbus*, *Crataegus* plus *Mespilus*, *Eriobotrya* plus *Rhaphiolepis*, *Pyrus* as sister to *Eriobotrya* plus *Rhaphiolepis*, *Cotoneaster* plus *Malus*, and *Docyniopsis* plus *Eriolobus*. We do not have a GBSSI-1A sequence for *Peraphyllum*, and *Amelanchier* was not grouped with *Malacomeles* on our tree, but all nodes between *Amelanchier* and *Malacomeles* are collapsed in the parsimony strict consensus tree. One intervening node between *Amelanchier* and *Malacomeles* is supported at 99% in the Bayesian analysis. The first branch within Pyrinae, which leads to *Stranvaesia*, is supported weakly (57% bootstrap) by parsimony and strongly (99% posterior probability) by Bayesian analysis. Three other deeper nodes within Pyrinae have less than 50% bootstrap values but more than 95% posterior probability values. There is one potentially informative GBSSI-1A indel, a 10-bp in the second intron in *Kageneckia*, *Lindleya*, *Cormus*, *Cotoneaster*, *Malus*, *Photinia*, *Pyrus*, and *Sorbus* that supports three of the well supported clades noted above but otherwise did not map well onto the GBSSI-1A tree.

Our sample for GBSSI-1B is only 18 genera; eight nodes are resolved on the parsimony GBSSI-1B tree (Fig. 3), and only 4 nodes are supported by Bayesian posterior probability values of at least 95%. *Amelanchier* and *Peraphyllum* form a clade with 99% bootstrap and 100% posterior probability, and *Aria* plus *Chamaemespilus* are also supported at 99% bootstrap and 100% posterior probability. *Cormus* and *Sorbus* form a clade that is sister to the remaining sample of Pyrinae, although support for monophyly of the remainder of our sample of Pyrinae is moderate (70% bootstrap and 90% posterior probability).

In GBSSI-1B, a 40-bp deletion occurred in all sampled taxa except *Kageneckia*, *Vauquelinia*, *Cormus*, and *Sorbus*, further supporting the clade of Pyrinae excluding the *Cormus-Sorbus* clade. Two other indels are shared by taxa that do not form clades for any data. A 12-bp insertion in the fourth intron is found

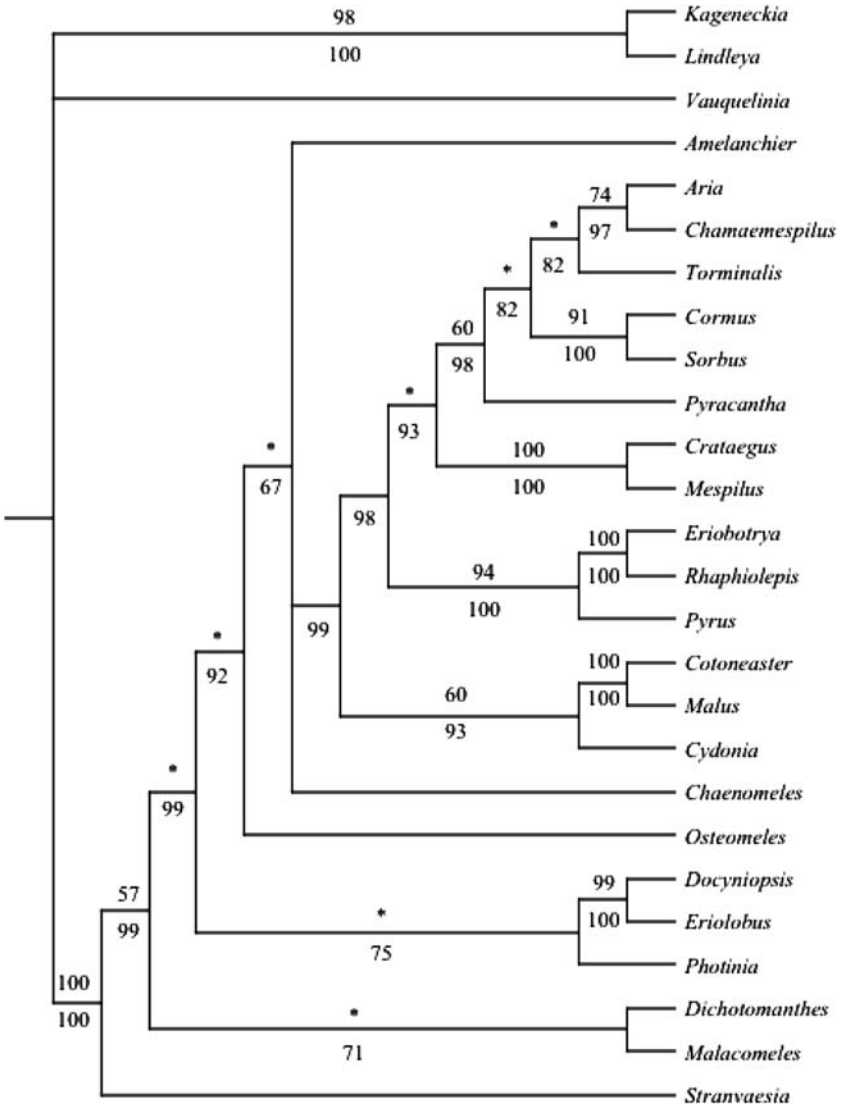

Fig. 2. The Bayesian 50% majority-rule consensus tree for GBSSI-1A from 26 genera of Pyreae. The parsimony strict consensus of 28 trees (843 steps, ci = 0.543, ri = 0.595) is less resolved than the Bayesian tree but does not differ otherwise in topology. Numbers above branches are parsimony bootstrap values, and numbers below branches are Bayesian posterior probabilities. Nodes indicated by an asterisk were not resolved in the parsimony strict consensus tree

in *Amelanchier* (all eight clones), *Aria*, *Chaenomeles* (both of the two clones for this locus), *Cotoneaster* (both clones), *Dichotomanthes* (all four clones), *Heteromeles*, and *Peraphyllum* (both clones). Finally, *Cormus*, *Malus*, and *Stranvaesia* shared a 7-bp insertion.

Analysis of a single GBSSI-2A sequence for each genus yielded the unexpected result of a strongly supported *Amelanchier-Malus* clade. Other data (see above and below) put *Amelanchier* with *Peraphyllum*, which is part of a clade of 13 genera (not including *Amelanchier*) in GBSSI-2A trees. Therefore, we analyzed all 62 clones of the Pyreae (Fig. 4), using two GBSSI-2 sequences of *Gillenia* as outgroups and got another unexpected result. When we analyzed one clone

