

# AMERICAN JOURNAL OF Botany

April 2011 • Volume 98 • Number 4



Official Publication of the Botanical Society of America, Inc.  
[www.amjbot.org](http://www.amjbot.org)

---

**Cover Illustration:** Pollination of *Collinsia sparsiflora* var. *sparsiflora* (Plantaginaceae) by nectar-feeding male *Tetralonia* sp. (Apidae: Eucerini) at the Land Trust of Napa County's Wantrup Wildlife Sanctuary in northern California. Species of *Collinsia* show remarkable variation in flower size and timing of self-pollination, with repeated evolution of small-flowered species from large-flowered species. In phylogenetic analyses of *Collinsia* in this issue, Baldwin et al. showed that evolution of strong intersterility barriers has generally lagged well behind diversification events and that *C. tinctoria* likely had a homoploid hybrid origin, with independent evolution of a chromosome arrangement otherwise known only in *C. sparsiflora* var. *sparsiflora*. Extensive sampling across the geographic and ecological distributions of previously recognized taxa of *Collinsia* revealed much cryptic diversity and evidence for phylogeographic breaks in the northwestern California Floristic Province and in the vicinity of California's Transverse Ranges, as previously reported for Californian animals. Edaphic endemism has arisen repeatedly in *Collinsia* and is evident in both recent and ancient lineages, including a previously unrecognized clade that has diversified across metamorphic exposures in the Sierra Nevada, near the western boundary of Yosemite National Park. See Baldwin et al.—Phylogenetic perspectives on diversification, biogeography, and floral evolution of *Collinsia* and *Tonella* (Plantaginaceae) on pp. 731–753. *Image credit:* W. Scott Armbruster.



**PHYLOGENETIC PERSPECTIVES ON DIVERSIFICATION,  
 BIOGEOGRAPHY, AND FLORAL EVOLUTION OF *COLLINSIA* AND  
*TONELLA* (PLANTAGINACEAE)<sup>1</sup>**

BRUCE G. BALDWIN,<sup>2,7</sup> SUSAN KALISZ,<sup>3</sup> AND W. SCOTT ARMBRUSTER<sup>4–6</sup>

<sup>2</sup>Jepson Herbarium and Department of Integrative Biology, 1001 Valley Life Sciences Building #2465, University of California, Berkeley, California 94720-2465 USA; <sup>3</sup>Department of Biological Sciences, University of Pittsburgh, 4249 Fifth Avenue, Pittsburgh, Pennsylvania 15260 USA; <sup>4</sup>School of Biological Sciences, King Henry Building, King Henry I Street, University of Portsmouth, Portsmouth PO1 2DY, UK; <sup>5</sup>Department of Biology, NTNU, Realfagbygget, Gløshaugen, N-7491, Trondheim, Norway; and <sup>6</sup>Institute of Arctic Biology, University of Alaska, P.O. Box 757000, Fairbanks, Alaska 99775-7000 USA

- *Premise of the study:* *Collinsia* was the subject of classic biosystematic studies by Garber and colleagues and is increasingly investigated to address major evolutionary questions. Lack of phylogenetic data from more than one gene region and one taxonomic exemplar has left relationships, diversity, and phylogeography of *Collinsia* in question and has limited understanding of its diversification.
- *Methods:* Phylogenetic analyses representing 179 populations of *Collinsia* and closely related *Tonella* were conducted based on DNA sequences of nuclear ribosomal transcribed spacers, the single-copy nuclear gene *CYCLOIDEA-1*, and part of the chloroplast *matK/trnK* intron region to reexamine systematic hypotheses and extend understanding of the importance of floral characters, chromosome evolution, interfertility, crossability, hybridization, edaphic factors, and ecogeographic barriers to diversification in the group.
- *Key results:* Informal “sections” of *Collinsia* are artificial, although pedicel length and other traditional deep-level taxonomic characters are more conservative evolutionarily than flower size. Evolutionary loss of crossability and interfertility in *Collinsia* appears to be largely a byproduct of divergence. Although most taxa appear to have arisen by divergent evolution, multiple lines of evidence indicate a homoploid hybrid constitution of *C. tinctoria*, possibly explaining an occurrence of convergent chromosome evolution. Phylogeographic and cryptic diversity is extensive.
- *Conclusions:* Diversity in *Collinsia* is greater than previously documented. Recently divergent lineages are often associated with distinct habitat (including soil) and geographic factors, different flower sizes, and contrasting chromosomal arrangements. Evidence for a hybrid constitution of diploid *C. tinctoria* is consistent with lack of strong intersterility barriers between closely related taxa.

**Key words:** California; *Collinsia*; chromosome evolution; cryptic diversity; endangered species; floral evolution; hybrid speciation; phylogeography; reproductive isolation; Transverse Range break.

The need for a detailed phylogenetic understanding of *Collinsia* Nutt. (Plantaginaceae) has grown with increasing attention to the genus as a subject of diverse studies in mating-system

<sup>1</sup> Manuscript received 8 September 2010; revision accepted 20 December 2010.

