

REEXAMINATION OF RELATIONSHIPS, HABITAT EVOLUTION, AND PHYLOGEOGRAPHY OF CHECKER MALLOWS (*SIDALCEA*; MALVACEAE) BASED ON MOLECULAR PHYLOGENETIC DATA¹

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Phylogenetic analysis of nuclear ribosomal DNA external and internal transcribed spacer region (ETS and ITS) sequences for *Sidalcea* (Malvaceae) resolved five major, well-supported lineages, three of which represent species groups that have each been noted for complex patterns of morphological variation: the *oregana*, *malviflora*, and *glaucescens* clades. Very low variation within each of the three groups in the sequenced regions is consistent with recent radiation of each clade. We reject the previously suggested hypothesis of monophyly for the annual species of *Sidalcea*. Based on our findings, the annual habit in *Sidalcea* arose at least four times, probably as an adaptation to seasonally dry habitats. The hypothesis that the perennial species *S. hickmanii* and *S. malachroides* represent basally divergent groups within *Sidalcea* is supported, but the more recently discovered *S. stipularis* represents an additional basally divergent lineage. The previous suggestion that the genus spread northward from Mexico along two major routes (through the Rocky Mountains and the Sierra Nevada foothills), with the Rocky Mountain species *S. candida* and *S. neomexicana* representing basally divergent lineages, is not supported. *Sidalcea neomexicana* is nested within the *malviflora* clade and is likely a lineage of relatively recent descent that originated in California and subsequently spread to the Rocky Mountains.

Key words: internal/external transcribed spacers; Malvaceae; nrDNA; phylogeny; phylogeography; *Sidalcea*.

The checker mallows (*Sidalcea*, Malvaceae) are a predominantly western North American group of about 25 species of annuals and perennials that have been regarded as taxonomically difficult (Roush, 1931; Hitchcock, 1957; Hill, 1993) because of complex patterns of morphological variation and putative hybridization. Although most *Sidalcea* species are diploid ($2n = 20$), the occurrence of different ploidy levels (tetraploids and hexaploids) in the genus could potentially explain at least part of the confusing morphological variation within the group. Variation in vegetative morphology causes special problems for species delimitation in *Sidalcea*. The leaves are palmately veined, as is common for Malvaceae, but blade shapes vary depending on leaf position along the stems (proximal cauline leaves are often less deeply lobed than leaves on the distal stems). Indumentum can be highly variable within species, both in density and type (but usually includes stellate hairs, as is common in Malvaceae). Flowers and inflorescences

of *Sidalcea* are less variable than vegetative characteristics but floral dimorphism due to gynodioecy (in which pistillate flowers are smaller than perfect flowers; see Ashman, 1994; Graff, 1999; Marshall and Ganders, 2001) has been a source of confusion for delimiting species. *Sidalcea* flowers are protandrous and, like other Malvaceae, the filaments of the stamens are united around the style. What is probably unique for *Sidalcea* is that the filaments are fused into two groups near their tips.

Roush (1931) postulated evolutionary trends for morphological characters of *Sidalcea* and also proposed a historical biogeographic hypothesis for the genus. She divided *Sidalcea* into three subgenera: “*Eusidalcea*” (comprising sections *Annuae* and *Perennes*), *Malvastralcea*, and *Hesperalcea*. Subgenus *Hesperalcea*, with the single species *S. malachroides*, was considered to be the most “primitive” group, retaining characteristics of the ancestor of *Sidalcea* such as suffrutescence, leafy branches, and only slightly lobed leaves. Subgenus *Malvastralcea* also contained only one species, *S. hickmanii*, which was suggested to be a “slightly more recent branch” than subgenus *Hesperalcea* and which had retained most “primitive” characteristics except for more wrinkled carpels, slight changes in stamens, and larger pollen. Section *Perennes* in subgenus “*Eusidalcea*” contained the rest of the perennial species known at that time (*S. stipularis* was described later, in 1974, by Howell and True).

Roush (1931) suggested that the ancestor to modern species of *Sidalcea* spread northward from Mexico along two major routes, one through the Rocky Mountains and one through the Sierra Nevada foothills in California. *Sidalcea candida* and *S. neomexicana*, both of which occur in the Rocky Mountains, were proposed to have descended from an ancestor that moved along the eastern route after the divergence of lineages that gave rise to *S. malachroides* and *S. hickmanii*. The other species of subgenus *Perennes* were suggested to constitute a much more recent group, whose ancestor dispersed along the route

¹ Manuscript received 14 May 2002; revision accepted 31 October 2002.

The authors thank H. Forbes and the U.C. Berkeley Botanical Garden, V. Oswald, S. Bainbridge, R. Halse, E. Gruber McEvoy, G. Clifton, and K. Uptain for plant material; B. Söderström, B. Guggolz, and P. Hubbard (LADWP) for help in the field; D. Hickson, E. Burres, R. Bittman (all CA Dept. of Fish & Game), C. Brown and colleagues (Cal Trans), J. Stebbins, S. Bainbridge, B. Ertter, T. Sasaki and A. Bradley (USDA Forest Service) for help with permits and/or locality information and access; S. R. Hill for assistance with voucher determinations; J. S. Farris for performing analyses with the program Xarn; and S. R. Hill and an anonymous reviewer for useful comments on the manuscript. This study was supported by a postdoctoral grant to K. A. from the Swedish Natural Science Research Council/Swedish Foundation for International Cooperation in Research and Higher Education and grants to K. A. from the Faculty of Science and Technology at Uppsala University, The Royal Swedish Academy of Sciences, The H. Axson Johnsson Foundation, and The Lawrence R. Heckard Endowment Fund of the Jepson Herbarium, U.C. Berkeley.

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through California. Section *Annuae*, which occurs only in California, was considered to have all advanced characteristics of the genus and hence to be a very recent offshoot from section *Perennes*. Hitchcock (1957) agreed that the annuals constitute a single lineage and excluded them from his study of *Sidalcea*.