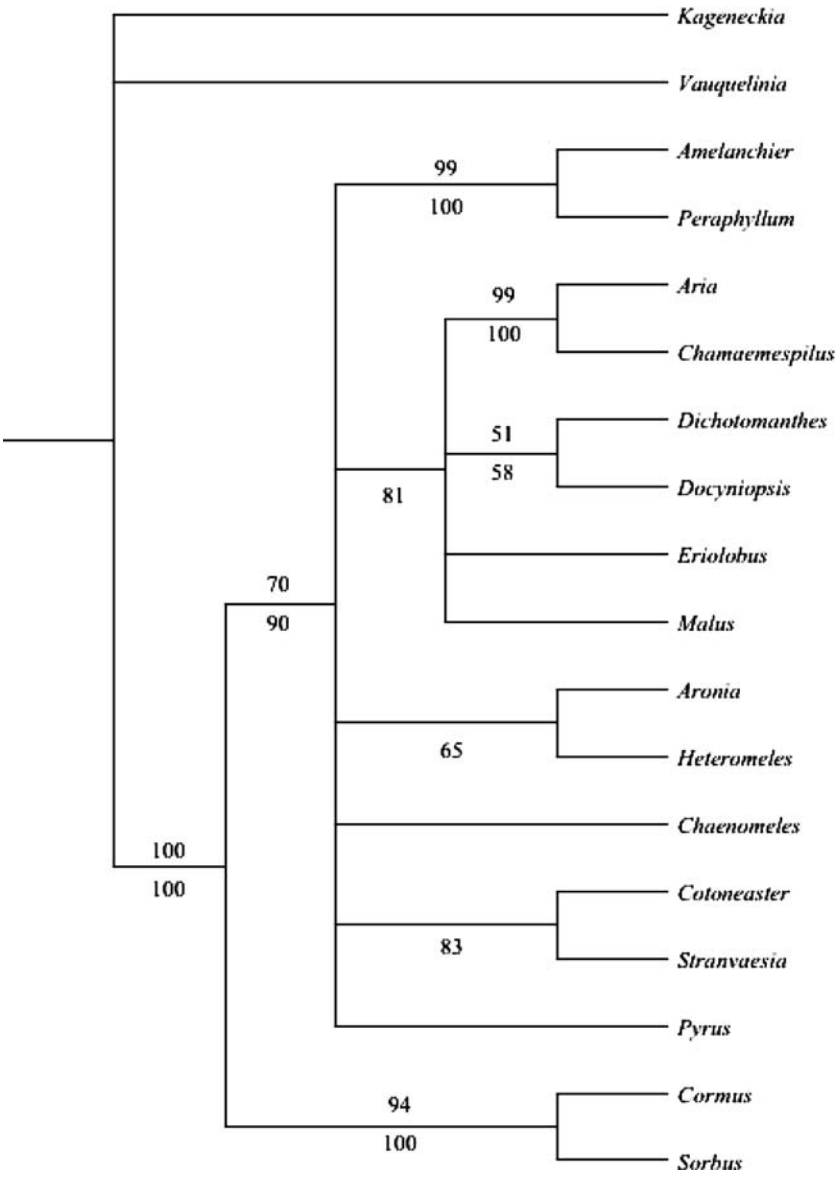

Fig. 3. The Bayesian 50% majority-rule consensus tree for GBSSI-1B from 18 genera of Pyreae. The parsimony strict consensus of 20 trees (724 steps, ci = 0.597, ri = 0.534) is less resolved than the Bayesian tree but does not differ otherwise in topology. The parsimony strict consensus tree for these data has the same topology as this tree. Numbers above branches are parsimony bootstrap values, and numbers below branches are Bayesian posterior probabilities. Nodes indicated by an asterisk were not resolved in the parsimony strict consensus tree

per genus, we used the one clone of *Osteomeles* for which we had a complete sequence (*Osteomeles*.b). Three of our six additional clones of *Osteomeles* form a clade with *Osteomeles*.b in the clade with *Cydonia*, *Dichotomanthes*, *Mespilus*, *Osteomeles*, *Photinia*, *Pseudocydonia*, plus *Pyracantha* (designated as clade Y in Fig. 4). The other three *Osteomeles* clones are a monophyletic group in the *Amelanchier-Malus* clade (clade X in Fig. 4). We are confident that the grouping of *Amelanchier*, *Malus*, and *Osteomeles*, which have not been considered to be closely related by any other molecular or non-

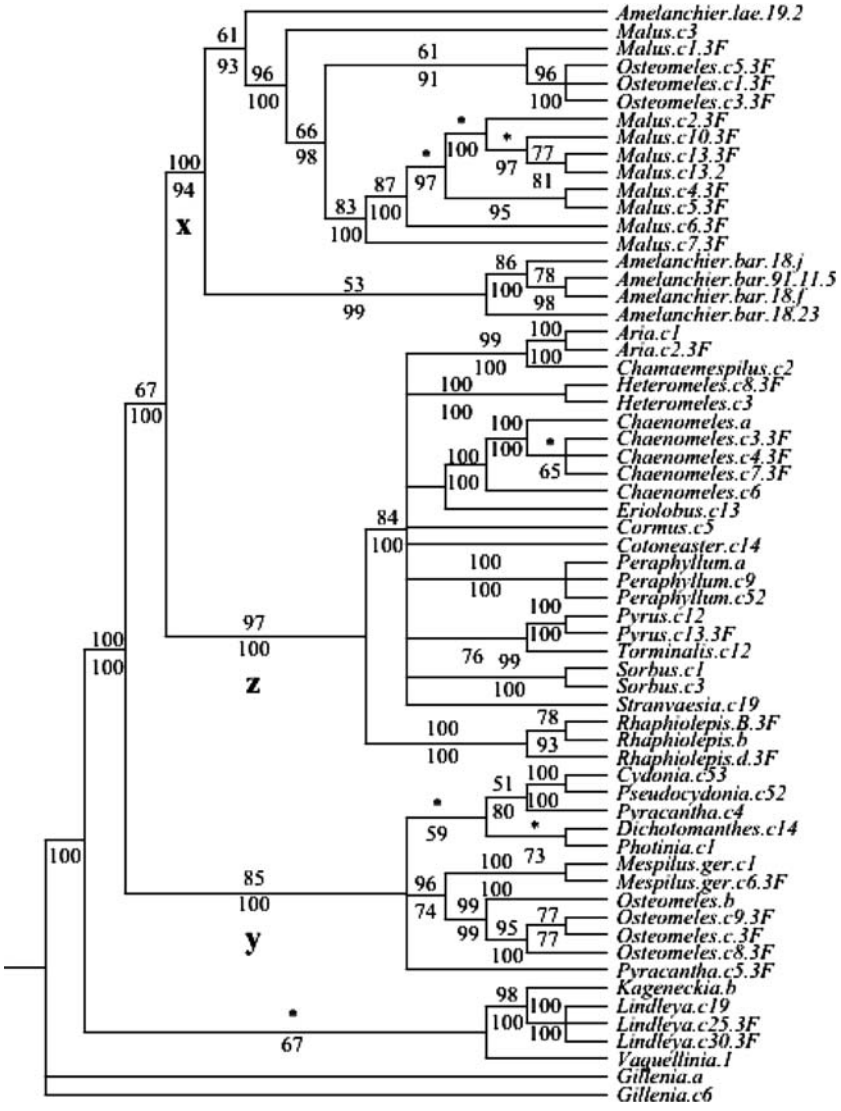

Fig. 4. The Bayesian 50% majority-rule consensus tree for GBSSI-2A from 25 genera of Pyrodae (Pyreae plus *Gillenia*), including all clones sequenced in full or in part for this locus. The clone designation follows the genus name; in *Amelanchier*, the first three letters of the specific epithet (*bartramiana* and *laevis*) are between the generic name and the clone designator. A"3F" following the clone designator indicates a partial sequence from primers 3F and 8R. The parsimony strict consensus of 1405 trees (1324 steps, ci = 0.638, ri = 0.820) is less resolved than the Bayesian tree but does not differ otherwise in topology. *Peraphyllum*.c9 and *Peraphyllum*.a are supported at 98% bootstrap support in maximum parsimony trees. Numbers above branches are parsimony bootstrap values, and numbers below branches are Bayesian posterior probabilities. Nodes indicated by an asterisk were not resolved in the parsimony strict consensus tree. Clades x, y, and z are discussed in the text

molecular data, is not the result of methodological error. We have a total of 18 clones for this locus for these three genera, and the clones were the result of multiple amplifications. *Amelanchier* clones designated with letters after the generic name were obtained at Harvard University, and clones designated with numbers were done at the University of Maine. Also, three individuals from two species represent *Amelanchier* in clade X (Fig. 4). Clade Y (Fig. 4) has 12 clones from its seven genera, and there are 25 clones for