This paper is dedicated to the memory of E. C. Neese, who was a major source of encouragement and help with collecting for this project. The authors especially thank M. S. Park, A. M. Randle, and C. Richey for their invaluable collections and interpretations of *Collinsia* and *Tonella* populations, M. S. Park for technical assistance, and B. L. Wessa and T. Yi for extensive help with generating molecular data. The authors also thank S. J. Bainbridge, B. A. Bennett, E. Elle, K. M. Hanley, C. Ivey, Å. Lankinen, W. Legard, J. McGraw, A. Moore, E. Painter, J. Paul, M. J. Sanderson, S. P. Schechter, M. Wetherwax, M. F. Wojciechowski, and J. W. Wright for help with collecting; the Land Trust of Napa County and Wantrup Wildlife Sanctuary for assistance in fieldwork; C. Christie, J. Davis, S. Matson, K. Morse, K. Robertson, and A. Schusteff for permission to reproduce their plant images in Figs. 1–6; and M. P. Simmons and two anonymous reviewers for helpful comments on the manuscript. This research was supported by grants from the National Science Foundation (DEB-0324733) to B.G.B., (DEB-0324764) and (DEB-0709638) to S.K., and (DEB-0324808) to W.S.A. and the Lawrence R. Heckard Endowment Fund of the Jepson Herbarium (to B.G.B.).

<sup>7</sup> Author for correspondence (bbaldwin@berkeley.edu)

doi:10.3732/ajb.1000346

biology and evolution (Weil and Allard, 1964; Rust and Clement, 1977; Kalisz, 1989; Charlesworth and Mayer, 1995; Mayer et al., 1996; Kalisz et al., 1999, 2004; Armbruster et al., 2002; Elle and Carney, 2003; Kalisz and Vogler, 2003; Elle, 2004; Parachnowitsch and Elle, 2004; Lankinen and Armbruster, 2007; Lankinen and Kiboi, 2007; Lankinen et al., 2007, 2009; Kennedy and Elle, 2008a, b; Lankinen, 2009; Madjidian and Lankinen, 2009; Randle et al., 2009) and in adaptation to serpentine and nonserpentine habitats (Wright et al., 2006a, b; Wright and Stanton, 2007; Lau et al., 2008; Schechter and Bruns, 2008). A preliminary phylogenetic hypothesis, based on nuclear rDNA internal transcribed spacer region (ITS) sequences, indicated that floral evolution of *Collinsia* has been dynamic, with repeated shifts in flower size and development (Armbruster et al., 2002) associated with differences in ability to autonomously self-pollinate (Randle et al., 2009). Additional sampling of populations across *Collinsia* revealed sufficient morphological and ecological variation to warrant a more extensive phylogenetic investigation and a reconsideration of the systematics and historical biogeography of the genus, which was the subject of intensive biosystematic and cytogenetic studies by Garber and colleagues in the 1950s and 1960s (see Garber, 1975).

Members of *Collinsia* are spring-flowering annuals that occur in a wide diversity of habitats from sea level to ~4000 m a.s.l.

in temperate North America, with most taxa confined to the California Floristic Province (CA-FP) (Raven and Axelrod, 1978) and only three that occur east of the Rocky Mountains (*C. parviflora*, *C. verna*, and *C. violacea*). All species except for the two central and eastern North American endemics (*C. verna* and *C. violacea*) occur at least in part in California. Newsom (1929), in the most recent, comprehensive taxonomic treatment of *Collinsia*, recognized 28 minimum-rank taxa (17 species plus 11 additional varieties). In subsequent treatments of the genus for the Pacific states or for California only, Pennell (1951), Munz (1968), and Neese (1993) adopted taxonomies similar to Newsom's, with 22–26 minimum-rank taxa (16–19 species), not counting the two exclusively central and eastern North American species. The only species of *Collinsia* described since Newsom's (1929) monograph, *C. antonina*, was treated as a synonym of *C. parryi* by Neese (1993) and as a distinct species by Baldwin and Armbruster (2002) based on morphological considerations and molecular (ITS) evidence for a closer relationship of *C. parryi* to *C. concolor* than to *C. antonina*.

*Tonella* Nutt. ex A. Gray, the putative sister genus of *Collinsia*, comprises two species of spring-flowering annuals of the far-western United States. One species occurs widely in the Pacific states (*T. tenella*; Fig. 1); the other is restricted to the Snake River drainage of the Pacific Northwest (*T. floribunda*). *Collinsia* and *Tonella* differ most conspicuously in whether the central lower lobe of the corolla is keeled and encloses the stamens and style (in *Collinsia*), much as in a papilionoid legume (see Figs. 2–6), or not (in *Tonella*).

Although no formal subgenera or sections of *Collinsia* have been described, relationships among the species have been implied or suggested on the basis of morphological and biosystematic criteria. Gray's (1880, 1886) primary separation of *Collinsia* into two informal groups differing in pedicel length in his key to species was adhered to by subsequent authors including Newsom (1929, p. 262), who noted that "... length of the pedicels forms the basis for the most conspicuous division of the genus into the sessile- and pedicel-flowered groups, and these in turn are easily arranged in series according to bearding of staminal filaments or size and shape of corollas." Gray (1880, 1886) and Newsom (1929) also referred to differences in seed size and shape (flattened vs. thickened) as important characteristics for distinguishing species and, in part, for diagnosing the sessile- vs. pedicel-flowered species groups.