Sidalcea has been treated within tribe Malveae (Malvaceae) since its description (e.g., Gray, 1849; Bentham and Hooker, 1862; Schumann, 1895; Fryxell, 1988). Malveae are a diverse tribe of approximate 70 genera with equivocal generic interrelationships. Traditionally, the tribe has been divided into subtribes, with *Sidalcea* in subtribe Malvinae, usually together with *Alcea*, *Callirhoë*, *Lavatera*, *Malva*, and *Napaea* (e.g., Bentham and Hooker, 1862; Schumann, 1895). Bates (1968) abandoned the subtribal taxonomy because he thought that too much emphasis had been placed on the occurrence of filiform style branches and stigmatic position, characteristics that had been used to group *Sidalcea* with the genera of Malveae-Malvinae. Bates (1968) and Bates and Blanchard (1970) took into account the chromosome numbers in the tribe and suggested informal groupings (alliances) within Malveae. Bates (1968) separated *Sidalcea* ($n = 10, 20, 30$) and *Napaea* ($n = 15$) from the genera of the Malveae-Malvinae and put them in the *Sidalcea* alliance. Later, Bates and Blanchard (1970) transferred *Napaea* to an alliance of its own, based on new chromosomal data and the hypothesized evolution of chromosome numbers. They also pointed to a possible relationship of *Sidalcea* to *Eremalche* ($n = 10, 20$) and *Urocarpidium* ($n = 10, 15$) of the *Sphaeralcea* ($n = 5, 10, 15, 25$) alliance, although they retained the *Sidalcea* alliance with *Sidalcea* as the sole member. Fryxell (1988, 1997) associated *Sidalcea*, *Callirhoë*, and *Napaea* in the *Sidalcea* alliance.

Published phylogenetic analyses of Malvaceae have included only a few taxa of Malveae but sampled members of the tribe have consistently formed a strongly supported monophyletic group (La Duke and Doebley, 1995; Judd and Manchester, 1997). Within the tribe, phylogenetic results have indicated problems with circumscriptions of the proposed alliances and subtribes. In a chloroplast DNA restriction site analysis of Malvaceae sensu stricto that included 14 genera of Malveae, La Duke and Doebley (1995) showed that the *Sphaeralcea* alliance was not monophyletic and did not find support for other subgroups previously proposed within Malveae.

Considering the highly variable and difficult morphology of *Sidalcea*, we suspected that examining rapidly evolving nuclear DNA regions would shed new light on historical relationships in the group. Internal and external transcribed spacers (ITS and ETS) of 18S–26S nuclear ribosomal DNA (rDNA) are often sufficiently variable to be useful for resolving phylogenetic questions at low taxonomic levels (see e.g., Baldwin et al., 1995; Baldwin and Markos, 1998; Andreasen and Baldwin, 2001). Accordingly, rDNA transcribed spacers were sequenced and analyzed to estimate the phylogeny of *Sidalcea* and to evaluate earlier proposed hypotheses of relationships and historical biogeography of the group.

MATERIALS AND METHODS

Taxon sampling and outgroup choice—The sister group of *Sidalcea* is unknown in the absence of a higher-level phylogenetic study including any members of the genus. However, monophyly of Malveae, the tribe that includes *Sidalcea*, has been upheld in phylogenetic analyses that included some other genera of the group (La Duke and Doebley, 1995; Judd and Manchester, 1997). Outgroup taxa (four sequences, each representing the genera *Sphaeralcea*, *Napaea*, *Eremalche*, and *Callirhoë*) were chosen from Malveae based

on morphology and on suggested close relationships of these taxa to *Sidalcea* (Kearney, 1951; Bates and Blanchard, 1970; Fryxell, 1988, 1997). We also attempted to use more distantly related genera, from other Malvaceae tribes (from e.g., Hibisceae and Gossypieae) as outgroup taxa.

Within the ingroup, we sampled from 24 species of *Sidalcea* sensu Roush (1931), Hitchcock (1957), and Hill (1993) and from as many intraspecific taxa as possible (taxa and voucher information has been archived at the Botanical Society of America website <http://ajbsupp.botany.org/v90>). For many of the taxa more than one population was included to permit investigation of potential geographic and interpopulational differences. Taxa represented in the analyses by more than one population have been distinguished by arabic numerals in text and figures and can be identified with their source at <http://ajbsupp.botany.org/v90>.

Laboratory procedures—DNA was extracted from fresh, recently pressed, or silica-gel dried plant material or from herbarium specimens. Total DNAs were isolated using a modification of the cetyltrimethylammonium bromide (CTAB) procedure (Doyle and Doyle, 1987) with phenol extraction and ethanol precipitation. When possible, fresh material from 10 or more plants per population was collected and pooled in the extractions to capture potential intrapopulational variation.

The ITS region was amplified with primers ITS-I (ITS-leu.1) and ITS4 and sequenced with ITS4 and ITS5-A, and for some polymerase chain reaction (PCR) products, ITS2 and/or ITS3 (White et al., 1990; Downie and Katz-Downie, 1996; Andreasen et al., 1999; Urbatsch et al., 2000) using standard procedures (see Andreasen and Baldwin, 2001). The primer Sid-F (Andreasen and Baldwin, 2001), approximately 550 base pairs (bp) upstream from 18S, was used together with 18S-E (Baldwin and Markos, 1998) to amplify an ETS fragment of approximately 540 bp with the same PCR conditions as for ITS, except that the annealing temperature used was 50°C instead of 48°C. Primer 18S-E and Sid-F also were used in the ETS sequencing reactions. The ITS and ETS amplification products were cycle sequenced with BigDye (Applied Biosystems, Foster City, California, USA) or Thermo Sequenase (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA) terminator cycle sequencing kits and resolved on polyacrylamide gels using an ABI Prism 377 Automated Sequencer (Applied Biosystems).

Four *Sidalcea* species, i.e., *S. ranunculacea*, *S. reptans*, *S. campestris*, and *S. cusickii*, were not included in the present analyses because they displayed complex patterns of different ITS and ETS copy types and of recombinants between copy types. These species, together with other polymorphic *Sidalcea* samples, are the subjects of a separate investigation (K. Andreasen and B. G. Baldwin, unpublished data). The exclusion of these species does not alter the conclusions presented below.