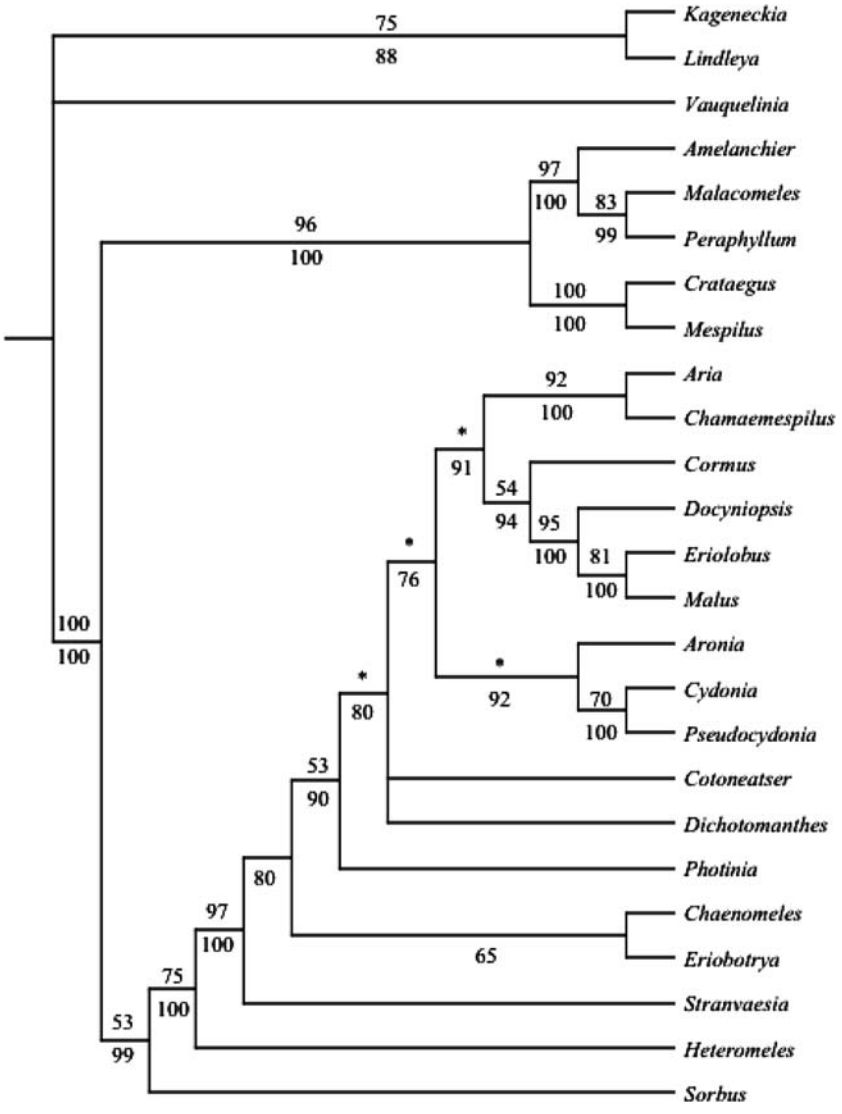

Fig. 5. The Bayesian 50% majority-rule consensus tree for GBSSI-2B from 25 genera of Pyreae. The parsimony strict consensus of 7 trees (908 steps, ci = 0.557, ri = 0.636) is less resolved than the Bayesian tree but does not differ otherwise in topology. Numbers above branches are parsimony bootstrap values, and numbers below branches are Bayesian posterior probabilities. Nodes indicated by an asterisk were not resolved in the parsimony strict consensus tree

the 13 genera forming clade Z (Fig. 4). Of the 62 GBSSI-2A sequences, 34 are additional representatives of 13 of the genera. Multiple clones of 12 of the genera formed strongly supported monophyletic groups, confirming relationships based on a single clone per genus. Clones of *Amelanchier*, *Malus*, and *Pyracantha* did not coalesce, but support for clades containing sequences of these genera plus those of other genera is not strong.

Because of the unexpected results in GBSSI-2A, we limit phylogenetic use of this locus to the observation of sister-group relationships between *Kageneckia* plus *Lindleya*, *Aria* plus *Chamaemespilus*, and *Cydonia* plus *Pseudocydonia* and to four potentially informative indels. A 21-bp insertion in the

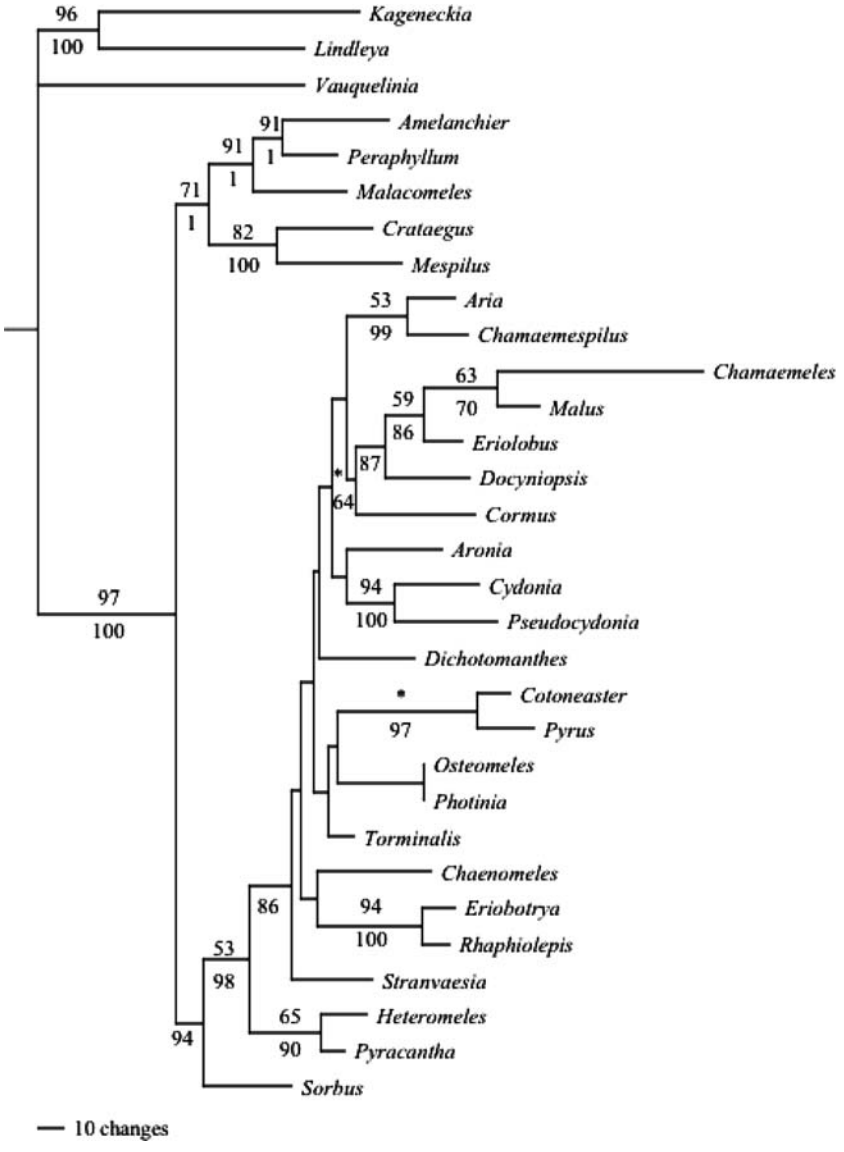

Fig. 6. A phylogram from the Bayesian analysis of the combination of nrITS plus GBSSI-2B from 31 genera of Pyreae. The parsimony strict consensus of 342 trees (1578 steps, ci = 0.492, ri = 0.514) is less resolved but otherwise topologically identical to the Bayesian tree. Numbers above branches are parsimony bootstrap values, and numbers below branches are Bayesian posterior probabilities (a posterior probability of 1 was used under nodes where there was not enough space for the value of 100). Nodes indicated by an asterisk were not resolved in the parsimony strict consensus tree

second intron is shared by *Kageneckia* and *Lindleya* but not by *Gillenia*, *Vauquelinia*, and all members of the Pyrinae. Two indels are shared by all members of clades Y and Z: a 3-bp deletion in intron 4, a 1-bp deletion in intron 4, and a 12-bp deletion in intron 6.

GBSSI-2B has more potentially informative characters, resolves more nodes, and has more coding region sequence divergence than any of the other three GBSSI loci (Table 2). It is also the most promising of the four loci of this gene in terms of topological congruence with other data. GBSSI-2B trees (Fig. 5)

resolve a clade consisting of *Amelanchier-Malacomeles-Peraphyllum*, with *Malacomeles* and *Peraphyllum* as sister taxa. This clade is sister to *Crataegus* plus *Mespilus*, and the clade of all five genera is sister to the remainder of Pyrinae. GBSSI-2B strongly supports a close relationship of *Malus* and two segregates of this genus, *Docyniopsis* and *Eriolobus*. This locus links *Aria* with *Chamaemespilus* and *Cydonia* with *Pseudocydonia*, and it resolves three of the first four branches within Pyrinae with at least 75% bootstrap and 100% posterior probability values. One indel, a 3-bp deletion in intron 7, occurred in all 11 clones of the *Amelanchier-Malacomeles-Peraphyllum-Crataegus-Mespilus* clade and *Sorbus* and in no other taxa of Pyreae.

The combined nrITS plus GBSSI-2B analysis contains all sampled genera of Pyrinae, including those not sequenced for nrITS (*Eriolobus*, *Heteromeles*, and *Stranvaesia*) and for GBSSI-2B (*Chamaemeles*, *Osteomeles*, *Pyracantha*, *Pyrus*, *Rhaphiolepis*, and *Torminalis*). The nrITS plus GBSSI-2B trees (Fig. 6) resolve only 13 nodes in parsimony analyses, compared to 15 for GBSSI-2B, and with mostly less parsimony bootstrap support. The only node that differs between GBSSI-2B trees and nrITS plus GBSSI-2B trees is the sister group of *Amelanchier*, which is *Malacomeles* in GBSSI-2B and *Peraphyllum* in the combined analysis. The maximum parsimony strict consensus tree is less resolved than but otherwise topologically the same as the Bayesian tree except that parsimony placed *Aronia* as sister to the *Aria-Chamaemespilus* clade. Bootstrap and posterior probability values are less than 50% for the relationships of *Aronia*. The nrITS region does not have any potentially informative indels of at least three bp in length.

