

Leavenworthia (Brassicaceae) Revisited: Testing Classic Systematic and Mating System Hypotheses

JAMES B. BECK,^{1,2,3} IHSAN A. AL-SHEHBAZ,² and BARBARA A. SCHAAL¹

¹Department of Biology, Washington University, St. Louis, Missouri 63130-4899;

²Missouri Botanical Garden, St. Louis, Missouri 63166-0299

³Author for correspondence (beck@biology.wustl.edu)

Communicating Editor: James F. Smith

ABSTRACT. The genus *Leavenworthia* (Brassicaceae) has long been a focus of research on mating system evolution, due to the presence of both self-incompatible and self-compatible species within the genus. A phylogenetic hypothesis invoking multiple transitions between mating systems has been proposed for *Leavenworthia*, but this hypothesis has not been subject to molecular phylogenetic analysis. DNA sequence variation from four non-coding chloroplast regions (the *trnL* intron; and the *trnT-trnL*, *trnL-trnE*, and *psbA-trnH* intergenic spacers) was used to reconstruct the generic phylogeny, to test the validity of several proposed species, and to assess the number of mating system transitions within the genus. The strict consensus tree largely reflected the long-standing phylogenetic hypothesis for *Leavenworthia*, although additional data are needed to fully validate the recognition of *L. crassa* and *L. alabamica*. Unexpected results included the placement of *L. uniflora* as sister to the rest of the genus, and the apparent hybridization between *L. exigua* and *L. torulosa*. Finally, our data strongly supported a minimum of three mating system transitions within *Leavenworthia*.

The long-standing relationship between systematics and evolutionary biology has only been strengthened by the theoretical and empirical advances of the past 50 years. The combination of cladistic methodology and the seemingly endless character state data available from gene sequences has allowed researchers to identify lineages (Hilu et al. 2003; Flynn et al. 2005), reconstruct character evolution (Whiting et al. 2003; Crayn et al. 2004), and infer fundamental evolutionary patterns (Pagel 1999; Harmon et al. 2003; Sargent 2004). In addition to generating and testing novel evolutionary hypotheses using these new methodologies, systematists are actively reevaluating countless ideas resulting from over 250 years of systematic research (Crawford and Mort 2003).

One such hypothesis is that of mating system evolution in the genus *Leavenworthia* Torrey (Brassicaceae). Reed Rollins' (1963) comprehensive monograph of *Leavenworthia* stands as one of the classic integrations of systematics and evolutionary biology. His detailed examination of morphology, cytology, geographic distribution, and mating system resulted in the description of two new species (*L. alabamica* and *L. crassa*) and a detailed phylogenetic hypothesis regarding the evolution of self-fertilization within the genus. Rollins noted a distinct morphological syndrome associated with the self-compatible species (introrse anthers; smaller, scentless flowers; smaller stigmas; shorter styles) and used the distribution of chromosome numbers and morphological characters to form a phylogenetic hypothesis (Fig. 1), one that included at least three transitions from self-incompatibility (SI) to self-compatibility (SC). Over the next 40 years *Leavenworthia* became the focus of a wide range of evolutionary studies including evolutionary ecology (Lloyd 1968a, 1968b, 1969; Solbrig and Rollins 1977; Lyons and Antonovics

1991; Charlesworth et al. 1994; Lyons 1996), molecular evolution (Charlesworth et al. 1998; Filatov and Charlesworth 1999), population genetics (Solbrig 1972; Charlesworth and Yang 1998; Liu et al. 1998, 1999; Innan and Tajima 2002), and development (Yoon 2003; Yoon and Baum 2004). This interest was largely due to the multiple hypothesized origins of SC within *Leavenworthia*, and the view that self-compatible species were derived from self-incompatible ancestors. These multiple, independent mating system transitions presented researchers with a framework to test hypotheses regarding phenotypic evolution and genetic diversity (Lloyd 1968a, 1969; Charlesworth and Yang 1998; Liu et al. 1998, 1999). However, this important framework remains largely untested, both in *Leavenworthia* and in many plant groups with SI systems. Self-incompatibility systems in Brassicaceae are homomorphic, i.e. no morphological differences exist between incompatible flowers. Homomorphic incompatibility can be further broken down into sporophytic SI (found in Brassicaceae), where determination of pollen compatibility is determined by the parental sporophyte; and gametophytic SI, where pollen compatibility is determined by the genotype of the pollen grain itself. Although common (occurring in 60–90 families; Weller et al. 1995), homomorphic incompatibility has been examined phylogenetically in only two studies (Goodwillie 1999; Stone 2002). Given that the growing body of *Leavenworthia* research is largely dependent on the taxonomic and evolutionary hypotheses laid out in Rollins' (1963) monograph, a rigorous evaluation of these ideas is needed. Unfortunately, the molecular phylogenetic information currently available (Charlesworth et al. 1998; Filatov and Charlesworth 1999; Liu et al. 1999) included limited sampling (three, five, and four of the eight recognized species, respectively),