Data analysis—Resolved sequences were aligned using the Clustal method or Goroh-Myers' comparative alignment option as implemented in the software Sequence Navigator (Applied Biosystems) and adjusted by eye. Gaps were coded as missing data and each potentially phylogenetically informative indel (regardless of length) was recoded as one binary character. Identical sequences were identified and only one sequence from each set of identical sequences was included in the analyses. PAUP* 4.0b2–8 (Swofford, 1998) was used for the parsimony analyses. Cladistic analyses of the combined and separate ITS and ETS aligned-sequence matrices were conducted using heuristic searches with at least 1000 random taxon-addition replicates, tree bisection-reconnection (TBR) branch swapping, and MULPARS option in effect. To estimate support for clades, jackknife (Farris et al., 1996) support was estimated with 10000 replicates, five random TBR replicates, and MULPARS off. For the combined analysis, Bremer/decay support (Bremer, 1994) analysis was carried out using PAUP*, with batch files generated by the program Autodecay 4.0 (Eriksson, 1999) and the same settings as for the heuristic searches above. The incongruence length difference test (ILD; Farris et al., 1994) was performed using the programs Xarn (Farris, 1991; Farris et al., 1994) or PAUP* (10000 replicates, 10 randomly selected addition sequences with TBR branch swapping) to compare the ITS and ETS matrices. Only phylogenetically informative characters were included in the test (see Cunningham, 1997).

RESULTS

ITS region sequences—ITS-1 in *Sidalcea* varied in size from 258 to 296 bp and ITS-2 varied between 206 and 226 bp. The 5.8S gene was invariantly 164 bp long and total length for the whole ITS region (ITS-1 + 5.8S + ITS-2) of *Sidalcea* was 645–686 bp. This size range is within the limits reported earlier for sequenced angiosperms (see e.g., Baldwin et al., 1995) and close to the upper end of the range for other sequenced Malvaceae genera (the whole ITS region, 588–689; ITS-1, 218–297; ITS-2, 206–229 [Seelanan et al., 1997]). Most length variation in *Sidalcea* was due to a 34-bp deletion in the middle of ITS-1 in *S. calycosa* and deletions at the 5' end of ITS-2 in *S. hartwegii* (20 bp) and *S. stipularis* (14 bp). *Sidalcea* and outgroups exhibited no extreme values in guanine and cytosine (GC) content. The GC values varied between 49% and 54% in the ingroup and between 50% and 55% in the outgroup.

The alignment of sequences from the ITS region of *Sidalcea* and the outgroup was straightforward and required the insertion of few gaps (EMBL accession number ALIGN_000457 for the aligned sequences; for the individual sequences see <http://ajbsupp.botany.org/v90>). Attempts to include more distantly related Malvaceae genera (e.g., *Hibiscus* and *Gossypium* sequences from GenBank) resulted in too much alignment uncertainty to allow their use as members of the outgroup. (Note: Seelanan et al. [1997] suggested that the ITS region is too variable to use for family-wide studies in Malvaceae.) The aligned sequences with gaps yielded a matrix of 692 bp.

Pairwise distances (HKY85 corrected) between the sequences varied between 0 and 11% within the ingroup and up to 15% between the most divergent pair of ingroup and outgroup taxa (*S. diploscypha* and *Callirhoë digitata*). Within *Sidalcea*, 154 (22%) of the characters were variable, and 98 (14%) potentially phylogenetically informative (plus 13 recoded gap characters). Eighty-two of the variable characters and 53 (18% of the ITS-1 positions) of the potentially phylogenetically informative characters (plus eight recoded gap characters) were found in ITS-1. ITS-2 contributed 68 variable and 42 (19% of the ITS-2 positions) potentially phylogenetically informative characters (plus five gap characters). Only three characters (2% of the 5.8S positions) were potentially phylogenetically informative in the 5.8S gene (all of them point mutations).

ETS sequences—The 3' region of the universal ETS (upstream from the 18S subunit) was highly uniform in length between the different *Sidalcea* accessions; all sequences were 540 bp except for those of the two *S. hartwegii* accessions, which were 539 bp. Length of the sequenced ETS region in the outgroup varied from 540 to 544 bp. The number of positions in the aligned matrix (see EMBL accession number ALIGN_000458 for the aligned sequences; for the individual sequences see <http://ajbsupp.botany.org/v90>) including the outgroups was 550 bp. Of these positions, 148 (27%) were variable within the ingroup and 101 (18%) were potentially phylogenetically informative (plus four recoded gap characters). Variation in the sequenced ETS region was not evenly distributed; a region about 100 bp long, starting approximately 390 bp from 18S instead seems to be more conservative and less variable than the rest of the region (Fig. 1). The GC content was 47–50% for *Sidalcea* and 48–52% for the outgroups. Pairwise distances (HKY85 corrected) varied from 0% to 13% within the ingroup and up to 21% between the most divergent

pair of ingroup and outgroup taxa (*S. calycosa* and *Callirhoë digitata*).

Phylogenetic analyses—The ITS analyses resulted in 132 minimum-length trees and the strict consensus tree is shown in Fig. 2. The basal topology and some species groups are highly supported, but intermediate branches received low support. Monophyly of *Sidalcea* is well supported by a jackknife value of 97%. The basalmost diverging lineage in *Sidalcea* is a strongly supported group (jackknife value [jk] 86%) consisting of *S. stipularis*, *S. malachroides*, and *S. hickmanii*. Relationships among the three species are unresolved, but the three exemplars representing two subspecies of *S. hickmanii* form a well-supported group. The two annual species *S. diploscypha* and *S. keckii* constitute a very strongly supported lineage (jk 100%) that diverged at the node immediately above the basal node in *Sidalcea*. The rest of the taxa in *Sidalcea* form a well-supported (jk 99%) clade. The two annual species *S. hartwegii* and *S. calycosa* are each strongly supported (jk 100%), as is a clade (jk 99%) consisting of *S. asprella*, *S. maxima*, *S. hirtipes*, *S. sp. nov.*, and a putative hybrid (*S. asprella* × *oregana*). The name *S. "gigantea"* has been preliminarily used for *S. sp. nov.*, a robust checker mallow that does not exactly fit the description of any other species of *Sidalcea* (G. Clifton, Deer Park, California, personal communication).

Parsimony analysis using the sequenced ETS region resulted in 110 minimum trees. The ETS strict consensus tree (Fig. 3) is somewhat more resolved at the intermediate nodes than is the ITS strict consensus tree (Fig. 2). None of these differences in the ingroup topology is well supported, however (jackknife values near or below 50%). The position of the root differs from the position found in the ITS trees. In the ETS trees, the root attaches at the branch leading to *S. malachroides* only; in the ITS trees the root is positioned at the branch uniting *S. malachroides* with *S. stipularis* and *S. hickmanii*.