Garber and colleagues (e.g., Garber, 1956, 1958, 1960; Garber and Gorsic, 1956; Ahloowalia and Garber, 1961; Bell and Garber, 1961; Garber and Dhillon, 1962; Garber and Unni, 1965) based their biosystematic and cytogenetic studies of *Collinsia* on the informal taxonomic framework of sessile- and pedicel-flowered "sections" and discovered that grouping of taxa by chiasma frequencies, patterns of crossability between taxa, and levels of chromosomal association, chiasma formation, and interfertility in hybrids were to some extent consistent with previously suggested relationships or groups in the mostly diploid ( $x = 7$ ) genus. They found evidence for extensive chromosomal evolution by reciprocal translocations and paracentric inversions, with most widely recognized taxa distinguished by at least one chromosomal interchange. Notwithstanding a sometimes striking lack of correlation between levels of interfertility and degree of chromosomal structural divergence between taxa, Garber and Dhillon (1962, p. 291) noted that crossability, interfertility, and chromosomal associations at meiosis I "... have, in most cases, provided a reasonable basis for recognizing taxa

in this genus" and for judging the relative merits of previous taxonomies of *Collinsia*.

Garber and colleagues inferred explicit chromosomal arrangements for most studied taxa of *Collinsia* on the basis of cytogenetic evidence for arm interchanges (Garber, 1960; Ahloowalia and Garber, 1961; Bell and Garber, 1961; Garber and Dhillon, 1962), yet an overall phylogenetic hypothesis based on those and other biosystematic data was not proposed. Garber (1960) and Ahloowalia and Garber (1961) suggested an incipient role for chromosomal repatterning in evolutionary divergence leading to speciation, although they cautioned about an absence of evidence for variation in chromosomal arrangements within natural populations, with one relatively recently documented exception (Dhillon, 1975). Polyploidy was regarded by Garber and colleagues as of relatively minor importance in speciation within *Collinsia*; despite repeated documentation of spontaneous polyploids or polyploid branches among progeny of artificial hybrids (e.g., Hiorth, 1933; Garber and Gorsic, 1956; Ahloowalia and Garber, 1961; Hayhome and Garber, 1968), stable polyploids have been documented in the wild only in the hexaploid *C. torreyi* s.s. ( $2n = 21_{II}$ ; Garber, 1958) and, subsequently, in Canadian (tetraploid) populations of *C. parviflora* ( $2n = 14_{II}$ ; Ganders and Krause, 1986) and other members of the *C. grandiflora*–*C. parviflora* clade (E. Elle, Simon Fraser University, personal communication).

Here, we examine *Collinsia* and its putative sister genus, *Tonella*, from a molecular phylogenetic perspective to resolve evolutionary patterns and processes and historical biogeography in the two genera, with attention to hypotheses from prior evolutionary studies based on morphological, biosystematic, and ecological data. In particular, we focus on the following questions: (1) Does the keeled floral lobe in *Collinsia* diagnose a monophyletic group or does *Tonella* belong within *Collinsia*, as suggested by Greene (1887) on the basis of floral polymorphism? (2) Does the traditional division of *Collinsia* into sessile- and pedicel-flowered groups reflect phylogeny or has pedicel length been less conservative evolutionarily? (3) Does crossability between taxa or levels of chromosomal association or interfertility in hybrids reflect relationships within *Collinsia*, as suggested by Garber and colleagues, or have rates of chromosomal evolution or of loss of crossability or interfertility been too irregular to reflect phylogeny? (4) Has hybridization had evolutionary consequences in *Collinsia*? (5) Does the history of chromosomal interchanges estimated by Garber and colleagues contain phylogenetic signal? (6) Have previously undetected "cryptic species" evolved within *Collinsia*, as suspected by Garber and Unni (1965)? (7) Do patterns and timing of diversification in *Collinsia* provide any insights into processes of speciation or the biogeographic history of the genus?

## MATERIALS AND METHODS

**Taxon sampling**—We sampled representatives of 179 populations of *Collinsia* and *Tonella*, with attention to capturing ecological, geographic, and morphological variation and taxonomic diversity in the two genera. All species and most or all varieties recognized by Newsom (1929), Pennell (1951), Munz (1968), and Neese (1993) were represented by samples from 2–21 populations each. The outgroup consisted of up to three additional taxa in *Chelone* L., *Keckiella* Straw, and *Penstemon* Schmidel that represent the three principal clades of the sister group to *Collinsia* and (putatively) *Tonella* in tribe Cheloneae, based on results of Wolfe et al. (2002). Collection and voucher data are presented in Appendix 1. All vouchers are at JEPS and UC.



Figs. 1–6 Flowers and inflorescences of *Collinsia* and *Tonella*. **1.** *T. tenella* (scale bar = 1 mm). Image by John Davis. **2.** *C. wrightii* (scale bar = 2 mm). Image by Keir Morse. **3.** *C. verna* (scale bar = 10 mm). Image by Kenneth Robertson. **4.** *C. corymbosa* (scale bar = 10 mm). Image by Steve Matson. **5.** *C. tinctoria* (scale bar = 10 mm). Image by Aaron Schusteff. **6.** *C. heterophylla* (scale bar = 10 mm). Image by Christopher Christie.