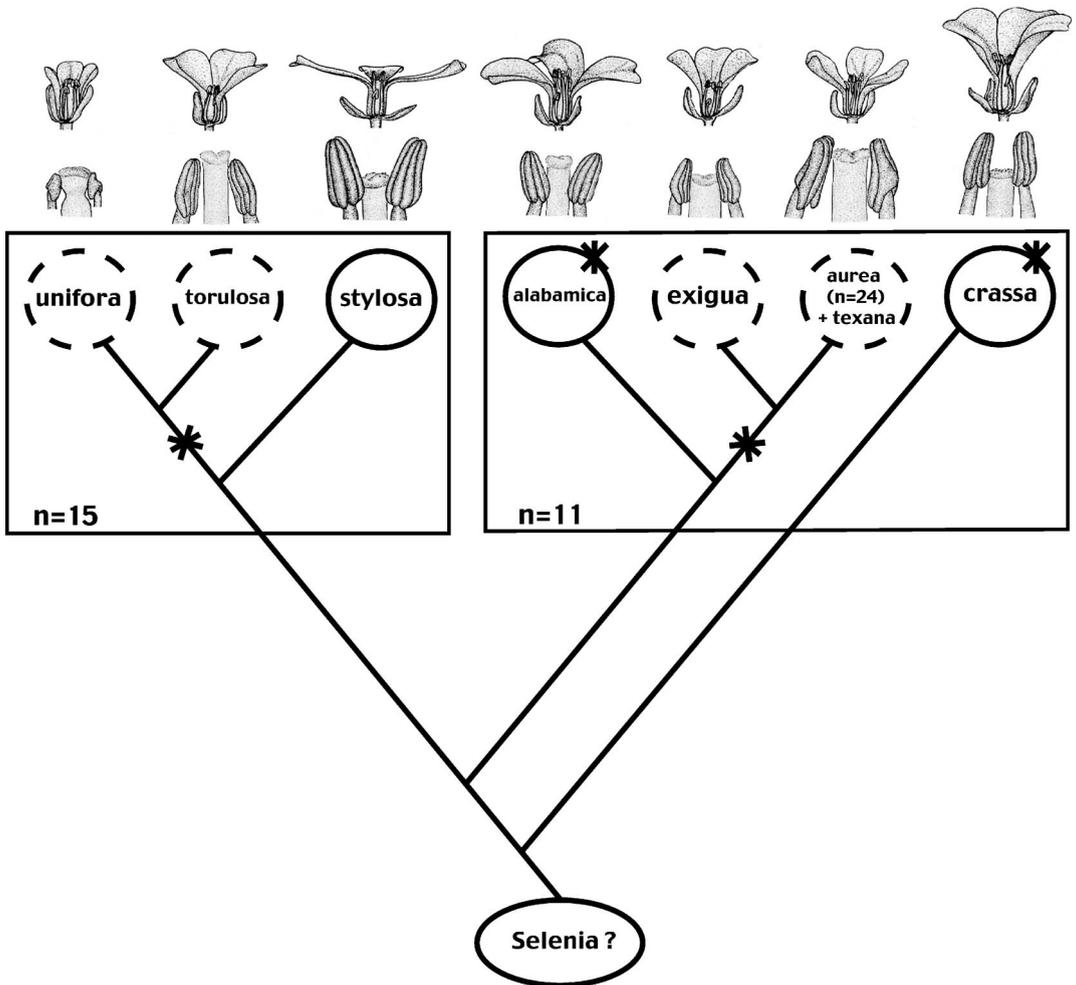


FIG. 1. *Leavenworthia* phylogeny as outlined by Rollins (1963). Dotted lines indicate SC species, solid lines indicate SI species. Drawings [originally from Rollins (1963), not to scale but relative sizes accurate] above each species show flowers and stigma/anther relationships; note smaller flowers and introrse anthers in self-compatible species. Asterisks note hypothesized shifts from SI to SC. Note that shifts occur within two species (*L. alabamica* and *L. crassa*). Groups of species sharing haploid chromosome numbers are delimited with boxes.

since these studies were not primarily focused on phylogenetic relationships. Incomplete lineage sorting at both nuclear loci studied, *Adh* and *PgiC*, further complicated phylogenetic inference. In this study we consider all eight recognized *Leavenworthia* species and test hypotheses regarding species identity and mating system transition by analyzing chloroplast DNA (cpDNA) sequence variation, which is less likely to be complicated by incomplete lineage sorting due to the shorter coalescence time of the chloroplast. Species of *Leavenworthia* are small (<10 cm), annual herbs which germinate in early fall, overwinter as rosettes, and flower from late February through April (Al-Shehbaz 1988). The genus is distributed across ten states of the southeastern United States (Fig. 2), found almost exclusively on limestone cedar glades. These glades are often wa-

terlogged during the winter and spring, and at least one species, *L. uniflora*, has been shown to be metabolically adapted to flooded conditions (Baskin and Baskin 1976). In his monograph Rollins (1963) considered *Leavenworthia* as composed of 11 taxa (seven species, four varieties), and the geographically disjunct, diploid populations of *L. aurea* have since been described as *L. texana* (Mahler 1987). Although intraspecific designations in two species (*L. crassa* var. *elongata* and *L. alabamica* var. *brachystyla*) have been associated with incipient mating system transitions (Rollins 1963; Lloyd 1968a), these relationships have not been well established and this study focuses only on relationships and mating system transitions between the eight accepted species of *Leavenworthia*. Assignments of mating system to each species (except for the then unde-