Additional analyses of GBSSI-1A, GBSSI-2B, and GBSSI-2B + nrITS data sets using *Gillenia* as outgroup (not shown) all agree with GBSSI-2A in supporting a sister relationship between *Kageneckia* and *Lindleya*. These analyses yield three different results concerning relationships of this pair of genera and *Vauquelinia* to one another and to Pyrinae. The GBSSI-2B data set agrees with GBSSI-2A in supporting a monophyletic group composed of all three dry-fruited genera (bootstrap 53%). However, the GBSSI-1A data set supports a sister relationship between *Vauquelinia* and Pyrinae (bootstrap 64%), and the GBSSI-2B + nrITS data set supports a sister relationship between the *Kageneckia-Lindleya* group and Pyrinae (bootstrap 81%).

Mapping non-molecular characters on a molecular tree. Of the 12 characters mapped to the nrITS plus GBSSI-2B phylogeny, four support the Pyrinae: 20 stamens, adnation between ovary and hypanthium > 0.5, presence of a pome fruit, and presence of prismatic crystals in axial parenchyma (Fig. 7). Whether a genus is a host for *Cercospora* fungi supports the split between the *Amelanchier-Malacomeles-Peraphyllum-Crataegus-Mespilus* clade (which are not hosts except for *Crataegus*) and the remaining Pyrinae (almost all of which are hosts; Fig. 7). Loss of the *Cercospora* character provides support for the *Aria-Chamaemespilus* clade. Having Krib's III homogeneous rays also supports the *Amelanchier-Malacomeles-Peraphyllum-Crataegus-Mespilus* clade, but arises in parallel in a number of other Pyrinae.

Ovary-hypanthium adnation is quite informative and shows a progression from free through partially adnate in *Vauquelinia*, to various levels of adnation within Pyrinae. Other characters that support a close relationship between *Vauquelinia* and Pyrinae include being a host for *Gymnosporangium* fungal species, production of flavone-C glycosides, and lacking crystals in ray parenchyma cells in wood (Fig. 7). Loss of the *Gymnosporangium* and flavone characters, while occurring variably throughout Pyrinae, supports the *Chamaemeles-Rhaphiolepis* and *Chamaemeles-Docyniopsis* clades, respectively. In both cases, however, there is a reacquisition of these characters in *Malus*. The complex character of ovule number per locule and their position within the locule is relatively conserved throughout the tree, as many taxa have two collateral ovules per locule. Clades that deviate

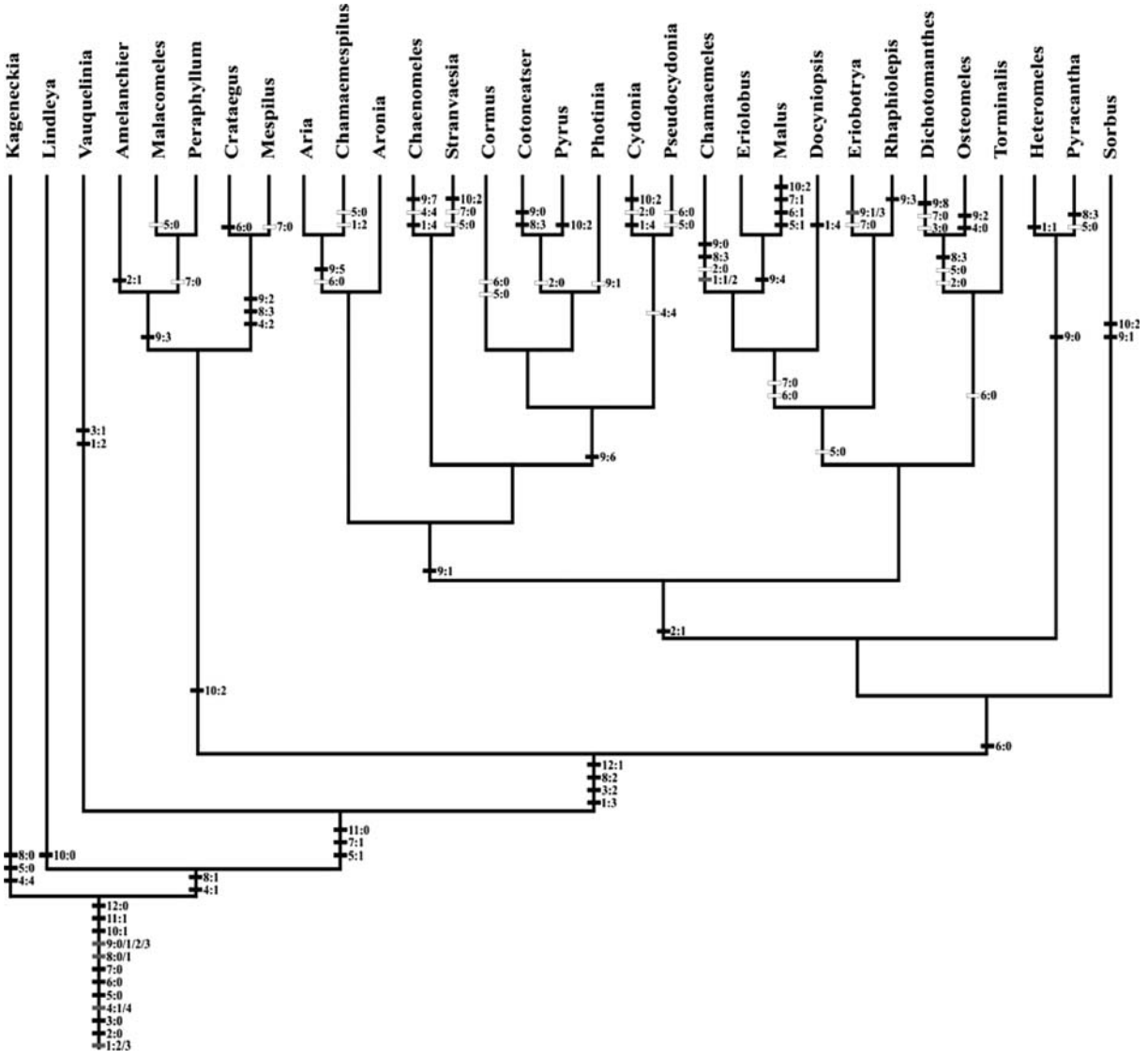

Fig. 7. Mapping of 12 non-molecular characters onto one most parsimonious tree from analysis of GBSSI-2B and nrITS, including 31 genera of Pyreae. Black boxes represent character state change, gray boxes polymorphic character states, and white boxes reversals to a previous character state. Characters and states as per Table 3

from this common state include *Crataegus-Mespilus* (two, superposed ovules) and *Cydonia-Pseudocydonia* (>two ovules in files). The latter state is also shared with *Chaenomeles* and *Kageneckia*. Fruit type is variably informative depending upon how fruits are coded. Pome fruits are synapomorphic for Pyrinae, but the presence of pyrenes is spread throughout the subtribe, and this pattern does not support the tribe Crataegeae Koehne, which was based on the possession of pyrenes. The groups determined by Rohrer et al. (1991) using phenetic methods do not support Pyrinae on the whole, but do provide support for some of the smaller clades obtained in our analyses: *Amelanchier-Peraphyllum* (group 3, minus *Rhaphiolepis*), *Crataegus-Mespilus* (group 2, minus *Osteomeles*), *Aria-Chamae-*

mespilus (group 5), *Cormus-Pseudocydonia* (group 6 plus *Photinia* from group 1), and *Eriolobus-Malus* (group 4). The remaining taxa are variously assigned to the other groups; group 1 being the least cohesive in our tree.

Discussion

Phylogenetic relationships in the Pyreae. We have markedly improved upon the study of Campbell et al. (1995), whose nrITS data identified only three clades with at least 80% bootstrap support: (1) *Amelanchier*, *Malacomeles*, plus *Peraphyllum*, (2) *Crataegus* plus *Mespilus*, and (3) *Eriobotrya* plus *Rhaphiolepis*. These clades also occur in the new results presented here. Campbell et al. (1995) also recovered an *Amelanchier-Malacomeles-Peraphyllum-Crataegus-Mespilus* clade in strict consensus trees for analyses of nrITS and of nrITS plus morphology. This clade also occurs here in trees based on cpDNA (Fig. 1), GBSSI-2B (Fig. 5), and nrITS plus GBSSI-2B (Fig. 6). These trees also contained a clade consisting of *Amelanchier*, *Malacomeles*, and *Peraphyllum,* although the sister-group relationships of these three genera differ among regions. *Peraphyllum* is sister to *Amelanchier* in cpDNA and nrITS trees and to *Malacomeles* in GBSSI-2B trees. Morphological affinities of *Amelanchier*, *Malacomeles*, and *Peraphyllum*, in particular the possession of pseudoberries with false septa separating the seeds, were noted by Jones (1946) and Robertson et al. (1991). This clade of three genera is sister to *Crataegus* plus *Mespilus* (Figs. 1, 5, 6).