**Gene regions sampled**—For all samples, we examined 18S-26S nuclear ribosomal DNA (nrDNA) sequences of the internal transcribed spacer region (ITS), i.e., ITS-1, 5.8S subunit, and ITS-2, and a 399–405 bp portion of the 3' end of the external transcribed spacer (ETS) immediately upstream of the 18S subunit. Chloroplast DNA (cpDNA) sequences of the 3' intron region of *trnK* and a portion of *matK* were obtained for the same, complete set of samples. For a more limited subset of samples representing major clades identified with ITS, ETS, and chloroplast data, we obtained sequences of a CYCLOIDEA-like locus, *CYCLOIDEA-1* (*CYC1*), a single-copy nuclear gene that is putatively homologous with TCP transcription factors involved in floral symmetry in *Antirrhinum majus* L. (see Hileman et al., 2003).

**DNA sequencing**—Total DNA was extracted from fresh, frozen, or silica-dried leaves of a single plant using a modification of Doyle and Doyle's (1987) method (adding a phenol extraction, RNase digestion, and two ethanol precipitations of DNA) or the DNeasy Plant Mini Kit (Qiagen, Valencia, California), following the manufacturer's protocol except with 1–2 h (rather than 10 min) of incubation (cell lysis step). Polymerase chain reaction (PCR) amplifications of the ITS region followed the methods of Baldwin and Wessa (2000) except for use of AccuPower PCR Premix (K-2016; Bioneer Corp., Chungbuk, Korea). Methods of Baldwin and Markos (1998) were used to amplify the intergenic spacer of nrDNA and sequence upstream from the 18S subunit to construct a primer, Col-ETS (5'-GGCATATTGGATCCCTGCT-3'), used for subsequent amplification and sequencing (with primer 18S-ETS; Baldwin and Markos,

1998) of 399–405 bp at the 3' end of the ETS. PCR conditions for amplification and sequencing of ETS sequences were identical to those for the ITS region except for annealing temperature (60°C). The cpDNA 3' intron segment of *trnK* and the 3' end of *matK* were amplified and sequenced using primers *matK* 8 and *trnK* 2r designed by Steele and Vilgalys (1994), with corrected reporting of *trnK* 2r by Johnson and Soltis (1994). PCR conditions for the cpDNA region were identical to those for the ITS region.

CYC-like sequences were amplified and sequenced using primers P1 and P2 (Vieira et al., 1999) and methods of Hileman and Baum (2003). Amplification products were cloned using the Zero Blunt TOPO PCR Cloning Kit for Sequencing (K2875, Invitrogen Corp., Carlsbad, California, USA). Cloning of PCR products was undertaken for ITS and ETS sequences only when sequences from pooled PCR products could not be resolved unambiguously or were highly polymorphic. All sequences of CYC-like genes were from individual cloned sequences. Of the two CYC-like paralogues identified among the cloned sequences of *Collinsia* and relatives by subsequent phylogenetic analysis (*CYC1* and *CYC2*), only *CYC1* yielded sufficient clones and variation for study here. Both DNA strands were sequenced for all samples. Exonuclease I and shrimp alkaline phosphatase were used to remove excess nucleotides from PCR products using the PCR Product Pre-Sequencing Kit (70995, United States Biochemical Corp., Cleveland, Ohio, USA). Sanger sequencing of PCR products was conducted at the UC Berkeley DNA Sequencing Facility (Barker Hall) with the same primers used for PCR (except ITS5 was used instead of ITS-1 for sequencing of the ITS region). GenBank accession numbers for sequences are provided in Appendix 1.

**Phylogenetic analyses**—DNA sequences were aligned manually based on the similarity criterion (Simmons, 2004), with special attention to codon structure in the length-variable *CYCI* and 3' end of *matK*. Gaps were treated as missing data. A concatenated matrix was produced for the entire data set. Lack of allelic or copy-type variation in more than one of the four gene regions (i.e., ITS, ETS, cpDNA, or *CYCI*) for all but one sample allowed for duplicate copies of sequences of three regions to be concatenated with each distinct cloned sequence from the fourth region (the lone exception, *Collinsia tinctoria* from Napa County, California was included only in separate analyses of ITS and ETS data; data not shown). Separate and simultaneous (combined) phylogenetic analyses of the four data sets were conducted to examine congruent and potentially conflicting signal in the different gene regions and to allow for analyses that either maximized taxon sampling or maximized sequence data (as noted above, the *CYCI* data set included samples from only a subset of populations examined for the other three gene regions). The incongruence length difference (ILD) test (Farris et al., 1995), as implemented in the program package PAUP\* version 4.0b10 (Swofford, 2002), as the partition homogeneity test, also was conducted to assess potentially conflicting phylogenetic signal across the ITS, ETS, cpDNA, and *CYCI* data sets, using 1000 replicates, with 10 random addition sequences per replicate and MulTrees set to 1.