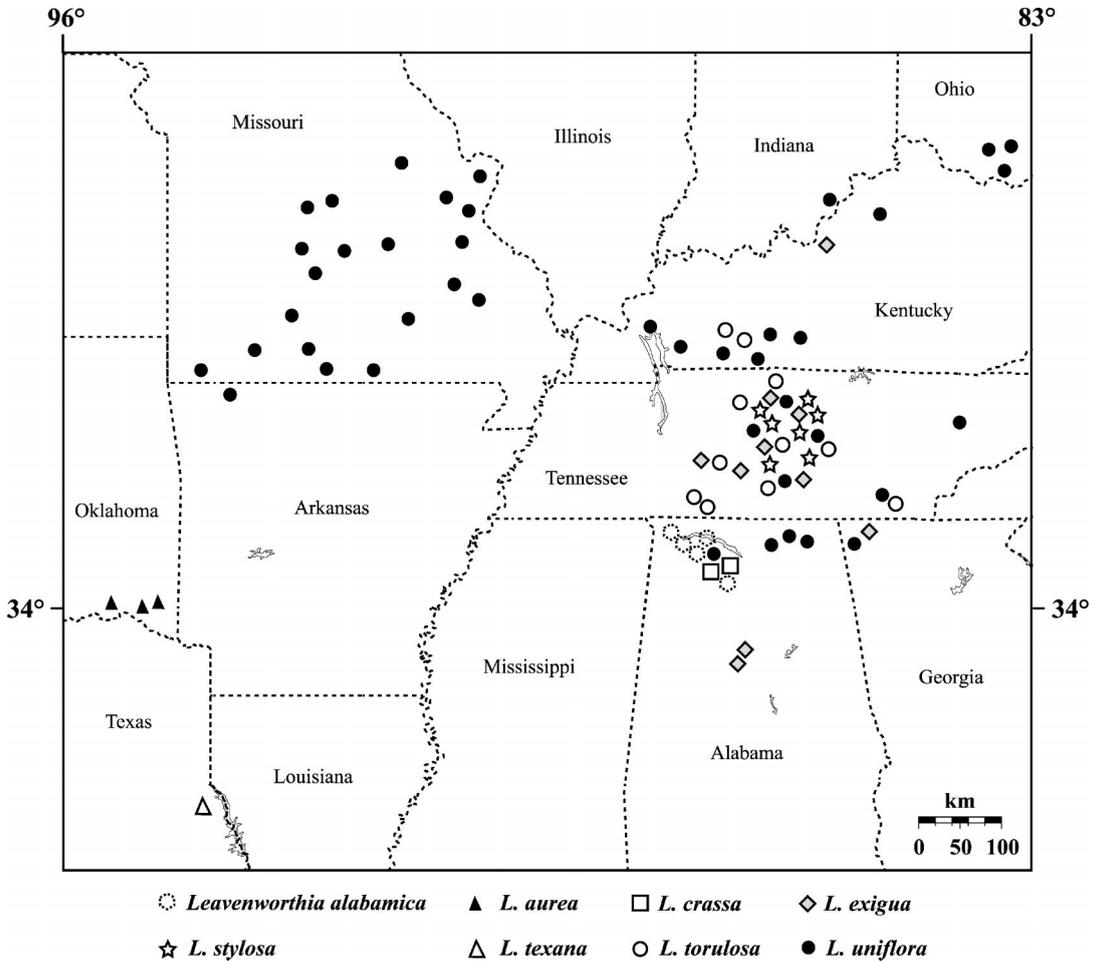


FIG. 2. Generalized distributions of the eight currently recognized species of *Leavenworthia* (adapted from Rollins 1963). The base map was created using software available at: <http://www.aquarius.geomar.de/omc/omc-intro.html>.