The ILD test demonstrated that the ITS and ETS matrices were significantly incongruent at the 0.05 level ($P = 0.02$). When the outgroup was excluded, however, the matrices were not significantly incongruent ($P = 0.2$). A combined analysis of the ITS and ETS data yielded 5831 minimum-length trees (consistency index [CI] = 0.63 excluding non-informative characters, rescaled consistency index [RC] = 0.50). Excluding the outgroup species and rerunning the analysis resulted in a strict consensus tree with the same ingroup topology. The strict consensus of the combined analysis (Fig. 4) is more resolved than either the ITS tree (Fig. 2) or the ETS tree (Fig. 3), but support for intermediate branches remained low. The outgroup attaches at the branch leading to the group comprising *S. malachroides*, *S. stipularis*, and *S. hickmanii*, and thus agrees with the root position found in the ITS trees. Six relatively strongly supported clades are indicated within *Sidalcea* and given informal names in Figs. 2–4 to facilitate discussion of the groups. The two basally divergent clades are indicated as “basal perennials” and “basal annuals.” Another well-supported clade is the *glaucescens* clade, which includes the *asprella* clade. The two remaining groups consist of the *S. oregana* samples and *S. nelsoniana* (the *oregana* group) and the subspecies of *S. malviflora* plus three other species (the *malviflora* clade).

DISCUSSION

Variation in ETS and ITS—Variability and phylogenetic utility of ETS sequences have not been widely investigated in

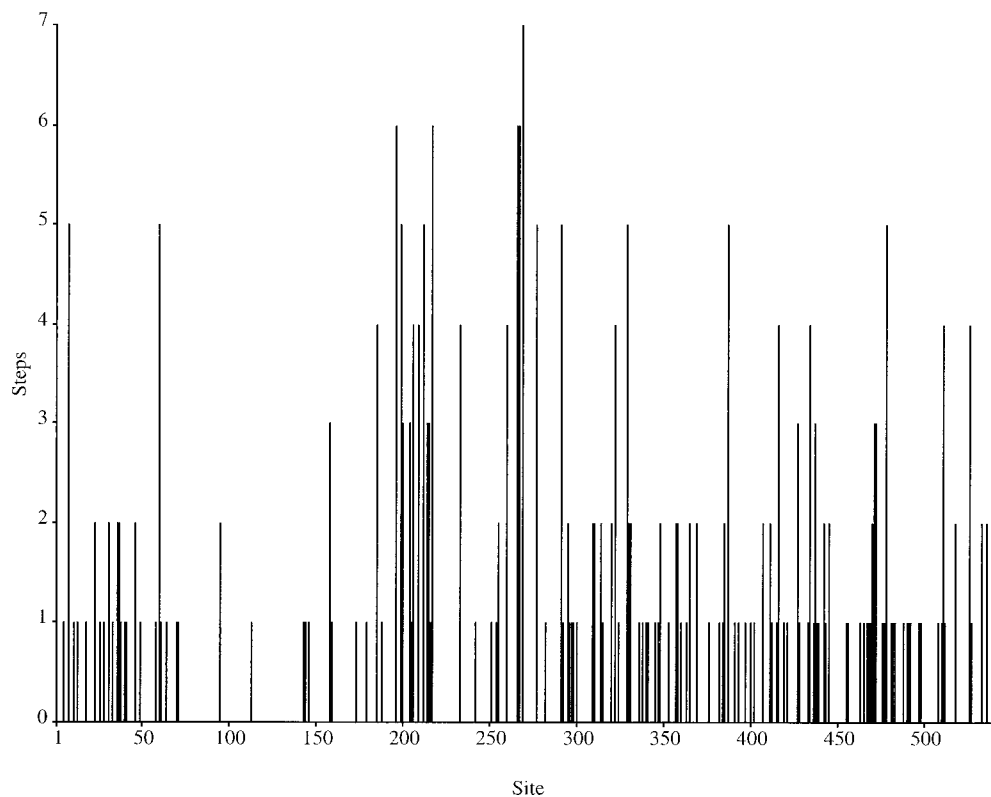


Fig. 1. The distribution and amount of variation in the amplified 3' region of the universal rDNA external transcribed spacer (ETS; upstream from the 18S subunit) in *Sidalcea*. The y-axis shows the number of changes (= steps) at each aligned position in the matrix. Outgroup taxa are not included. The changes were calculated on one of the most parsimonious trees, chosen at random.

plants outside Asteraceae (see e.g., Poaceae, McIntyre et al., 1988; Brassicaceae, Bennett and Smith, 1991; Solanaceae, Volkov et al., 1996; Fabaceae, Bena et al., 1998; Chandler et al., 2001; Myrtaceae, Wright et al., 2001). In *Calycadenia* (Asteraceae) the ETS region approximately 1–700 bp upstream from the 18S subunit was found to contain about 1.5 times the number of variable and potentially informative sites found in ITS-1 and ITS-2 combined (Baldwin and Markos, 1998). In the present study the number of variable and potentially informative sites were similar for ETS and ITS. The ratio of average pairwise distances between *Osmadenia* (the sister-group of *Calycadenia*) and each taxon in *Calycadenia* was 1.4 (ETS) to 1 (ITS) (Baldwin and Markos, 1998). The ETS/ITS ratio for pairwise distances (HK85 corrected) in *Sidalcea* was on average one for comparisons between *Eremalche* and representatives of the different clades in *Sidalcea*. It thus appears that the variation of the 3' portion of the universal ETS region in *Sidalcea*, in contrast to *Calycadenia* and various other plant groups (e.g., *Medicago* [Fabaceae], Bena et al., 1998; *Argyranthemum*, *Asteriscus*, and *Helianthus* [Asteraceae], Linder et al., 2000; *Lessingia* [Asteraceae], Markos and Baldwin, 2001), is not greater than in the internal transcribed spacers. Similar results, showing the same general ETS region to be equally or even less variable than ITS, have been found in some investigated genera of Asteraceae, i.e., *Arnica* (K. Andreassen and B. G. Baldwin, unpublished data), *Lasthenia* (Chan et al., 2001), *Cirsium*, (D. Kelch, University of California, Berkeley, personal communication), and *Stephanomeria* (Lee et al., 2002). Regardless of the relative levels of variation in ETS and ITS sequences, combining data from both sources has

been shown to enhance tree support and resolution and is thus a useful strategy for investigating phylogeny of young groups such as *Sidalcea*.