Phylogenetic criteria used to estimate relationships and clade support included maximum parsimony (MP), using PAUP\*, and Bayesian Markov chain Monte Carlo (MCMC) inference (BI; Yang and Rannala, 1997), using the programs MrBayes 3.1 or 3.2 (Ronquist and Huelsenbeck, 2003) and BEAST v1.5.4 (Drummond and Rambaut, 2007). For computational efficiency, samples with identical sequences were treated as one operational taxonomic unit (OTU). For BI analyses, parameters for each data partition (nrDNA, cpDNA, or *CYCI*) were obtained using the program MrModeltest 2.3 (Nylander 2004) or, for BEAST runs, Modeltest 3.7 (Posada and Crandall, 1998) using the Akaike information criterion (Akaike, 1974). Chosen parameters were as follows: nrDNA and *CYCI*: GTR + I +  $\Gamma$ ; cpDNA: GTR + G for MrBayes or TIM + G for BEAST, with editing of the BEAST XML file. For each MCMC analysis of separate or combined (partitioned) data in MrBayes, four independent runs were conducted, each using three "heated" chains and one "cold" chain, for 10–15 million generations (saving one tree every 1000 generations). All trees obtained prior to a decrease in the standard deviation of split frequencies below 0.01 were discarded as burn-in. To obtain ultrametric trees, sequence data were analyzed using BEAST under a relaxed clock (uncorrelated log normal [Drummond et al., 2006]) and Yule process of speciation, with four gamma categories and constrained monophyly of the ingroup. In the absence of a known fossil record for *Collinsia* or *Tonella*, we estimated maximum ages for nodes by calibrating the most recent common ancestor (MRCA) of *Collinsia* and *Tonella* at 15 million years ago (Ma), under the assumption that diversification of this principally western North American clade of drought-avoiding spring annuals would not have begun prior to the mid-Miocene onset of summer-drying in the region (Axelrod, 1992; Flower and Kennett, 1994; see Baldwin and Sanderson, 1998). To do so, we selected a uniform prior distribution for the ingroup MRCA, with a lower setting of 14.999 and an upper setting of 15.001. Each of four independent MCMC analyses were run for 10 million generations (saving one tree every 1000 generations) when the program Tracer v1.5 (Rambaut and Drummond, 2009) indicated that the effective sample size of the posterior distribution was >1000 across runs, with a burn-in of 25%. (Note: Veracity of a mid-Miocene calibration as a maximal age for the MRCA of *Collinsia* and *Tonella* was upheld by results of BI analysis of the expanded nrDNA + cpDNA data set using BEAST under a relaxed clock [75 million generations  $\times$  4 chains, sampling every 10000 generations, with 10% burn-in for each chain], with specification of a normally distributed prior for the mean substitution rate of the ITS-1 + ITS-2 partition [instead of a nodal calibration] that corresponded to the mean  $[4.13 \times 10^{-9}$  substitutions/site/year or sub/site/y] and standard deviation  $[1.81 \times 10^{-9}$  sub/site/y] of ITS rates estimated from calibrated trees for a wide diversity of herbaceous angiosperms [Kay et al., 2006]: The resulting mean age estimate for the MRCA of *Collinsia* and *Tonella* was 12.5 Ma [95% highest probability density or HPD = 5.8–20.6 Ma], with an estimated mean ITS rate of  $5.92 \times 10^{-9}$  sub/site/y [95% HPD = 2.92–8.95  $\times 10^{-9}$  sub/site/y]). For MP analyses of the nrDNA + cpDNA + *CYCI* (all partitions) data set, full heuristic searches were conducted using 1000 random addition sequences, with tree-bisection-reconnection (TBR) branch swapping and MulTrees on. All characters were given equal weight except for higher weights in some analyses for the single, ordered character of recoded chromosomal-interchange data (see below), which involved a step matrix. MP analyses of the expanded taxon data sets of nrDNA, cpDNA, or nrDNA + cpDNA were conducted using 1000 random addition sequences, with TBR branching swapping and MulTrees off, followed by TBR branch-swapping on saved trees with

MulTrees on. Clade reliability based on MP was estimated using nonparametric bootstrapping (Felsenstein, 1985), with 10000 replicates, 10 random addition sequences per replicate, and MaxTrees set to 1. For the BI analyses, posterior probabilities for each clade were obtained from a 50% majority-rule consensus of retained trees (minus burn-in). Sequence matrices and trees are deposited at TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S11078>).

Character evolution and biogeographic history were estimated in the program package Mesquite 2.6 (Maddison and Maddison, 2009), using parsimony mapping for those polymorphic (in part) or multistate data. Pedicel/ sessile flowers, stamen bearding, and seed shape for each species were scored as binary characters, as by Newsom (1929) for seed and stamen traits and by Garber and collaborators for pedicels (e.g., Garber, 1975), except for scoring of *C. greenei* and *C. multicolor* as polymorphic for sessile and pedicel flowers (see Discussion). The same characters were scored for *C. antonina* and *C. "metamorphica"* (not studied by Newsom or Garber) from herbarium specimens. Species floral size was determined by growing plants of all species from seed to flowering in a common greenhouse environment at the University of Pittsburgh. Floral measurements of corolla size were taken on 3–6 individuals/species. Mean flower size was calculated for each species and a grand mean flower size for the genus was determined. Flower size was also treated as a binary character: species means that were greater or smaller than the grand mean of the genus were scored as large- or small-flowered, respectively. Chromosome-arm arrangements proposed for different species of *Collinsia* by Garber and colleagues (Garber, 1960; Ahloowalia and Garber, 1961; Bell and Garber, 1961; Garber and Bell, 1962) were treated as states of an ordered character and recoded in a step matrix for assessing genomic evolution by reciprocal translocations in a parsimony context. Evolution of intersterility was also explored in a phylogenetic context by considering pairwise data from crosses of Garber and colleagues (see Garber, 1975) with respect to depth of divergence of taxa in the smaller taxon-set (all-partition) trees. For biogeographic analysis, two coding schemes were used, one with taxa scored as occurring in one or more of six areas (CA-FP, Pacific Northwest outside of CA-FP, Great Basin, Mojave/Sonoran Desert, Rocky Mountains, or Central-Eastern North America) and the other with taxa included in one or more of 11 areas (northwestern CA-FP, Cascade Range of CA-FP, Sierra Nevada, Great Central Valley, central western California, southwestern California, Great Basin, Mojave/Sonoran Desert, Pacific Northwest outside of CA-FP, Rocky Mountains, or Central-Eastern North America), with boundaries of regions within the CA-FP based on geographic subdivisions in *The Jepson Manual* (Hickman, 1993) and extra-Californian CA-FP boundaries based on Raven and Axelrod's (1978) delimitation of the region. Coding of each OTU was based on collection locality of that sample for the expanded-taxon (nrDNA and cpDNA) data set.