scribed *L. texana*) were originally made by Rollins (1963) by assessing the seed set of hand selfed plants. These original designations have been supported by additional experimental work (Lloyd 1968a; Lyons and Antonovics 1991; Charlesworth et al. 1994; Charlesworth and Yang 1998; Liu et al. 1998). For example, Charlesworth and Yang (1998) reported that individuals of the self-incompatible species *L. stylosa* do not set seed when hand pollinated with "self" pollen and set very low levels of seed in pollinator-free greenhouses. In contrast, individuals of the self-compatible species *L. uniflora*, *L. torulosa*, and *L. exigua* were highly self-compatible when pollinated with "self" pollen and set high (95%, 66%, and 68%, respectively) levels of seed in pollinator-free greenhouses. Finally, these mating system assignments are consistent with analyses of allozyme and DNA sequence variation within species (Solbrig 1972; Solbrig and Rollins 1977; Lyons and Antonovics 1991; Charlesworth and Yang 1998; Liu et al. 1998). As noted above, a number of studies (Rollins

1963; Lloyd 1968a; Liu et al. 1998) have identified a continuum of compatibility within both *L. alabamica* and *L. crassa*, with some populations self-compatible, some largely self-incompatible, and some intermediate. Given their "outcrosser" phenotype of large, open, scented flowers with long styles it is reasonable to assume that SI is the ancestral state within each species, a notion shared by Rollins (1963) and Lloyd (1968a). For this reason both *L. alabamica* and *L. crassa* are considered self-incompatible for the purposes of this analysis, and the origin of SC within each species are considered additional evolutionary events. Although never examined experimentally, *Leavenworthia texana* is thought to be a self-compatible species, given its phenotype and likely sister relationship with *L. aurea*. *Leavenworthia texana* is thought to be the diploid progenitor of the tetraploid *L. aurea*, and the two species are morphologically very similar, sharing the SC phenotypic syndrome discussed above. Regarding outgroup choice, although historically (Michaux 1803) allied with

TABLE 1. Relative phylogenetic information in the four chloroplast regions analyzed in this study. * Includes one inversion event.

Sequence characteristic	<i>trnT-trnL</i> spacer	<i>trnL</i> intron	<i>trnL-trnF</i> spacer	<i>psbA-trnH</i> spacer
Aligned length (bp)	706	518	633	292
Analyzed characters, including indels	463	486	456	202
Variable characters, including indels (%)	33 (7.1)	18 (3.7)	55 (12.0)	21 (10.4)
Parsimony informative characters, including indels (%)	14 (3.0)	4 (0.8)	13 (2.9)	8 (3.8)
Number of indels (parsimony informative)	10 (6)	4 (0)	10 (2)	12* (5*)

Cardamine L., recent workers (Torrey 1837; Rollins 1963; Al-Shehbaz 1988) have hypothesized that the small (four sp.) North American genus *Selenia* Nuttall is sister to *Leavenworthia*. Molecular evidence (Koch et al. 2000, 2001) has indicated that *Leavenworthia* is part of a strongly supported clade consisting of approximately 10 genera informally known as the "Cardaminoide lineage" (Price and Sweeney 1998). Additional data from two chloroplast regions strongly placed *Leavenworthia* sister to *Selenia* (Price and Sweeney, unpubl. data; Beilstein, in prep.), and *Selenia* is therefore used as an outgroup in the current study. Although the mating system of *Selenia* species has not been examined experimentally, the bright yellow, scented flowers exhibited by all four species (Martin 1940; Rollins 1993) provides a strong indication of SI, and this character-state assignment is used in this analysis.

Specifically, we wish to evaluate the basic phylogenetic hypothesis laid out by Rollins (1963), as well as the status of *L. alabamica* and *L. crassa*, the two species described therein. Additionally, we test Rollins' assertion that SC has arisen multiple times within *Leavenworthia*.

MATERIALS AND METHODS

Sampling, DNA Extraction, Amplification, and Sequencing. Sample information appears in Appendix 1. Total DNA was extracted from herbarium or silica-dried leaf material using a modified CTAB protocol (Doyle and Doyle 1987). Sequence information was gathered from four non-coding cpDNA regions (the *trnL* intron; and the *trnT-trnL*, *trnL-trnF*, and *psbA-trnH* intergenic spacers). With the exception of the *psbA-trnH* intergenic spacer, these regions have a well-established record of providing phylogenetic signal at the familial (Hall et al. 2002), generic (Mummenhoff et al. 2001), and intraspecific (Widmer and Baltisberger 1999) level in Brassicaceae. The *trnT-trnL* intergenic spacer, *trnL* intron, and *trnL-trnF* intergenic spacer were PCR-amplified using the primer pairs "a-b," "c-d," and "e-f" respectively (Taberlet et al. 1991). The *trnH-psbA* intergenic spacer was PCR-amplified using the primers "trn H" and "psb A" (Hamilton 1999). All reactions were performed under standard conditions. Products were visualized and purified via agarose gel electrophoresis with either a QIAquick (Qiagen, Valencia, CA) or Viogene (Viogene U.S.A., Sunnyvale, CA) gel extraction kit. Products were dye-labeled using a Big Dye Terminator Kit (Applied Biosystems, Foster City, CA), and analyzed on either an ABI 373 (Applied Biosystems, Foster City, CA) or MJ Research BaseStation (MJ Research, Waltham, MA). Sequences were deposited in the EMBL database (Appendix 1).