Even more so than in the ITS region (see also Baldwin et al., 1995) variation in the sequenced ETS region is predominantly present as point mutations rather than indels in *Sidalcea*. The few ETS indels that were present were found in sequences of *S. diploscypha* and *S. keckii* and were only one or two base pairs long. In contrast to the conserved length of the sequenced ETS region, certain parts of the ITS-1 and ITS-2 seem to have been under relaxed evolutionary constraints, e.g., at the 5' end of ITS-2, where long (nonhomologous) deletions occur both in *S. hartwegii* and *S. stipularis*. These ITS-2 deletions occur in regions that are highly variable among angiosperms (Hershkovitz and Zimmer, 1996) and are likely to be under less functional constraints than are other regions of the spacer.

Outgroups and incongruence—No higher-level phylogenetic analysis of Malvaceae has included *Sidalcea*; consequently, the sister group to *Sidalcea* is unknown. For the outgroup, we used taxa that have been suggested to be closely related to *Sidalcea* based on morphology. The outgroup attached at different branches of the ingroup in the ITS and ETS analyses. In the combined-data analysis and in the analysis of ITS alone, the outgroup attached at the branch separating *S. malachroides*, *S. hickmanii*, and *S. stipularis* from other taxa of *Sidalcea*; in the ETS analysis the root was positioned at the *S. malachroides* branch. The different positions of the outgroup in the ETS and ITS trees and the significant result in

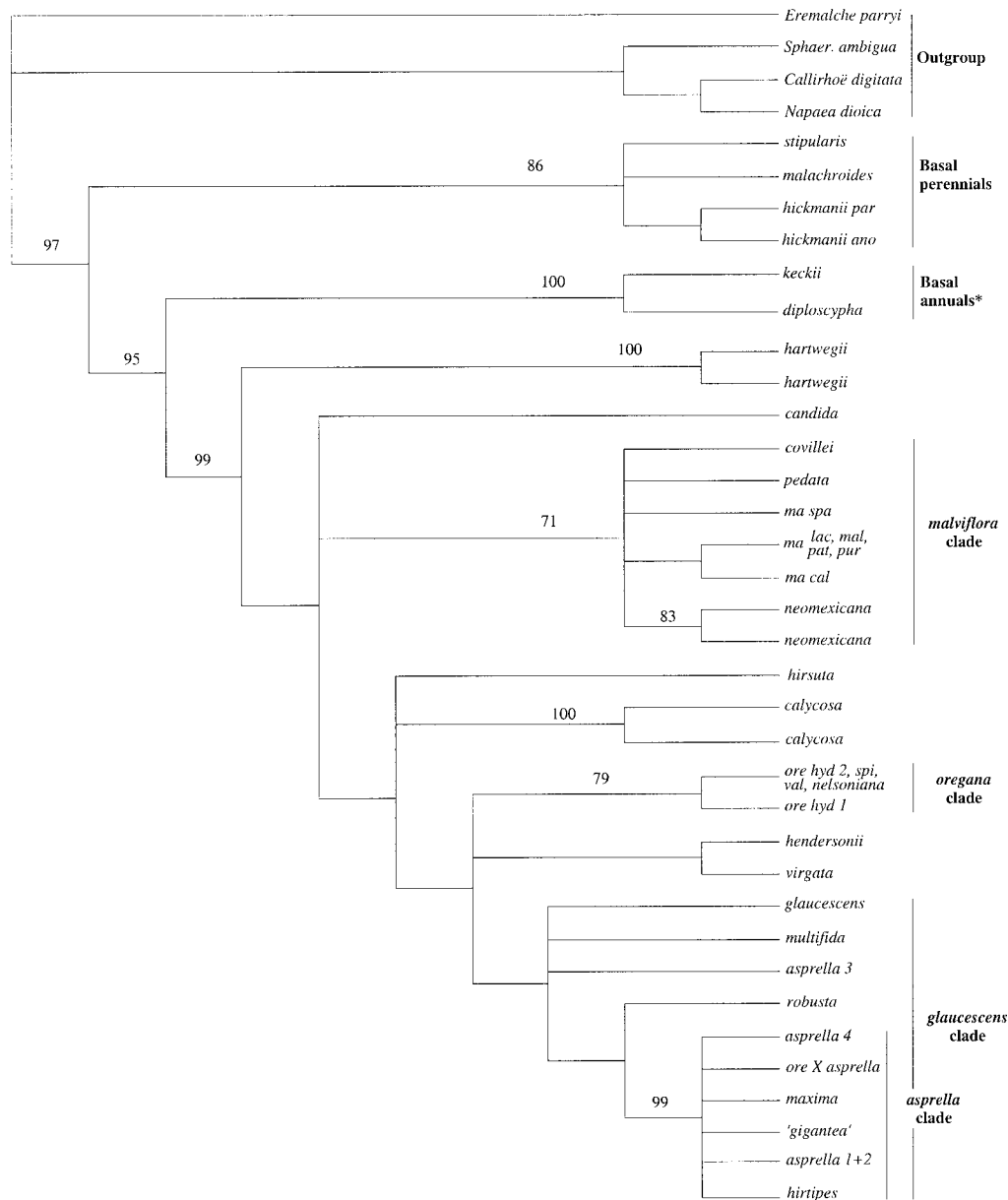


Fig. 2. Strict consensus of 132 maximally parsimonious trees from phylogenetic analysis of the ITS sequence data for *Sidalcea* (CI = 0.62, excluding non-informative characters; RC = 0.56). Population numbers are indicated when sequences differ within taxa. Numbers on branches indicate nodes with jackknife support over 70%.

Figure Abbreviations: ano, anomala; cal, californica; hyd, hydrophila; lac, laciniata; ma, malviflora; mal, subsp. malviflora; ore, oregana; par, parishii; pat, patula; pur, purpurea; Sphaer., Sphaeralcea; spa, sparsifolia; spi, spicata; val, valida; CI, consistency index; RC, rescaled consistency index.

the ILD test only when the outgroup taxa were included may be an indication that the outgroup taxa introduced problematic homoplasy into the analysis. This potentially disruptive homoplasy was not so extensive, however, as to make sequence alignment difficult or to result in a different ingroup topology when the outgroup taxa were added to the analysis.