## RESULTS

**Phylogenetic analyses**—Partition homogeneity test results indicated that ITS and ETS data are not significantly heterogeneous ( $P = 0.412$ ), so the two nrDNA regions were treated as a common partition in most subsequent analyses. All pairwise comparisons among nrDNA, cpDNA, and *CYCI* data suggested significant heterogeneity ( $P < 0.05$ ), so the three regions were treated as separate partitions in subsequent phylogenetic analyses of separate and combined data. Incongruence in clade composition between trees based on different partitions was limited to clades with <90% MP bootstrap support in one or both trees. Clades supported by two or more partitions with  $\geq 90\%$  MP bootstrap support included each of the following groups: *Collinsia*, *Tonella*, *C. bartsiiifolia* + *C. corymbosa*, *C. concolor* + *C. parryi* s.s. (i.e., excluding *C. antonina*), *C. grandiflora* + *C. parviflora*, *C. linearis* s.s. (i.e., excluding the *C. "metamorphica"* complex) + *C. rattanii*, the *C. "metamorphica"* clade, *C. sparsiflora* var. *collina* + *C. sparsiflora* var. *sparsiflora* (including var. *arvensis*), and *C. verna* + *C. violacea*. Simultaneous phylogenetic analysis of nrDNA, cpDNA, and *CYCI* data yielded trees (Fig. 7) with generally higher MP bootstrap and BI posterior probabilities than trees based on individual gene regions (Figs. 8–10). Groups resolved at  $\geq 90\%$  MP bootstrap support in the all-partitions trees (Fig. 7) that were less robust

in results of separate data partitions (Figs. 8–10) include a west–east clade, (*C. grandiflora* + *C. parviflora*) + (*C. verna* + *C. violacea*), and a clade containing the sessile-flowered taxa, i.e., *C. bartsifolia*, *C. corymbosa*, *C. heterophylla*, *C. multicolor*, and *C. tinctoria*, plus *C. antonina*, *C. greenei*, *C. parryi*, and *C. sparsiflora*. Groups of diminished bootstrap support in the all-partitions trees that were supported at  $\geq 90\%$  MP bootstrap support in trees based on one or more individual gene regions were (1) the clade containing all sessile-flowered taxa + *C. antonina* and *C. parryi* (see Fig. 11) and (2) a (nrDNA and nrDNA+cpDNA) clade containing the montane species *C. childii* and the *C.* “metamorphica” complex (Figs. 8, 12), not recovered in the all-partitions maximum-clade-credibility (MCC) tree (Fig. 7). Posterior probabilities of BI trees from simultaneous analysis of all partitions were  $\geq 95\%$  for all clades supported by  $\geq 90\%$  bootstrap

support in separate or combined-data MP analyses except for the nrDNA clade containing *C. childii* and the *C.* “metamorphica” clade; the BI combined all-partition analysis instead resolved a clade of 96% posterior probability with the *C.* “metamorphica” clade sister to a clade that included the sessile-flowered taxa plus *C. antonina*, *C. greenei*, *C. parryi*, and *C. sparsiflora*.

Separate analyses of the expanded taxon-set for nrDNA or cpDNA data (Figs. 8, 9) yielded trees congruent with results from the smaller taxon-set analyses of each of those molecular regions (results not shown). All species of *Collinsia* as treated by Newsom (1929) and Munz (1968) were resolved as clades except for species in each of three large- and small-flowered species pairs: *C. grandiflora* + *C. parviflora*, *C. linearis* + *C. rattanii*, and *C. concolor* + *C. parryi*. Plants referable to *C. austromontana*, recognized as a species by Pennell (1951) and