Sequence Alignment and Phylogenetic Analysis. Sequences were manually aligned in Se-Al (Rambaut 1995). All gaps (except as noted below) were coded as binary characters following the "simple indel coding" method of Simmons and Ochoterena (2000) and were appended at the end of the NEXUS file. Regions con-

taining gaps, stretches of poor sequence, and areas of uncertain alignment (discussed below) were not analyzed. The aligned matrix was submitted to TreeBASE (study accession S1367). A heuristic parsimony search with 100 random addition replicates was performed using PAUP* 4.0b10 (Swofford 2002) with the following parameters: starting trees obtained by stepwise addition, TBR branch swapping, "MulTrees" turned on, and steepest descent not in effect. Ten thousand bootstrap replicates were conducted with PAUP* 4.0b10 using identical parameters. Decay indices were calculated in PAUP* 4.0b10 using a command file generated in MacClade 4.0 (Maddison and Maddison 2000). The strict consensus tree was drawn using WinClada (Nixon 2002).

The status of *L. alabamica* and *L. crassa* was assessed by comparing the shortest trees conforming to the non-monophyly of each species to the most parsimonious trees (MPTs) obtained in the original, unconstrained search. Constraint topologies conforming to the monophyly of these two species were constructed in MacClade 4.0 (Maddison and Maddison 2000). These two constraint topologies enforced only either a monophyletic *L. alabamica* or *L. crassa*. These constraint topologies were used to limit subsequent parsimony searches, instructing the algorithm to find the shortest tree *not* conforming to the constraint (the shortest tree that contained a non-monophyletic *L. alabamica* or *L. crassa*, respectively). Parameters for constrained searches were otherwise identical to the unconstrained search. All MPTs for each constrained scenario were compared to each unconstrained MPT using a one-tailed Wilcoxon signed-ranks test (Templeton 1983) employed in PAUP* 4.0b10. Although use of the Wilcoxon signed-ranks test for comparison of a posteriori (unconstrained MPT) and a priori (constrained MPT) topologies has been questioned (Goldman et al. 2000), this approach has been shown to be equally or more conservative relative to alternative tests (Melville et al. 2001; Townsend and Larson 2002). Alternative hypotheses (the non-monophyly of each species) were rejected if trees conforming to them were significantly longer than either of the overall MPTs.

To identify the number of mating system transitions within the evolutionary history of *Leavenworthia*, topologies conforming to alternative scenarios of mating system evolution were generated and compared to the MPTs. The "loose convex" command employed in PAUP* 4.0b10 was used to constrain parsimony searches to search for trees conforming to one, two, and three origins of SC within *Leavenworthia*. These constrained trees were then compared to the MPTs as detailed above.

RESULTS

Phylogeny of *Leavenworthia*. Statistics regarding size, composition, and phylogenetic signal in each of the cpDNA regions appear in Table 1. Relative phylogenetic signal within these regions largely corresponded to that seen in other studies (Renner et al. 2000; Chanderbali et al. 2001; Mummenhoff et al. 2001; Borsch et al. 2003). Polynucleotide repeats in the *trnT-trnL* spacer approximately 185 and 400 bp from primer "a" interrupted sequencing reactions in 75% of samples, a situation noted previously in Brassicaceae (Mummenhoff et al. 2001). This approximately 180 bp

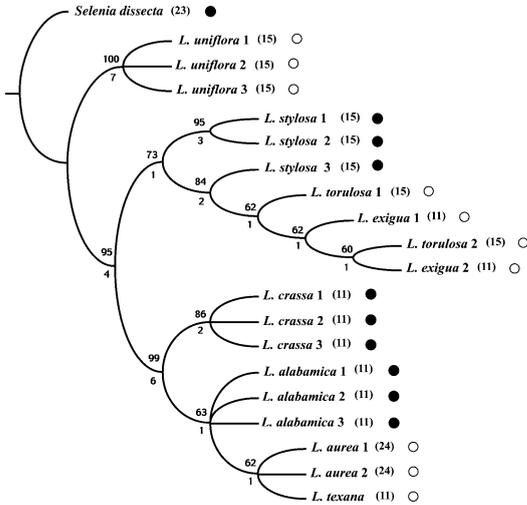


FIG. 3. Strict consensus of the two most parsimonious trees ($L = 136$) obtained with a heuristic search of sequence data from the *trnL* intron; and the *trnT-trnL*, *trnL-trnF* and *psbA-trnH* intergenic spacers. Bootstrap values (10,000 replicates) appear above branches, decay values appear below branches. Numbers in parentheses indicate haploid chromosome numbers. Open circles denote samples from self-compatible species, filled circles denote samples from self-incompatible species.