Clade support and unequal evolutionary rates—Lack of support for many clades of *Sidalcea* may reflect rapid radiation of lineages without sufficient time between branching events for mutations to accumulate. Annual clades are marked by many more mutations than are most perennial clades, as is reflected by the high support for these branches (Figs. 2, 3,

and 4) relative to branches leading to perennial species. Comparisons of evolutionary rates between perennial and annual lineages in *Sidalcea* established that both ITS and ETS have evolved significantly faster in the annuals than in the perennials (Andreasen and Baldwin, 2001).

Phylogeny and polyploidy in *Sidalcea*—The molecular evidence for monophyly of checker mallows (100% jk; Fig. 4), given the outgroup taxa sampled, supports the interpretation that the most conspicuous morphological feature unique to *Sidalcea*, i.e., the numerous stamens forming two distinct groups (an inner and an outer whorl), is synapomorphic for the group. The occurrence of different ploidy levels (tetraploids and

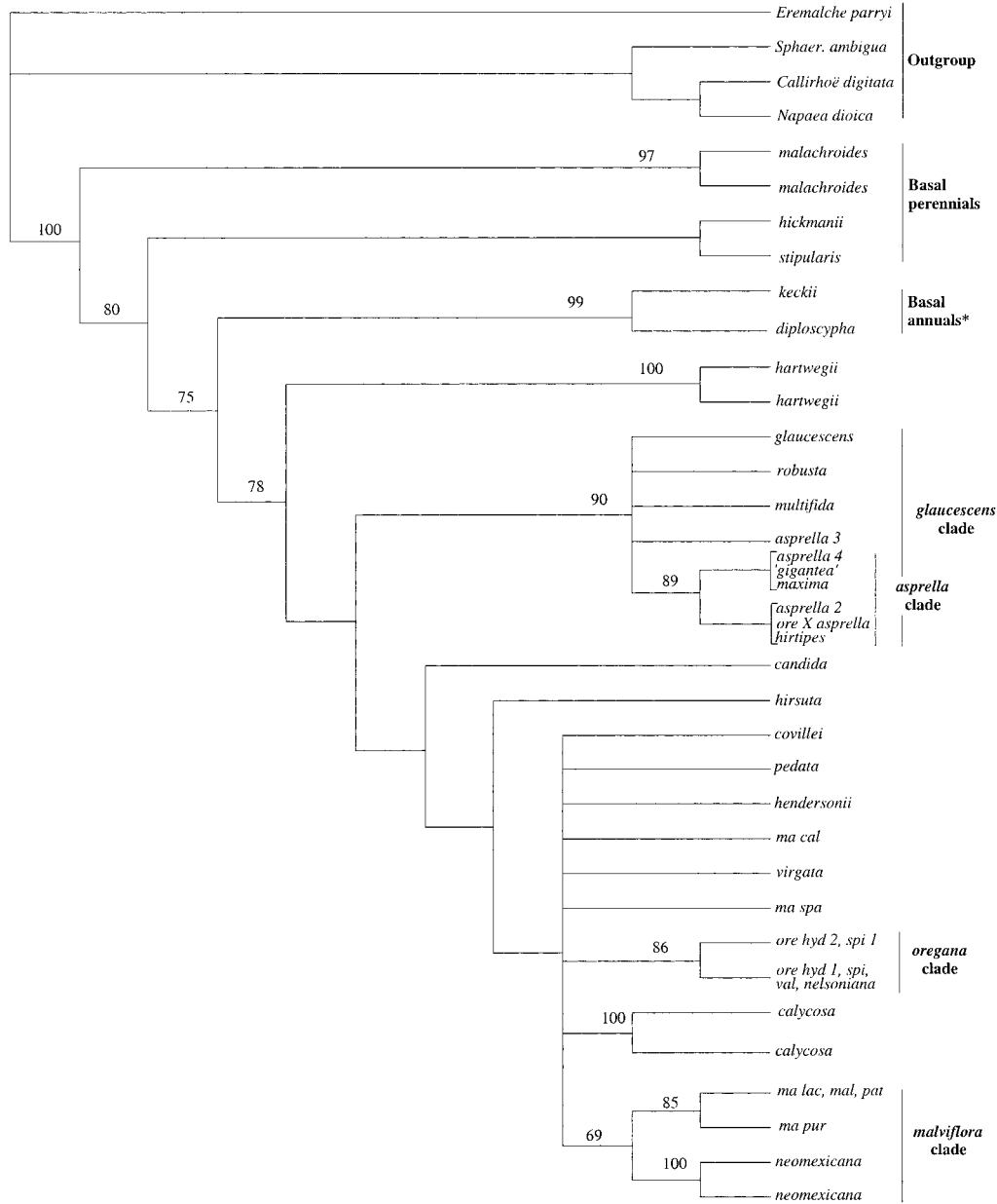


Fig. 3. Strict consensus of 110 maximally parsimonious trees from phylogenetic analysis of the ETS sequence data for *Sidalcea* (CI = 0.67, excluding non-informative characters, RC = 0.53). Population numbers are indicated when sequences differ within taxa. Numbers indicate nodes with jackknife support over 68%.

hexaploids) in different clades in the trees (see Fig. 4) demonstrates multiple origins of polyploidy in *Sidalcea*. If a polyploid taxon displays heterogenous sequences, one possible explanation is allopolyploidy. As mentioned above, heterogenous sequences were excluded from the analyses and the samples were cloned and sequenced to investigate the origin of the polymorphisms (K. Andreasen and B. G. Baldwin, unpublished data). When nuclear sequences (such as ITS and ETS) from polyploids are not heterogenous, the plants may be autopolyploid. Alternatively, biased concerted evolution may have homogenized divergent parental ITS and ETS copies, with only one copy remaining (or PCR drift or selection may have occurred). To investigate these possibilities further,

additional data from an independent DNA region, nuclear or chloroplast, are needed.