The *Amelanchier-Malacomeles-Peraphyllum-Crataegus-Mespilus* clade is the largest, more or less consistently supported clade within Pyrinae. Members of this clade (except for *Crataegus*), *Kageneckia*, *Lindleya*, and *Vauquelinia* do not serve as hosts for the fungus *Cercospora*, whereas many other Pyrinae are hosts (Fig. 7; Farr 1989, Farr et al. 2005). The possibility that the *Amelanchier-Malacomeles-Peraphyllum-Crataegus-Mespilus* clade is sister to the remainder of Pyrinae is consistent with host relationship to *Cercospora* and topologies from cpDNA and GBSSI-2B. Members of the *Amelanchier-Malacomeles-Peraphyllum-Crataegus-Mespilus* clade share a three-base deletion in intron 7 of GBSSI-2B that is also in *Sorbus*. They also share the same character states in ray composition in wood (Kribs' homogeneous to heterogeneous: Zhang et al. 2001), which are also present in *Stranvaesia*, *Pyrus*, *Cydonia*, *Malus*, and *Sorbus* (Fig. 7).

cpDNA (Fig. 1), GBSSI-1A (Fig. 2), and nrITS (tree not shown) unequivocally support a close relationship between *Eriobotrya* and *Rhaphiolepis*, two genera of evergreen trees or shrubs that bear fruits with one to three large seeds and that are mostly southeast Asian in distribution (Robertson et al. 1991). In Campbell et al.'s (1995) nrITS tree *Vauquelinia* was nested within this clade, but the new sequence of *Vauquelinia* used in the present study lies outside Pyrinae in nrITS trees, along with the two other dry-fruited genera, *Kageneckia* and *Lindleya*.

The only other clade within Pyrinae identified by nrITS in Campbell et al. (1995) with more than 50% bootstrap support was the *Cydonia-Pseudocydonia* clade, which is strongly supported by GBSSI-2A (Fig. 4) and GBSSI-2B (Fig. 5). We did not obtain GBSSI-1A and GBSSI-1B sequences for *Pseudocydonia*. The character state of more than two ovules per locule in Pyrinae is found only in *Cydonia*, *Pseudocydonia*, *Chaenomeles*, and *Docynia* (not included in this study). Robertson et al. (1991) considered *Pseudocydonia* to be intermediate between *Cydonia* and *Chaenomeles*, and other authors have put it into one of these two genera. *Cydonia* is strongly linked by our cpDNA data to *Dichotomanthes*, another monotypic genus from China. Nuclear data do not specify with any certainty the relationships of *Dichotomanthes*, whose flowers are unusual for the solitary, superior carpel and whose fruits are unusual because the carpel is not completely enclosed by the hypanthium.

GBSSI-2A trees, for which two *Gillenia* sequences were used as outgroups, indicate

that *Kageneckia* and *Lindleya* are sister taxa. This relationship is corroborated by a 21-bp insertion in the second intron of GBSSI-2A and possibly by the presence of crystals in the ray cells of *Kageneckia* and *Lindleya* (the latter character has not been studied in *Gillenia*.).

Another clade that is supported by our data includes *Docyniopsis*, *Eriolobus*, and *Malus*. This clade is supported by GBSSI-2B (Fig. 5), but support diminishes when this locus is combined with nrITS (Fig. 6). This combination of data also inserts *Chamaemeles* into this clade as sister to *Malus*. We do not have GBSSI sequences of *Chamaemeles*, a native of the Canary Islands, and the long branch that leads to it in nrITS trees may be responsible for falsely uniting it with *Malus* in trees based on nrITS alone and in combination with GBSSI-2B. However, these genera, except *Malus*, are united by not being hosts for either *Cercospora* or *Gymnosporangium* fungi (Fig. 7). Furthermore, they are linked with the clade containing *Eriobotrya* and *Rhaphiolepis*. Analyses of cpDNA did not put *Chamaemeles* near any members of the *Docyniopsis-Eriolobus-Malus* clade, but cpDNA did unite *Docyniopsis* and *Malus* in their own clade that is part of a part of a larger clade that includes *Eriolobus* (Fig. 2). GBSSI-1A unites *Docyniopsis* and *Eriolobus*, but *Malus* is very far removed and strongly linked to *Cotoneaster*. This unexpected relationship between *Malus* and *Cotoneaster* is confirmed by a second *Malus* GBSSI-1A clone, which, together with the first *Malus* GBSSI-1A clone, forms a clade that is sister to *Cotoneaster*.

Docyniopsis, *Eriolobus*, and *Malus* plus *Docynia* (not included in this study) are the only genera of Pyreae with dihydrochalcones (Challice 1973). These genera (except for *Malus*) plus *Chamaemeles* have lost the ability to produce flavone C-glycosides (Fig. 7; Challice 1974). Given the plausibility of the *Docyniopsis-Eriolobus-Malus* clade, we are suspicious of strongly supported relationships of *Malus* to *Cotoneaster* (GBSSI-1A) and to *Amelanchier* plus *Osteomeles* (GBSSI-2A). Paralogy (duplication) of GBSSI-2A possibly explains this aberrant relationship, and it may be that a similar history produced the questionable relationship of *Malus* in GBSSI-1A, but we did not find accessions harboring strongly divergent sequences of this locus, like those in *Osteomeles* for GBSSI-2A.

We thus find support, from cpDNA and nuclear DNA as well as non-molecular data, for three clades within Pyrinae: the *Amelanchier-Malacomeles-Peraphyllum-Crataegus-Mespilus* clade, the *Eriobotrya-Rhaphiolepis* clade, and the *Docyniopsis-Eriolobus-Malus* clade. Other relationships within Pyrinae are not as widely supported and must be considered tentative. Nuclear and morphological data, but not cpDNA, support the *Cydonia-Pseudocydonia* clade. Similarly, as already noted, one genome identifies support for relationships of *Chamaemeles* (nrITS) and *Dichotomanthes* (cpDNA), whereas the other genome is not helpful, leaving us uncertain about the position of these two genera.

The data are also unclear about relationships of the remaining 14 genera of Pyrinae. GBSSI-2B placed *Heteromeles* and *Stranvaesia* as isolated, early branches in the Pyrinae. Otherwise, none of our data supported clear relationships of these two genera nor of *Aronia*, *Chaenomeles*, *Osteomeles*, *Photinia*, and *Pyracantha*. Uncertainty for many genera is tied to incongruence between cpDNA and GBSSI loci and among GBSSI loci. We have already discussed *Malus*, which links with *Docyniopsis* in cpDNA, *Cotoneaster* in GBSSI-1A, *Amelanchier* and *Osteomeles* in GBSSI-2A, *Eriolobus* and *Docyniopsis* in GBSSI-2B, and *Chamaemeles* in nrITS (as seen in trees based on the combination of nrITS and GBSSI-2B). At least two data sets differ in their estimate of the relationships of seven other genera: *Sorbus*, *Cormus*, *Pyrus*, *Cotoneaster*, *Aria*, *Chamaemespilus*, and *Torminalis*. *Sorbus* is sister to *Pyrus* in cpDNA trees, sister to *Cormus* in GBSSI-1A and GBSSI-1B trees, and the second branch in GBSSI-2B trees. Posterior probability values are 99 or 100% for all these relationships (except cpDNA), and bootstrap support ranges from strong

(GBSSI-1A), to weak (GBSSI-2B), to less than 50% (cpDNA and GBSSI-1B). Like GBSSI-2B, GBSSI-1B placed *Sorbus* (with *Cormus*) as the first branch in Pyrinae.

In addition to being grouped with *Sorbus*, *Cormus* is sister to the *Docyniopsis-Eriolobus-Malus* clade in GBSSI-2B trees, and *Pyrus* is sister to the *Eriobotrya-Rhaphiolepis* clade in GBSSI-1A trees. *Cotoneaster* moves from the sister to *Malus* in GBSSI-1A to link with *Pyrus* in trees based on the combination of nrITS and GBSSI-2B. As already noted, relationships of *Cydonia* differ strongly between cpDNA and nuclear data. This conflict between genomes may be a record of past gene flow, a possibility considered more fully below in the case of *Aria*, *Chamaemespilus*, and *Torminalis*.

Why is the phylogeny of the Pyrinae so uncertain? Supertribe Pyrodae and tribe Pyreae are clearly monophyletic (Potter et al. in press), as is subtribe Pyrinae (Figs. 1–6). Relationships within Pyrinae, however, are uncertain, possibly because evolution of this group may have involved processes that can confound phylogenetic inference. Four processes that could have occurred in the Pyrinae are intergeneric hybridization, rapid and ancient radiation, slow divergence, and gene duplication followed by paralog extinction. Here we discuss how each of these processes could have affected evolution of Pyrinae.