region was not analyzed. Also not analyzed was the 3' end of the *trnL-trnF* spacer, which was composed of a large repeat region of 1–14 copies of an 11 bp repeat motif followed by 51 bp of typical sequence. This length variation was due to the presence of pseudo-genized copies of the adjacent *trnF* coding region (Koch et al. 2005) and was therefore considered likely homoplastic. The total aligned sequence matrix consisted of 2,185 characters, including 35 insertion/deletion events and one inversion. A total of 578 characters were excluded due to gaps, alignment ambiguity, or poor sequence quality. After exclusion the analyzed matrix contained 16 (0.0005%) data cells coded as missing and 39 (0.0012%) data cells coded as uncertain. The analyzed matrix of 1,607 characters yielded 127 (7.9%) variable and 39 (2.4%) parsimony-infor-

mative characters. Each of 100 random addition replicates recovered the same island of two MPTs (length 136, consistency index = 0.95, retention index = 0.95). The strict consensus is shown in Fig. 3. The consistency index (excluding uninformative characters = 0.85) reflected the low degree of homoplasy in this data set, a factor that contributed to the moderate to high bootstrap support for clades with modest decay values. The strict consensus tree exhibited a well-supported (100% bootstrap) clade comprising all three *L. uniflora* samples that was sister to the remainder of the ingroup. The remainder of the ingroup was subdivided into two clades: one comprising the $n = 15$ species (*L. stylosa* and *L. torulosa*) and *L. exigua* ($n = 11$), the other a well-supported (99% bootstrap) clade comprising the remaining $n = 11$ species (*L. alabamica*, *L. crassa*, *L. aurea*, and *L. texana*). The clade comprising *L. torulosa* and *L. exigua* rendered *L. stylosa* paraphyletic, and both *L. torulosa* and *L. exigua* were paraphyletic with respect to each other.

The shortest tree not exhibiting a monophyletic *L. crassa* was 138 steps long, which was not significantly longer than the MPTs (Table 2). A clade comprising all *L. alabamica* samples was not supported in either MPT, and a search for longer trees not exhibiting this clade was therefore not conducted.

Mating System Transitions. Heuristic searches constrained to recover MPTs corresponding to one or two origins of SC recovered MPTs that were significantly longer than either unconstrained MPT (Table 2). A search constrained to recover MPTs corresponding to three origins recovered the unconstrained MPTs.

DISCUSSION

Phylogenetic Structure of Leavenworthia. The *Leavenworthia* strict consensus tree (Fig. 3) is largely reflective of both Rollins' original hypotheses and a more recent cladistic analysis of morphological characters (Price 1992), with a few notable exceptions. Instead of being derived from within the $n = 15$ clade as proposed by Rollins (1963), *L. uniflora* is sister to the rest of the genus. Previous morphological (Rollins

TABLE 2. Results of the Wilcoxon signed-ranks tests. One-tailed probabilities are shown. The unconstrained topology refers to either of the two MPTs, comparisons between either MPT and trees resulting from an individual constraint were identical. N is the number of characters differing in number of changes on paired trees in each comparison. P-values for the *L. crassa* constraint are approximate due to the small N value.

Constraint	# trees recovered	Tree length	N	P	Significantly longer?
Unconstrained (MPT)	1	136	—	—	—
<i>L. alabamica</i> NOT monophyletic	1	136	—	—	no
<i>L. crassa</i> NOT monophyletic	8	138	2 or 4	0.079 or 0.159	no
<i>L. alabamica</i> + <i>L. stylosa</i> monophyletic	12	146	10 or 12	<0.005*	yes
One origin of SC	12	151	17 or 19	<0.005*	yes
Two origins of SC	6	143	9 or 11	<0.05*	yes
Three origins of SC	1	139	—	—	no

1963) and isozyme (Solbrig 1972) studies are consistent with this placement, as they both identified *L. uniflora* as having the most divergent phenotype relative to the rest of the genus. Regarding the paraphyletic nature of *L. stylosa*, Rollins (1963) noted considerable intra-specific variation in both siliqua length and flower color, variation that often existed as local geographic trends. However, this variation was not considered significant enough to warrant designating intraspecific taxa, a notion shared by Al-Shehbaz (1988). The paraphyletic nature of *L. stylosa* is likely due to incomplete lineage sorting following the origin of *L. torulosa*, a common progenitor/descendant scenario discussed by Rieseberg and Brouillet (1994). The paraphyletic nature of *L. stylosa* therefore represents a useful system in which to study the transition from SI to SC, potentially providing a way to identify populations and genotypes that contributed to the self-compatible *L. torulosa*.