Basal perennials—Roush’s (1931) hypothesis that the perennial species *S. hickmanii* and *S. malachroides* represent basally divergent groups within *Sidalcea* is upheld by our rDNA trees (the “basal perennials”; jk 76% in Fig. 4). Hitchcock (1957) also supported the view that these two taxa were more closely related to each other than to other *Sidalcea* species. *Sidalcea stipularis*, the third species in the “basal perennials,” was discovered subsequent to Roush’s and Hitchcock’s studies (Howell and True, 1974) and represents an additional basally divergent lineage. Until now, no phylogenetic placement has

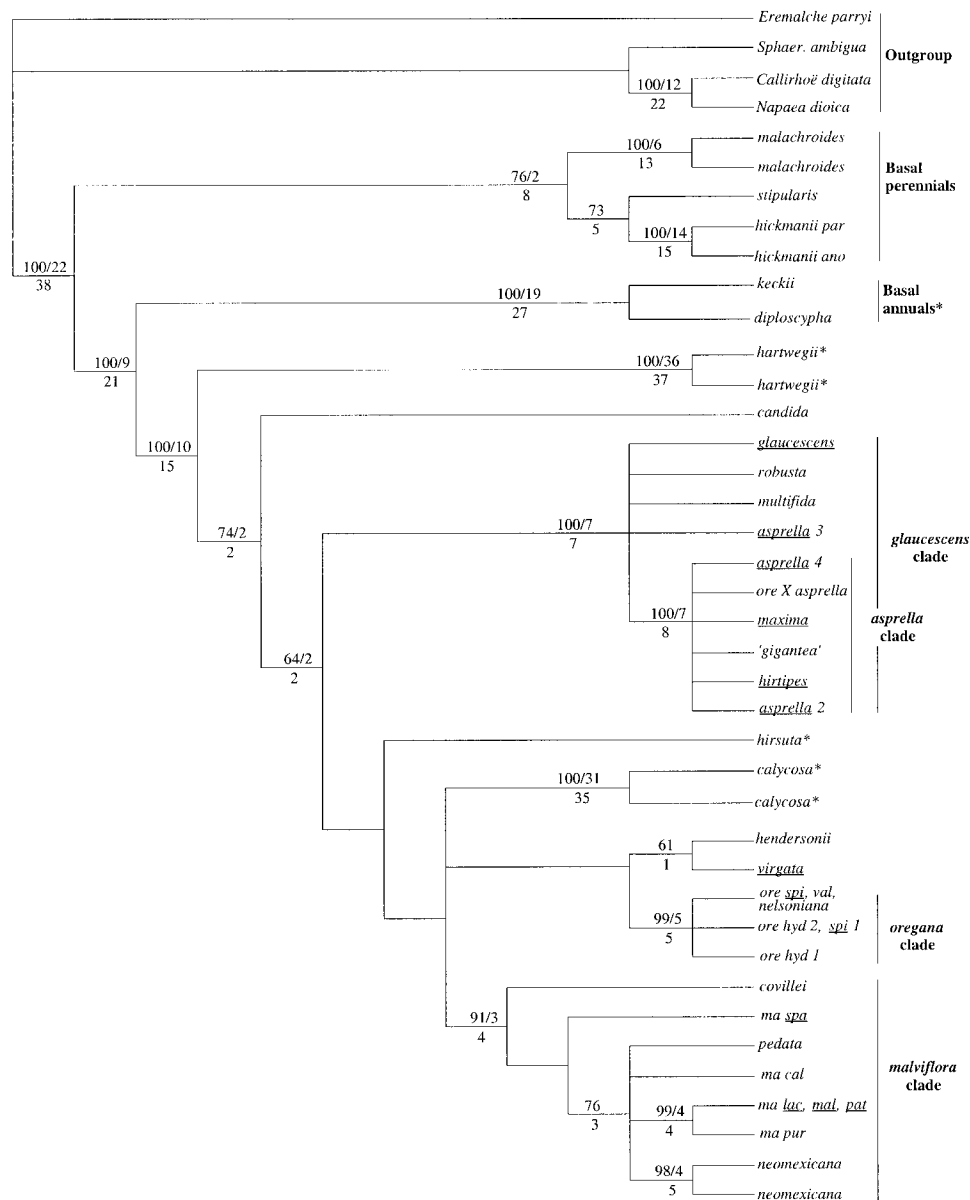


Fig. 4. Strict consensus of 5831 maximally parsimonious trees from phylogenetic analysis of the combined ITS and ETS sequence data for *Sidalcea*. Population numbers are indicated when sequences differ within taxa. Numbers indicate nodes with jackknife support over 60% and decay/Bremer support values over 1. Numbers below branches indicate branch lengths in one of the maximally parsimonious trees. Annuals are indicated by asterisks, and taxa with at least some polyploid populations are underlined.

been suggested for the morphologically distinct *S. stipularis*, but the beakless carpels, the tribracteolate calyx (ebracteolate or sometimes uni- or bi-bracteolate in *S. malachroides*), and the similarity of lower and upper leaves are characteristics otherwise found within *Sidalcea* only in *S. hickmanii* and *S. malachroides*.

Annuals—The suggested monophyly of the annual species of *Sidalcea* (Roush, 1931; Hitchcock, 1957) is not consistent with the results presented here (Figs. 2, 3, and 4; annuals are indicated by asterisks in Fig. 4); neither is the idea that the annuals represent young lineages relative to the perennials (Roush, 1931). Based on the rDNA data, we conclude that the annual habit arose at least four times, probably as an adaptation to seasonally dry habitats. Alternately, if the annual habit

is interpreted as plesiomorphic (the reconstruction of the ancestral state within *Sidalcea* is equivocal), then the annuals constitute a paraphyletic group.

The malviflora clade—The seven subspecies of *S. malviflora* (subsp. *californica*, *dolosa*, *laciniata*, *malviflora*, *patula*, *purpurea*, and *sparsifolia*) form a clade along with *S. covillei*, *S. pedata*, and *S. neomexicana* (the *malviflora* clade; jk 91%, Fig. 4), although relationships among the taxa are not well supported. The subspecies of *S. malviflora* are of coastal or southern distribution in California and *S. neomexicana* and *S. pedata* occur in the same areas as some of the southern subspecies. *Sidalcea neomexicana* is the most widely distributed species of *Sidalcea*. Besides occurring in southern California, *S. neomexicana* occurs in Oregon, Nevada, Utah, Colorado, New

Mexico, and Arizona in the U.S.A. and in northern Mexico. *Sidalcea covillei*, on the other hand, is a rare species, occurring only in Owens Valley, Inyo County, California. The affinity between *S. malviflora* and *S. neomexicana* was suggested earlier (Roush, 1931; Hitchcock, 1957) but, according to Hitchcock, *S. covillei* and *S. pedata* are closer to the “*S. oregana-spicata* complex” than to *S. malviflora*. Roush (1931), however, recognized an affinity among *S. covillei*, *S. malviflora*, and *S. neomexicana*, but treated *S. covillei* as a variety of *S. neomexicana*. The position of *S. malviflora* subsp. *sparsifolia* basally to the clade (jk 76%) consisting of the other subspecies of *S. malviflora* plus *S. pedata* and *S. neomexicana*, provides evidence for paraphyly of *S. malviflora* and may justify treatment of *S. malviflora* subsp. *sparsifolia* as a separate species. The position of *S. malviflora* subsp. *californica* is not resolved in the combined analysis but it is sister to the majority of the subspecies of *S. malviflora* in the ITS analysis.