The chloroplast is maternally inherited in Pyrinae (Ishikawa et al. 1992, Oddou-Muratorio et al. 2001), and, in comparison with nuclear data, can be used to detect hybridization (Linder and Rieseberg 2004). Clear, well-supported conflict between cpDNA and nuclear tree topologies occurs for *Aria*, *Chamaemespilus*, and *Torminalis*, three segregates of *Sorbus*. *Chamaemespilus* and *Torminalis* are sister taxa with 99% bootstrap support and 100% posterior probability in cpDNA trees (Fig. 1). This relationship appears, with less support, in trees based on the *atp*B-*rbc*L intergenic spacer, *mat*K, and *trn*L-*trn*F (trees not shown). *Torminalis* and *Eriolobus* form a clade on the *ndh*F trees, but only with 65% bootstrap support. In contrast, *Aria* and *Chamaemespilus* are sister taxa in GBSSI trees, with modest to strong support from GBSSI (Figs. 2–5) and the combination of GBSSI-2B plus nrITS tree (Fig. 6). This result represents a possible case of chloroplast (Rieseberg and Soltis 1991) or nuclear capture (Petit et al. 1997).

A scenario that is consistent with the cpDNA and nuclear DNA data is that *Chamaemespilus* arose through hybridization, obtaining its chloroplast genome from *Torminalis* and its nuclear genome from *Aria*. *Aria* and *Torminalis* have long been known to hybridize frequently in Europe in current times (Godron 1874), but gene flow has been shown to be in the other direction (Nelson-Jones et al. 2002), with *Aria* the cpDNA parent in 75% of matings (Oddou-Muratorio et al. 2001). *Chamaemespilus* may have arisen on multiple occasions, from crosses in both directions. *Chamaemespilus* is diploid, triploid or tetraploid, agamospermous (Liljefors 1934, 1953), and shares non-molecular character states with both *Aria* and *Torminalis*. The flesh of the fruit is heterogeneous in *Aria* and *Chamaemespilus*, which led Robertson et al. (1991) to consider these two genera to be a ''natural unit.'' Flavonoid chemistry of *Chamaemespilus*, on the other hand, is more similar to *Torminalis* than to *Aria* (Challice and Kovanda 1978). Non-molecular characters thus suggest that *Chamaemespilus* received genes from both *Aria* and *Torminalis*.

The lack of support from the data for deep branches in the Pyrinae molecular phylogeny could be explained by a rapid radiation or cladogenesis early in the history of the group. Short internal branches created by rapid cladogenesis leave a meager record of diversification that is further potentially obscured by long terminal branches. This explanation was supported in a study attempting to resolve early metazoan evolution: ''lack of phylogenetic resolution is a positive signature of closely spaced cladogenetic events'' (Rokas et al. 2005). This explanation was also supported

by Fiala and Sokal's (1985) simulations. They suggested that decreased stemminess (shorter internodes, longer terminal branches) reduces the accuracy with which a phylogeny can be estimated, hence the resolution that can be obtained. A rapid, ancient radiation of Pyrinae appears to be recorded by fossil data. Wolfe and Wehr (1988) observed that Rosaceae underwent "a major generic-level diversification during the Eocene" in northwestern North America. Fossils of *Amelanchier*, *Crataegus* and *Photinia* as well as close relatives of *Malus* and *Sorbus* are known from the early middle Eocene (48–50 million years ago); *Heteromeles* and *Sorbus* appeared later in the Eocene.

We speculate that this rapid radiation could have been associated with acquisition, in the ancestors of the pome-fruited clade, of a fleshy fruit and the animal dispersal syndrome that such a fruit makes possible. In this connection, we note the considerable variation in fruit composition (multiple origins of polypyrenous drupes), and in fruit size, texture, and seed number that occurs both between and within genera in this clade. All of these features have the potential to have been (and to be) specializations for different vertebrate vectors of seed dispersal.

The short internal branches generated by a rapid, ancient radiation provide little signal to determine branching order and relationships during the radiation. Because of conflict among our data sets involving branches nearer the tips of the trees, we have not been able to combine full data sets to achieve greater resolution of deep branches that more data potentially produce. We did attempt to combine GBSSI data sets after excluding taxa that are in conflict with the hope of resolving deeper branches. Unfortunately, this approach did not yield additional resolution, perhaps because of other conflict between pruned data sets. Bayesian and parsimony trees are topologically congruent, and therefore it does not appear that long-branch attraction strongly influences inferences of relationships among genera of Pyrinae.

Our efforts to reconstruct the phylogeny of Pyrinae have also been hampered by low sequence divergence, especially in the chloroplast DNA, wherein it is about 1% or less and only 2% of the sites are potentially informative for phylogeny (Table 2). Sequence divergence within Pyrinae is an order of magnitude less than that within Rosoideae for cpDNA and 42% less in nrITS. Sequence divergence that is significantly lower than found in Rosoideae may reflect a difference in habit. Wilson et al. (1990), for example, reported low chloroplast sequence divergence in palms. All Pyrinae are shrubs or small to medium-sized trees, and most of the Rosoideae we included in our comparison are herbaceous.

Other indications that genera of Pyreae have not diverged greatly from one another genetically are interfertility (Robertson et al. 1991), meiotic pairing of chromosomes from different genera (Sax 1932), graft compatibility (Robertson et al. 1991), and alignability of GBSSI introns between all genera, including *Gillenia*, within each GBSSI locus as well as between GBSSI-1A and GBSSI-1B and between GBSSI-2A and GBSSI-2B.

Sampling paralogs, as may have happened in the GBSSI-2A phylogeny (Fig. 4), could confound phylogeny reconstruction. It is possible that clades X, Y, or Z of the GBSSI-2A phylogeny reflect the true phylogeny of the Pyrinae and are not the product of gene duplication. Genera that are strongly supported by other nuclear data as sister taxa – *Aria* plus *Chamaemespilus* and *Cydonia* plus *Pseudocydonia* – are sister taxa in GBSSI-2A trees. On the other hand, in addition to *Amelanchier* and *Peraphyllum* not being close to one another on the GBSSI-2A tree, clades Y and Z do not correspond to any clades recovered from any other data, and one would expect *Mespilus*, which is in clade Y, and *Peraphyllum*, which is in clade Z, to be more closely related. The presence of two divergent copies of GBSSI-2A in *Osteomeles* (Fig. 4) but not in other genera is most easily explained by gene duplication in the ancestor of Pyrinae and loss of one copy in all genera except *Osteo-*

meles. Clades Y and Z of GBSSI-2A are both strongly monophyletic (Fig. 4) and one of them may be a duplicate, with all paralogs having gone extinct.

Divergence between clades X, Y, and Z could also be the result of recombination between GBSSI-2A and GBSSI-2B. If this were the case, we would expect that the recombined region (or regions) of one or more of these clades would be more closely related to the same region (or regions) of GBSSI-2B than to GBSSI-2A. We performed parsimony analyses of seven parts of GBSSI-2: the 3′ region of exon 1 through intron 2 (641 aligned sites), exon 3 through exon 4 (328 sites), intron 4 through exon 5 (288 sites), intron 5 through exon 6 (228 sites), intron 6 through exon 7 (239 sites), intron 7 through exon 8 (317 sites), and intron 8 through the 5′ region of exon 9. In all analyses of these seven parts clades x, y, and z are monophyletic, showing that recombination has not occurred.

We did find an apparent recombinant GBSSI-2A sequence. In the clone *Amelanchier*.B18.f, the first approximately 71% of the region 5′ (extending part way into exon seven) nests in GBSSI-2A, and the last 29% of the region at the 3′ end nests in GBSSI-2B.

The apparent duplication representing clades X and Y (Fig. 4) was followed by considerable divergence; mean sequence divergence between clones of *Osteomeles* in clades X and Y (Fig. 4) is 0.602 ($\pm$0.006). This apparent duplication likely occurred early in the diversification of the Pyreae because divergence between clades X, Y, and Z, which ranges from 0.039 ($\pm$0.005; clade Y to Z) to 0.041 ($\pm$0.002; clade X to clade Z), is close to that among all genera of GBSSI-2B (0.042 $\pm$ 0.011). This assumes that rates of evolution are roughly equivalent between GBSSI-2A and GBSSI-2B. There has been a long time for paralog extinction, which may have been driven by reduction of genetic redundancy (Sang 2002). This hypothesized GBSSI-2A duplication and loss have obscured some of the phylogenetic signal from that locus.

Sampling only one species for most of the genera may have added to a possible problem of long-branch attraction and thereby contributed to the lack of resolution of our trees.