Perhaps most surprisingly, instead of being derived from within the $n = 11$ clade *L. exigua* is placed within the $n = 15$ clade, intimately related to *L. torulosa*. The presence of a *L. torulosa*-like chloroplast genome in *L. exigua* appears to be the result of previous hybridization for two reasons. 1) Sequence variation at two nuclear loci strongly places *L. exigua* within the $n = 11$ clade (J. Beck, unpubl. data) and 2) *Leavenworthia exigua* and *L. torulosa* are sympatric in the Central Basin of Tennessee (Fig. 2), commonly occurring in close proximity (J. Beck, pers. obs.). Although currently unable to hybridize (Rollins 1963), given this sympatry and the edaphic requirements of the entire genus, it is likely that these two species have shared the same restricted habitat for a considerable period, providing ample time for their lineages to exchange genes. Furthermore, Tsitrone et al. (2003) have recently shown that such "chloroplast capture" can theoretically occur quickly (approx. 1000 generations) in a population, and that SC in the recipient species (in this case the self-compatible *L. exigua*) can greatly speed this process. As noted above, *L. exigua* is primarily distributed in central Tennessee, but with disjunct populations in Georgia, Kentucky (var. *laciniata*), and Alabama (var. *lutea*). Two distinct hypotheses exist regarding the extent of cedar glades and the organisms that occupied them during the Pleistocene glacial cycles. Delcourt et al. (1986) used paleoecological data to reconstruct vegetative and hydrological conditions over the past 30,000 yrs in the Ozark Plateau of Missouri and the Interior Low Plateau of Tennessee and Kentucky. Their reconstructions suggested that during the last glacial interval, 24,000 yrs before present to 12,500 yrs before present, suitable glade habitat in these regions was restricted to areas south of 34° N latitude (which would include the extant Alabama and perhaps the Georgia populations of *L. exigua* – see Fig. 2). Alternatively, Baskin and Baskin (1986) cited examples of numerous glade taxa

that currently reside in glade-like areas of Canada and the northern U.S., areas that they deem comparable to Kentucky and Tennessee during the last glacial interval. They suggested that glade taxa therefore persisted north of 34° N latitude. Assuming that the *L. exigua*/*L. torulosa* hybridization event predated the last glacial retreat, these two scenarios predict differing patterns regarding the presence of *L. torulosa* chloroplasts across the current range of *L. exigua*. Under the Delcourt et al. scenario, *L. exigua* and *L. torulosa* shared the same small refugial areas south of 34° N latitude, and small population sizes therein likely drove chloroplast capture to completion. Many or all populations of *L. exigua* would therefore carry the *L. torulosa* chloroplast. Alternatively, the Baskin and Baskin scenario predicts that Tennessee and Kentucky populations of *L. exigua* could have persisted in place during the last glacial interval, and would exhibit *L. torulosa* chloroplasts only due to rare long-distance dispersal from refugial populations in Alabama and Georgia. Samples of *L. exigua* from both Tennessee and Alabama were included in this study, both of which carry *L. torulosa* chloroplasts. Sequence data from the Kentucky populations and dense sampling within all areas would indicate if chloroplast capture has gone to completion in *L. exigua*, potentially providing an insight into the history of eastern North American cedar glades.

The problematic placement of *L. exigua* does support Rollins' (1956) segregation of this species from *L. aurea*. The morphologically similar *L. aurea* originally included plants from southeastern Oklahoma (the type locality), San Augustine Co. Texas, and the "eastern" populations broadly distributed in Alabama, Georgia, Kentucky, and Tennessee. Rollins (1956) designated the eastern populations as *L. exigua*, based on a broad range of morphological characters. A recent investigation (Mahler 1987) concluded that among the remaining populations, the Texas populations are diploid ($n = 11$) distinguishing them from the polyploid ($n = 24$) Oklahoma populations (*L. aurea*). Mahler named these diploid Texas plants *L. texana*. Given that the *L. aurea*/*texana* lineage apparently did not receive the *L. torulosa* chloroplast genome as did the *L. exigua* lineage, the distinct nature of these two lineages seems justified. The polyphyletic nature of *L. torulosa* is only marginally supported and could be due to incomplete lineage sorting following this proposed hybridization episode. The nature of such apparent hybridization and other discordance between the chloroplast and nuclear phylogenies is currently being investigated and will be the subject a future paper.

With regards to *L. alabamica* and *L. crassa*, the results of the Wilcoxon signed-ranks tests (Table 2) indicate that trees corresponding to the non-monophyly of either species are not significantly longer than either MPT, highlighting the need to further document these

lineages. However, with regards to *L. alabamica*, Rollins' decision to distinguish these Alabama populations from *L. stylosa* (where previous workers had placed them) is strongly supported by our data. The shortest trees exhibiting a clade comprising the three *L. alabamica* samples and *L. stylosa* samples one and two was significantly longer than either MPT (Table 2). A similar test comparison was not possible for *L. crassa*, as Rollins was apparently the first to collect this species, and therefore no prior taxonomic hypothesis exists. While it is clear that additional data are needed to fully validate *L. alabamica* and *L. crassa*, the monophyly of *L. crassa* (86% bootstrap) and the distinctiveness of *L. alabamica* relative to *L. stylosa* indicate that these lineages serve as good working hypotheses.