The *asprella* clade—Hitchcock’s (1957) treatment of *S. asprella* as a subspecies of *S. malviflora* resulted in a polyphyletic *S. malviflora* based on our analyses. In contrast to the subspecies of *S. malviflora* recognized here (in the *malviflora* clade), *S. asprella* has a more inland distribution and also occurs at higher altitudes, in the Sierra Nevada and northward to northwestern Oregon. *Sidalcea asprella* forms the informal *asprella* clade (jk 100%; Fig. 4), together with *S. sp. nov.* (“*gigantea*”), *S. hirtipes*, *S. maxima*, and a proposed hybrid plant (*S. asprella* × *S. oregana*).

The *glaucescens* clade—Taxa in the *asprella* clade are part of a larger group, the *glaucescens* clade (jk 100%; Fig. 4). The *glaucescens* clade also contains *S. glaucescens*, *S. multifida*, *S. robusta*, and *S. asprella* population 3 [= *S. malviflora* (DC.) Benth. subsp. *nana* (Jeps.) C. L. Hitchc.], which all have inland and largely overlapping distributions. *Sidalcea asprella* 3 represents a taxon that was described by Hitchcock (1957) as *S. malviflora* subsp. *nana* but was later treated by Hill (1993) as a synonym of *S. malviflora* subsp. *asprella*, as was subsp. *elegans* (= *S. asprella* 2 in our analysis). Various taxa in the *glaucescens* clade are characterized by, or at least have a tendency towards, glaucous stems and leaves. *Sidalcea glaucescens* has the widest distribution, occurring in mountain meadows of the Sierra Nevada; *S. robusta* is the most narrowly distributed member of the group, found only in Butte County. The *glaucescens* clade corresponds to a group recognized by Roush (1931) as the “*S. asprella*-*S. glaucescens* affinity.” According to her, *S. asprella*, *S. glaucescens*, *S. multifida*, and *S. robusta* all belong to that group. *Sidalcea glaucescens* and *S. multifida* are morphologically similar, e.g., in glaucescence and leaf shape, and have been noted to intergrade (Hitchcock, 1957; Hill, 1993), with a “striking transition” from pedately dissected leaves to those typical of *S. glaucescens* (Hitchcock, 1957). Hitchcock even suggested that *S. multifida* might be treated as a subspecies of *S. glaucescens*; the near identity of ITS and ETS sequences of the two taxa does not detract from his proposal. *Sidalcea glaucescens* has been suggested to interbreed with *S. asprella* and *S. multifida* (Hitchcock, 1957; Hill, 1993) and Hitchcock (1957) pointed to the affinity of *S. glaucescens* to “*S. malviflora* subsp. *nana*” and of “subsp. *asprella*” sensu Hitchcock to *S. robusta*. Our results illustrate that the taxa in question are so closely related and minimally divergent from one another that hybridization or incomplete

lineage sorting could account for mosaic variation among them.

The *oregana* clade—Another well-supported group is the *oregana* clade (jk 99%; Fig. 4), which consists of *S. oregana* subsp. *spicata*, *hydrophila*, and *valida*, and also the endangered *S. nelsoniana*, from Oregon. *Sidalcea nelsoniana* has been considered to be “not greatly unlike” *S. oregana* subsp. *spicata* (Hitchcock, 1957, p. 65), as is also true for ETS and ITS sequences of the two taxa. Although the *oregana* clade is strongly supported, rDNA sequences of the component taxa are minimally divergent and no phylogenetic resolution was obtained within the group, in accord with a hypothesis of recent diversification from a common ancestor.

Historical biogeography of *Sidalcea*—Roush (1931) suggested that the ancestor to the modern species of *Sidalcea* spread northward from Mexico along two major routes: one through the Rocky Mountains and one through the Sierra Nevada foothills. *Sidalcea candida* and *S. neomexicana*, in the Rocky Mountains, supposedly descended from an ancestor that moved along the eastern (Rocky Mountain) route after the divergence of lineages that gave rise to *S. malachroides* and *S. hickmanii*. Our results leave open the possibility that this scenario may apply to *S. candida*, except for the positions of the “basal annuals” and *S. hartwegii*, which also branch off before *S. candida* in our trees. *Sidalcea neomexicana*, however, is well nested within the *malviflora* clade and must represent a lineage of relatively recent descent. The simplest biogeographic interpretation of our results is that *S. neomexicana* originated in California and subsequently became established widely in southwestern North America and northern Mexico.

Conclusions—Improved phylogenetic resolution and support in rDNA trees of *Sidalcea* that include ETS and ITS sequences compared to trees based on ITS data alone confirm the phylogenetic utility of the 3’ portion of the universal ETS for a young plant lineage in Malvaceae. Our results provide no support for Roush’s (1931) sections *Annuae* and *Perennes* and indicate instead that life-form evolution in *Sidalcea* has been highly dynamic. Our results are inconsistent with Roush’s (1931) biogeographic scenario of an early divergence of the Rocky Mountain species *S. neomexicana* but are consistent with subgenera *Hesperalcea* and *Malvastralcea* being basally divergent lineages within *Sidalcea*. Three of the five major, strongly supported clades of *Sidalcea* resolved in our analyses displayed low levels of sequence variation (and phylogenetic resolution) that are consistent with recent radiation of each group. Future phylogenetic studies in *Sidalcea* probably would benefit from analysis of rapidly evolving low-copy nuclear DNA regions, both to evaluate the present results from nrDNA and, if possible, to enhance phylogenetic resolution and support, especially within the most recently diverged clades. Phylogenetic understanding of *Sidalcea* should prove to be valuable for resolving evolutionary changes in sexual system in the genus, upon comprehensive characterization of patterns of sexual expression throughout the (often gynodioecious) checker mallows (see Ashman, 1994; Graff, 1999; Marshall and Ganders, 2001).

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