Taxonomic implications. The lack of divergence among genera of Pyrinae indicated by low sequence divergence, GBSSI intron alignability, present-day intergeneric hybridization, chromosomal homology, and graft compatibility is also evident in the morphological similarity of some genera of Pyrinae. Morphological similarity led Vidal (1965) and Kalkman (1973) to merge *Stranvaesia* into *Photinia*. Robertson et al. (1991) also included *Aronia* in *Photinia*. None of our molecular data, however, place these pairs of genera together or even near one another on our trees. We therefore recommend maintaining these genera until more data are available.

Another challenge to phylogeny reconstruction of Pyrinae, intergeneric hybridization, has also played a role in the taxonomic history of the group. Interfertility was a justification for the broad definition of *Sorbus* to include other genera with which it crosses in the wild, namely *Aria*, *Chamaemespilus*, *Cormus*, and *Torminalis* (Robertson et al. 1991). *Cormus* and *Sorbus* are a strongly supported lineage in GBSSI-1A, -1B, but not 2B. *Aria* and *Chamaemespilus* are together with posterior probabilities greater than 95% and 74 to 99% bootstraps at GBSSI loci. *Torminalis* is not closely associated with any other *Sorbus* s. l. genera in nuclear gene trees. *Sorbus* s. l. is a clade in the Bayesian GBSSI-1A tree but with only 82% posterior probability. Hence our data do not support a broad definition of *Sorbus*.

The most extreme expression of the interfertility justification for merging genera of Pyrinae was that of Sax (1931) who said, ''perhaps all of the Pomoideae [Pyrinae] could be classed as one genus and most of the present genera could be regarded as genetic species''; genetic species were defined as a ''group of individuals of common descent which possess genetically similar sets of chromosomes''. One could put *Docyniopsis* and *Eriolobus* back into

Malus or unite *Crataegus* and *Mespilus* (Lo et al. in press), but otherwise we argue for the status quo. Most genera of Pyreae are well marked morphologically (Robertson et al. 1991), and major realignments of genera are therefore not justifiable on the basis of molecular and morphological data.

In summary, the results presented here have made possible some resolution of relationships within Pyrinae. These data confirm previous suggestions about relationships of the dry-fruited genera and added some resolution to relationships among genera of Pyrinae. Many relationships remain unresolved, despite the considerable amount of data generated for this study, leading to the conclusion that the evolutionary history of Pyrinae involved rapid, ancient radiation. These results have also provided insights into the possible impacts of intergeneric hybridization and gene duplication on the evolution of this group. Additional DNA sequences from both the chloroplast and nucleus could identify lineages of hybrid origin and further resolve the phylogeny of the Pyrinae. The combination of hybridization and a rapid radiation or cladogenesis, however, makes full resolution of this group a difficult task.

We thank Wes Wright for help with DNA sequencing; the University of Maine DNA Sequencing Facility; the Arnold Arboretum (Jamaica Plain, MA), James S. Henrickson, Kenneth R. Robertson, University of California Botanical Garden (H. Forbes, Curator), the New England Wild Flower Society "Garden in the Woods" (C. Lowe, Director of Horticulture), the Dale Bumpers Small Farms Research Center (Booneville, Arkansas) and the Yucca Do Nursery (Waller, Texas) for plant samples; Craig W. Greene for help in the field; Harald Schneider and one anonymous reviewer for many helpful suggestions; Volker Wissemann for organizing the symposium on the Rosaceae at the International Botanical Congress in Vienna that stimulated this work; and the Natural Sciences and Engineering Research Council of Canada (NSERC) grants to RCE (238505) and to TAD (A3430), the National Science Foundation (BSR-9806945), and the Maine Agricultural and Forestry Experiment Station for funding. This is the Maine Agricultural and Forestry Experiment Station external publication number 2894.

References

Aldasoro J. J., Aedo C., Navarro C. (2005) Phylogenetic and phytogeographical relationships in Maloideae (Rosaceae) based on morphological and anatomical characters. Blumea 50: 3–15.

Campbell C. S., Baldwin B. G., Donoghue M. J., Wojciechowski M. F. (1995) A phylogeny of the genera of Maloideae (Rosaceae): Evidence from Internal Transcribed Spacers of nuclear ribosomal DNA sequences and congruence with morphology. Amer. J. Bot. 82: 903–918.

Challice J. (1973) Phenolic compounds of the subfamily Pomoideae: A chemotaxonomic survey. Phytochemistry 12: 1095–1101.

Challice J. S. (1974) Rosaceae chemotaxonomy and the origins of the Pomoideae. Bot. J. Linn. Soc. 69: 239–259.

Challice J., Kovanda M. (1978) Flavonoids as markers of taxonomic relationships in the genus *Sorbus* in Europe. Preslia 50: 305–320.

Chevreau E., Lespinasse Y., Gallet M. (1985) Inheritance of pollen enzymes and polyploid origin of apple (*Malus* x *domestica* Borkh.). Theor. Appl. Genet. 71: 268–277.

Donoghue M. J., Sanderson M. J. (1992) The suitability of molecular and morphological evidence in reconstructing plant phylogeny. In: Soltis D. E., Soltis P. S., Doyle J. J. (eds.) Molecular systematics of plants. New York, Chapman and Hall, pp. 340–368.

Doyle J. J., Doyle J. L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

Evans R. C. (1999) Molecular, morphological, and ontogenetic evaluation of relationships and evolution in Rosaceae. Ph.D. thesis, Botany Department, University of Toronto.

Evans R. C., Alice L. A., Campbell C. S., Kellogg E. A., Dickinson T. A. (2000) The granule-bound starch synthase (GBSSI) gene in Rosaceae: multiple putative loci and phylogenetic utility. Molec. Phylogenet. Evol. 17: 388–400.

Evans R. C., Campbell C. S. (2002) The origin of the apple subfamily (Rosaceae: Maloideae) is

clarified by DNA sequence data from duplicated GBSSI Genes. Amer. J. Bot. 89: 1478–1484.
Evans R. C., Dickinson T. A. (1999) Floral ontogeny and morphology in subfamily Spiraeoideae Endl. (Rosaceae). Int. J. Pl. Sci. 160: 981–1012.
Evans R. C., Dickinson T. A. (2005) Floral ontogeny and morphology in *Gillenia* (''Spiraeoideae'') and subfamily Maloideae C. Weber (Rosaceae). Int. J. Pl. Sci. 166: 427–447.
Farr D. F. (1989) Fungi on plants and plant products in the United States. APS Press, St. Paul, Minn.
Farr D. F., Rossman A. Y., Palm M. E., McCray E. B. (2005) Fungal databases, Systematic Botany & Mycology Laboratory. Agricultural Research Service, United States Dept. of Agriculture.
Felsenstein J. (1978) Cases in which parsimony and compatibility methods will be positively misleading. Syst. Zool. 27: 401–410.
Felsenstein J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.
Fiala K. I., Sokal R. R. (1985) Factors determining the accuracy of cladogram estimation: evaluation using computer simulation. Evolution 39: 609–622.
Fishbein M., Hibsch-Jetter C., Soltis D. E., Hufford L. (2001) Phylogeny of Saxifragales (angiosperms, eudicots): analysis of a rapid, ancient radiation. Syst. Biol. 50: 817–847.
Fishbein M., Soltis D. E. (2004) Further resolution of the rapid radiation in Saxifragales (angiosperms, eudicots) supported by mixed-model Bayesian analysis. Syst. Bot. 29: 883–891.
Godron D. A. (1874) De l'hybridité dans le genre Sorbier. Rev. Sci. Nat. 4: 443–447.
Graham S. (1997) Phylogenetic analyses of breeding-system evolution in heterostylous monocotyledons. Ph.D. thesis, Botany Department, University of Toronto.
Hasegawa M., Kishino H., Yano T. (1985) Dating of the human-ape split by a molecular clock of mitochondrial DNA. J. Molec. Evol. 21:160–174.
Huelsenbeck J. P. (1995) Performance of phylogenetic methods in simulation. Syst. Biol. 44: 17–48.
Hutchinson J. (1964) The genera of flowering plants, vol. 1, Dicotyledons. Clarendon Press, Oxford.
Ishikawa S., Kato S., Imakawa S., Mikami T., Shimamoto Y. (1992) Organelle DNA polymorphisms in cultivated apple and rootstocks. Theor. Appl. Genet. 83: 963–967.
Jones G. N. (1946) American species of *Amelanchier*. Urbana, Illinois.
Kalkman C. (1973) The Malesian species of the subfamily Maloideae (Rosaceae). Blumea 21: 413–442.
Kalkman C. (2004) Rosaceae. In: Kubitzki K. (ed.) The families and genera of vascular plants. Springer, Berlin, pp. 343–386.
Kovanda M. (1965) On the generic limits in the Maloideae. Preslia 37: 27–34.
Liljefors A. (1934) Űber normale und apospore Embryosackentwicklung in der Gattung *Sorbus*, nebst einigen Bemerkungen űber die Chromosomenzahlen. Svensk Bot. Tidskr. 28: 290–299.
Liljefors A. (1953) Studies on propagation, embryology, and pollination in *Sorbus*. Acta Horti Berg. 16: 277–329.
Linder C. R., Rieseberg L. H. (2004) Reconstructing patterns of reticulate evolution in plants. Amer. J. Bot. 91: 1700–1708.
Lo E., Stefanovic S., Dickinson T. A., in press. *Crataegus* and *Mespilus* (Pyreae, Rosaceae) – two genera or one? Syst. Bot.
McDade L. A. (1995) Hybridization and phylogenetics. In: Hoch P. C., Stephenson A. G. (eds.) Experimental and molecular approaches to plant biosystematics. St. Louis, Missouri Botanical Garden, pp. 305–331.
Morgan D. R., Soltis D. E., Robertson K. R. (1994) Systematic and evolutionary implications of *rbc*L sequence variation in Rosaceae. Amer. J. Bot. 81: 890–903.
Nelson-Jones E. B., Briggs D., Smith A. G. (2002) The origin of intermediate species of the genus *Sorbus*. Theor. Appl. Genet. 105: 953–963.
Oddou-Muratorio S., Petit R. J., Guerroue B. L., Guesnet D., Demesure B. (2001) Pollen- versus seed-mediated gene flow in a scattered forest tree species. Evolution 55: 1123–1135.
Petit R. J., Pineau E., Demesure B., Bacillieri R., Ducousso A., Kremer A. (1997) Chloroplast DNA footprints of postglacial recolonization by oaks. Proc. Natl. Acad. Sci. USA 94.
Phipps J. B., Robertson K. R., Rohrer J. R., Smith P. G. (1991) Origins and evolution of subfamily Maloideae (Rosaceae). Syst. Bot. 16: 303–332.
Posada D., Crandall K. A. (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Potter D., Eriksson T., Evans R. C., Oh S.-H., Smedmark J., Morgan D., Kerr M., Robertson K. R., Arsenault M., Dickinson T. A., Campbell C. S., In press. Phylogeny and classification of Rosaceae. Pl. Syst. Evol. 266: 5–43.