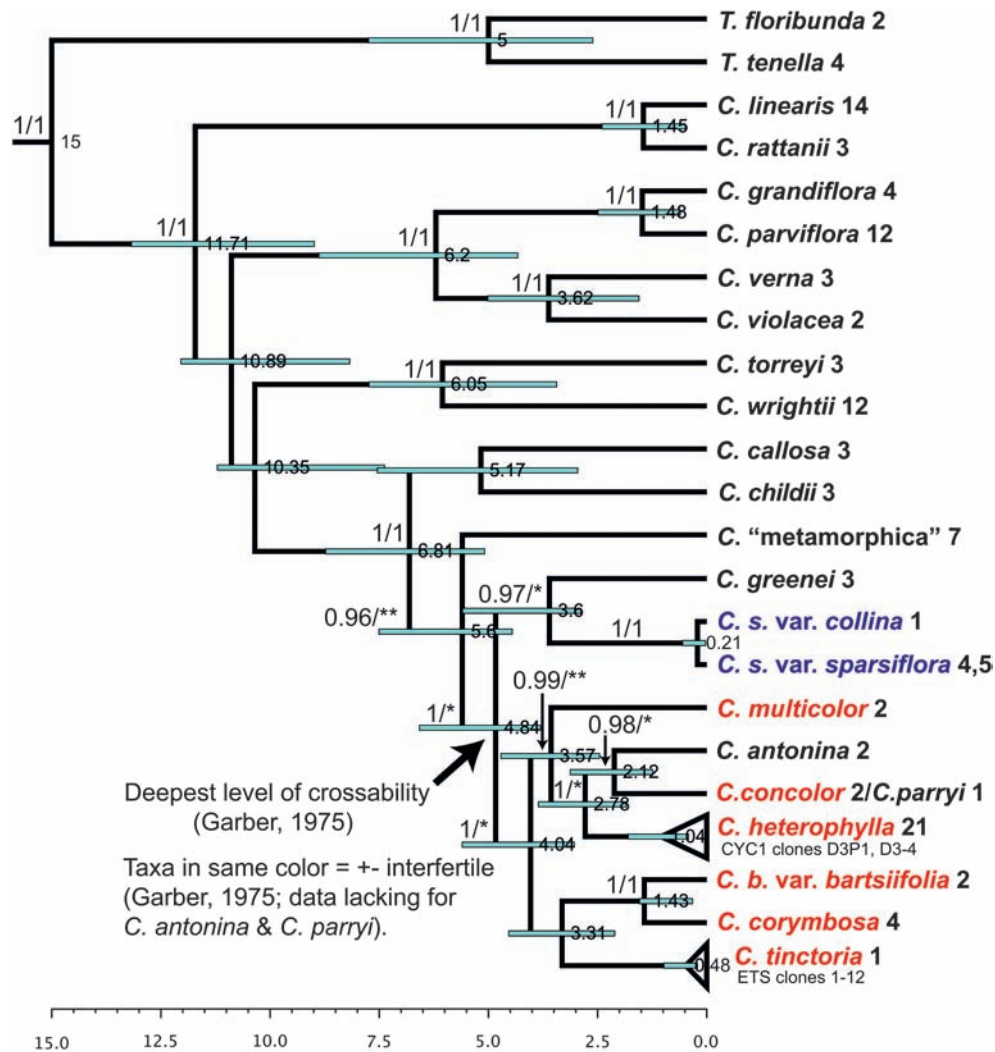


Fig. 7. Chronogram and maximum-clade-credibility tree of *Collinsia* and *Tonella* based on Bayesian phylogenetic analysis of combined sequences of nrDNA external and internal transcribed spacer (ETS and ITS) regions, 3' *matK*/3' *trnK* intron chloroplast DNA (cpDNA), and the single-copy nuclear gene *CYCLOIDEA 1* (*CYC1*) using BEAST. Branch lengths are scaled to time, in million years, with maximal basal calibration at 15 Ma (see Materials and Methods); estimated maximum ages are given to the right of the node (blue-green bars delimit the 95% highest probability density for ages). Numbers following taxon names refer to population number (see Appendix 1). Bayesian posterior probabilities (left of slash) and maximum parsimony (MP) bootstrap values (right of slash) are along or left of tree nodes, for nodes supported at  $\geq 0.95$  posterior probability. One asterisk (\*) = clade with  $< 90\%$  support in 0.5 majority-rule maximum parsimony bootstrap tree. Two asterisks (\*\*) = clade not resolved in 0.5 majority-rule MP bootstrap tree. Outgroup not shown. Abbreviations: *b.* = *bartsifolia*. *C.* = *Collinsia*. *s.* = *sparsiflora*. *T.* = *Tonella*.