Mating System Transitions. The Wilcoxon signed-ranks tests clearly demonstrate multiple (likely three) transitions between mating systems within *Leavenworthia*. This inference is consistent with other molecular phylogenetic studies (Schoen et al. 1997; Goodwillie 1999; Stone 2002) that have examined mating system evolution at the generic level, all of which identify multiple transitions. If one adds the two suggested incipient transitions to SC within *L. alabamica* and *L. crassa* (Rollins 1963), the total of five mating system transitions within this genus of eight species is truly remarkable. It should be noted that the proposed hybridization event (*L. exigua*/*L. torulosa*) would have no impact on our inference of multiple transitions within *Leavenworthia*, as the topological position of self-compatible species relative to self-incompatible species would remain the same. Regarding the direction of mating system transition, although both parsimony and likelihood based reconstructions on the chloroplast MPTs unambiguously identify only transitions from SI to SC (analysis not shown), a definitive reconstruction of these changes should wait until incongruence between the chloroplast and nuclear phylogenies has been fully assessed.

ACKNOWLEDGEMENTS. The authors thank Deborah Charlesworth for generously sharing seeds; Andrea Shea Bishop for help with fieldwork in Tennessee; Mike Dyer and the Jeanette Goldfarb Plant Growth Facility (WU) for greenhouse assistance; and the curators of BRIT, G, MO, NY, UNA, US, and VDB for herbarium loans. A special thanks to Ken Kozak for help with topology testing and ancestral character-state reconstruction. This study was funded by a NSF Graduate Research Fellowship DGE-0202737, the Graduate Studies Program at the Missouri Botanical Garden, and the Division of Biology and Biomedical Sciences at WU.

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APPENDIX 1

Sample information. Taxon (sample number), voucher: country, state, county, *trnT-trnL* GenBank, *trnL* GenBank, *trnL-trnF* GenBank, *psbA-trnH* GenBank.

Leavenworthia alabamica Rollins (1), Rollins 8315 (G): USA, AL, Franklin Co. AM072903, AM072842, AM072863, AM072883; *Leav-*

leavenworthia alabamica Rollins (2), Beck 486 (MO): USA, AL, Franklin Co. AM072904, AM072843, AM072864, AM072884; *Leavenworthia alabamica* Rollins (3), Beck 488 (MO): USA, AL, Lawrence Co. AM072905, AM072844, AM072865, AM072885

Leavenworthia aurea Torrey (1), Kral 63232 (VDB): USA, OK, Choctaw Co. AM072906, AM072845, AM072866, AM072886; *Leavenworthia aurea* Torrey (2), Kral 63234 (VDB): USA, OK, McCurtain Co. AM072907, AM072846, AM072867, AM072887

Leavenworthia crassa Rollins (1), Rollins 6023 (G): USA, AL, Morgan Co. AM072909, AM072848, AM072869, AM072889; *Leavenworthia crassa* Rollins (2), Beck 512 (MO): USA, AL, Morgan Co. AM072910, AM072849, AM072870, AM072890; *Leavenworthia crassa* Rollins (3), Beck 490 (MO): USA, AL, Morgan Co. AM072911, AM072850, AM072871, AM072891

Leavenworthia exigua Rollins (1), Beck 501 (MO): USA, TN, Marshall Co. AM072902, AM072841, AM072862, AM072882; *Leavenworthia exigua* Rollins (2), Webb 4927 (G): USA, AL, Marshall Co. AM072901, AM072840, AM072861, AM072881

Leavenworthia stylosa Gray (1), Beck 511 (MO): USA, TN, Wilson Co. AM072896, AM072835, AM072856, AM072876; *Leavenworthia*

stylosa Gray (2), Beck 492 (MO): USA, TN, Wilson Co. AM072898, AM072837, AM072858, AM072878; *Leavenworthia stylosa* Gray (3), Beck 513 (MO): USA, TN, Davidson Co. AM072897, AM072836, AM072857, AM072877

Leavenworthia torulosa Gray (1), Rollins & Channell 5657 (G): USA, TN, Rutherford Co. AM072899, AM072838, AM072859, AM072879; *Leavenworthia torulosa* Gray (2), Beck 496 (MO): USA, TN, Davidson Co. AM072900, AM072839, AM072860, AM072880

Leavenworthia texana Mahler, Correll & Correll 29080 (G): USA, TX, San Augustine Co. AM072908, AM072847, AM072868, AM072888

Leavenworthia uniflora (Michx.) Britton (1), Beck 515 (MO): USA, TN, Davidson Co. AM072894, AM072833, AM072854, AM072874; *Leavenworthia uniflora* (Michx.) Britton (2), Beck 514 (MO): USA, MO, Taney Co. AM072893, AM072832, AM072853, AM072873; *Leavenworthia uniflora* (Michx.) Britton (3), Beck 516 (MO): USA, AL, Morgan Co. AM072895, AM072834, AM072855, AM072875

Selenia dissecta Torrey & A. Gray, Crutchfield & Johnston 5855 (G): Mexico, Nuevo Leon, AM072892, AM072831, AM072852, AM072872