Rambaut A. (2002) Se-Al: sequence alignment editor. University of Oxford, Oxford, England.

Raspé O. A. Jacquemart L., De Sloover J. (1998) Isozymes in *Sorbus aucuparia* (Rosaceae: Maloideae): genetic analysis and evolutionary significance of zymograms. Int. J. Pl. Sci. 159: 627–636.

Raspé O., Kohn J. R. (2002) S-allele diversity in *Sorbus aucuparia* and *Crataegus monogyna* (Rosaceae: Maloideae). Heredity 88: 458–465.

Rieseberg L. H., Soltis D. E. (1991) Phylogenetic consequences of cytoplasmic gene flow in plants. Evol. Trends Pl. 5: 65–84.

Robertson K. R., Phipps J. B., Rohrer J. R., Smith P. G. (1991) A synopsis of genera of the Maloideae (Rosaceae). Syst. Bot. 16: 376–394.

Roemer M. J. (1847) Familiarum naturalium regni vegetabilis synopses monographicae. III. Rosiflorae. Amygdalacearum et Pomacearum. Weimar, Landes-Industrie-Comptoir.

Rohrer J. R., Robertson K. R., Phipps J. B. (1991) Variation in structure among fruits of Maloideae (Rosaceae). Amer. J. Bot. 78: 1617–1635.

Rohrer J. R., Robertson K. R., Phipps J. B. (1994) Floral morphology of Maloideae (Rosaceae) and its systematic relevance. Amer. J. Bot. 81: 574–581.

Rokas A., Kruger D., Carroll S. B. (2005) Animal evolution and molecular signature of radiation compressed in time. Science 310: 1933–1938.

Ronquist F., Huelsenbeck J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Sang T. (2002) Utility of low-copy nuclear gene sequences in plant phylogenetics. Crit. Rev. Biochem. Molec. Biol. 37: 121–147.

Savile D. B. O. (1979) Fungi as aids in higher plant classification. Bot. Rev. 45: 380–495.

Sax K. (1931) The origin and relationships of the Pomoideae. J. Arnold Arbor. 12: 3–22.

Sax K. (1932) Chromosome relationships in the Pomoideae. J. Arnold Arbor. 13: 363–367.

Sax K. (1933) The origin of the Pomoideae. Proc. Amer. Soc. Hort. Sci. 30: 147–150.

Simmmons M. P., Ochoterena H. (2000) Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49: 369–381.

Spjut R. W. (1994) A systematic treatment of fruit types. Bronx, New York.

Sterling C. (1964) Comparative morphology of the carpel in the Rosaceae. III. Pomoideae: *Crataegus*, *Hesperomeles*, *Mespilus*, *Osteomeles*. Amer. J. Bot. 51: 705–712.

Sterling C. (1965a) Comparative morphology of the carpel in the Rosaceae. IV. Pomoideae: *Chamaemeles*, *Cotoneaster*, *Dichotomanthes*, *Pyracantha*. Amer. J. Bot. 52: 47–54.

Sterling C. (1965b) Comparative morphology of the carpel in the Rosaceae. V. Pomoideae: *Amelanchier*, *Aronia*, *Malacomeles*, *Malus*, *Peraphyllum*, *Pyrus*, *Sorbus*. Amer. J. Bot. 52: 418–426.

Sterling C. (1965c) Comparative morphology of the carpel in the Rosaceae. VI. Pomoideae: *Eriobotrya*, *Heteromeles*, *Photinia*, *Pourthiaea*, *Raphiolepis*, *Stranvaesia*. Amer. J. Bot. 52: 938–946.

Sterling C. (1966) Comparative morphology of the carpel in the Rosaceae. VII. Pomoideae: *Chaenomeles*, *Cydonia*, *Docynia*. Amer. J. Bot. 53: 225–231.

Swofford D. L. (2001) PAUP*: Phylogenetic Analysis Using Parsimony. Sunderland, MA, Sinauer Associates, Inc.

Talent N., Dickinson T. A. (2005) Polyploidy in *Crataegus* and *Mespilus* (Rosaceae, Maloideae): evolutionary inferences from flow cytometry of nuclear DNA amounts. Canad. J. Bot. 83: 1268–1304.

Vidal J. E. (1965) Notes sur quelques Rosacées Asiatique (II) (*Photinia*, *Stranvaesia*). Adansonia 5: 221–237.

Weeden N., Lamb R. (1987) Genetics and linkage analysis of 19 isozyne loci in apple. J. Amer. Hort. Soc. 112: 865–872.

Wilson M. A., Grant B., Clegg M. T. (1990) Chloroplast DNA evolves slowly in the palm family (Arecaceae). Molec. Biol. Evol. 7: 303–314.

Wolfe J. A., Wehr W. (1988) Rosaceous *Chamaebatiaria*-like foliage from the Paleogene of western North America. Aliso 12: 177–200.

Yang Z. (1994a) Phylogenetic analysis using parsimony and likelihood methods. J. Molec. Evol. 39:294–307.

Yang Z. (1994b) Estimating the pattern of nucleotide substitution. Molec. Evol. 39: 105–111.

Zhang L. Q., Pond S. K., Gaut B. S. (2001) A survey of the molecular evolutionary dynamics

of twenty-five multigene families from four grass taxa. J. Molec. Evol. 52: 144–156.
Zhang, S.-Y. (1992) Wood anatomy of the Rosaceae. Rijksherbarium/Hortus Botanicus, Leiden.

Addresses of the authors: Christopher S. Campbell (e-mail: Christopher_Campbell@umit.maine.edu) and Mathew P. Arsenault, Department of Biological Sciences, University of Maine, Orono, Maine 04469-5735, USA. Rodger C. Evans, Department of Biology, Acadia University, Wolfville, Nova Scotia, B4P 2R6, Canada. David R. Morgan, Department of Biology, University of West Georgia, Carrollton, Georgia 30118, USA. Timothy A. Dickinson, Royal Ontario Museum, Botany Section, 100 Queen's Park, Toronto, M5S 2C6, Canada.

Verleger: Springer-Verlag GmbH, Sachsenplatz 4–6, 1201 Wien, Austria – Herausgeber: Prof. Dr. F. H. Hellwig, Institut für Spezielle Botanik, Botanischer Garten und Herbar Haussknecht, Universität Jena, Philosophenweg 16, 07743 Jena, Germany. – Redaktion: Philosophenweg 16, 07743 Jena, Germany. – Satz und Umbruch: Scientific Publishing Services (SPS) Ltd., Chennai, India. – Offsetdruck: Krips bv, Kaapweg 6, 7944 HV Meppel, The Netherlands – Verlagsort: Wien – Herstellungsort: Meppel – Printed in The Netherlands.