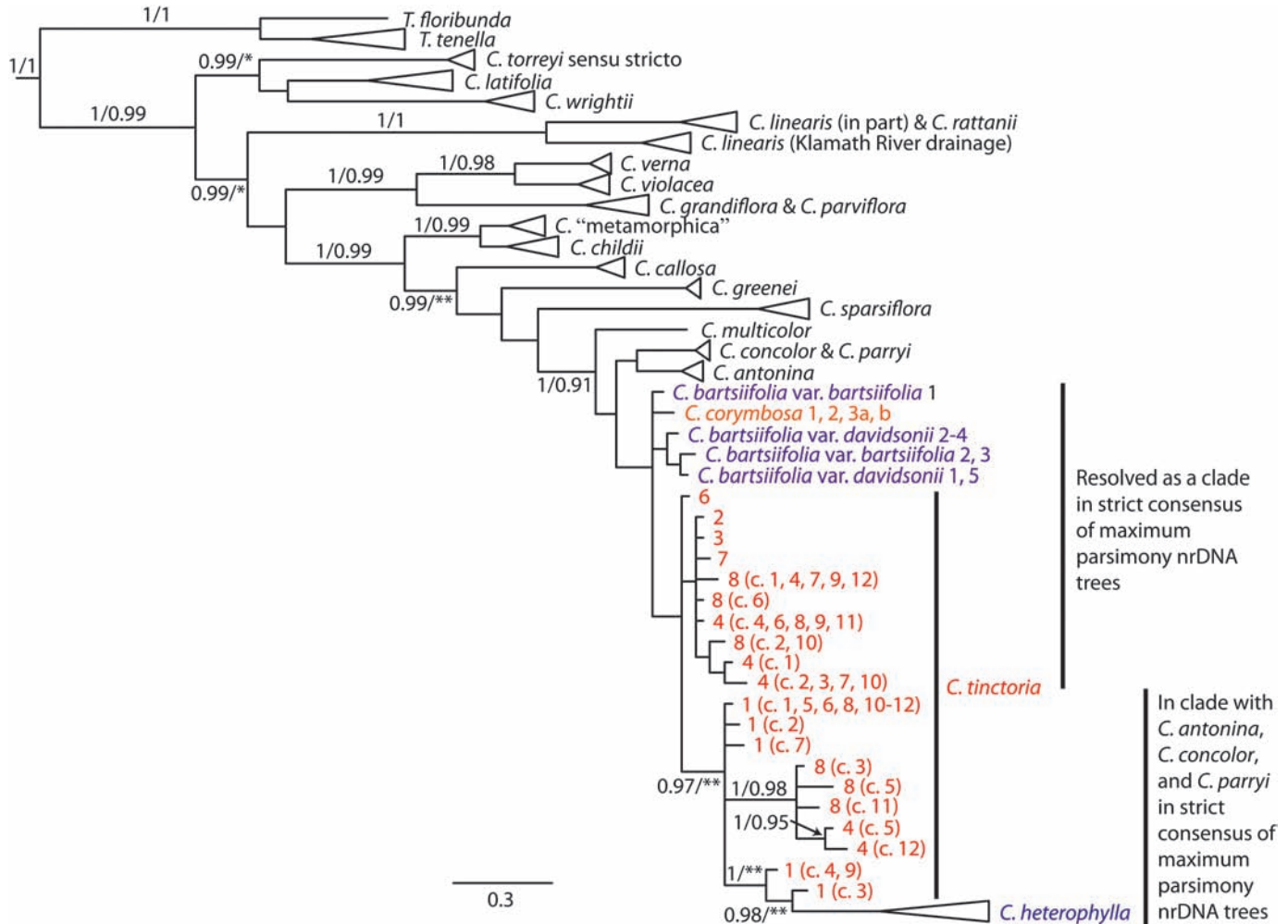


Fig. 8. Phylogram of 0.5 majority-rule consensus tree for *Collinsia* and *Tonella* based on Bayesian phylogenetic analysis of nrDNA ETS and ITS sequences using MrBayes (scale in expected substitutions per site). Numbers following taxon names (or, for *C. tinctoria*, at branch tips) refer to population number (see Appendix 1); all sampled populations are represented for taxa without indicated numbers. Numbers in parentheses refer to different cloned sequences for *C. tinctoria* (ETS clones for sample 1; ITS clones for samples 4 and 8). Bayesian posterior probabilities (left of slash) and maximum parsimony (MP) bootstrap values (right of slash) are given for nodes supported at  $\geq 0.95$  posterior probability. One asterisk (\*) = clade with  $< 90\%$  support in 0.5 majority-rule MP bootstrap tree. Two asterisks (\*\*) = clade not resolved in 0.5 majority-rule MP bootstrap tree. Outgroup not shown. Taxa in colored font pertain to *Hybridization and evolution* section of Discussion. Abbreviations: *C.* = *Collinsia*. *c.* = clone(s). *T.* = *Tonella*.

originally as a variety of *C. heterophylla* by Newsom (1929), were nested among samples of *C. heterophylla* s.s. in the expanded taxon-set trees. *Collinsia parryi* sensu Neese (1993) was resolved as nonmonophyletic, as in the all-partitions trees (Fig. 7), with Monterey County populations (= *C. antonina*) in a clade outside the *C. concolor* + *C. parryi* clade. *Collinsia linearis* sensu Neese (1993) also was resolved as polyphyletic, as in the all-partitions trees (Fig. 7), with populations from the Sierra Nevada in a separate, distantly related lineage (= *C. "metamorphica"* clade).

Sequences of *Collinsia tinctoria* in the expanded taxon-set trees were placed in distinct clades in cpDNA and nrDNA analyses. In the cpDNA trees (Fig. 9), one sample of *C. tinctoria* (population 7) was resolved within *C. heterophylla*, and the others were resolved as a clade closely related to *C. bartsiiifolia* and *C. corymbosa* (plus other taxa). Extensive polymorphism of directly sequenced PCR products of *C. tinctoria* ITS or ETS was reflected by placement of subsets of clones (from single plants)

of *C. tinctoria* nrDNA in different clades in the ITS, ETS, and ITS + ETS (nrDNA) trees. The nrDNA sequences of *C. tinctoria* were resolved either as successively diverging along a branch connecting *C. bartsiiifolia* + *C. corymbosa* with *C. heterophylla* (in BI trees; Fig. 8), or some sequences were resolved together in a clade with *C. bartsiiifolia* and *C. corymbosa* and the rest were in a clade with *C. heterophylla* (in MP trees; see Fig. 8). Such polymorphism for nrDNA copy types that belong to distinct clades was not detected in any other taxon of *Collinsia* except in one population of *C. heterophylla*, from Santa Barbara County, California (see Appendix 1 and Discussion).

#### Character-evolution analyses and biogeographic history—

Mapping of morphological characters on the all-partition MCC tree (Fig. 7) using the parsimony criterion yielded unambiguous evidence of the following evolutionary changes: (1) pedicel to sessile flowers (Fig. 11), with either two origins of sessile flowers (one represented by *Collinsia concolor*)

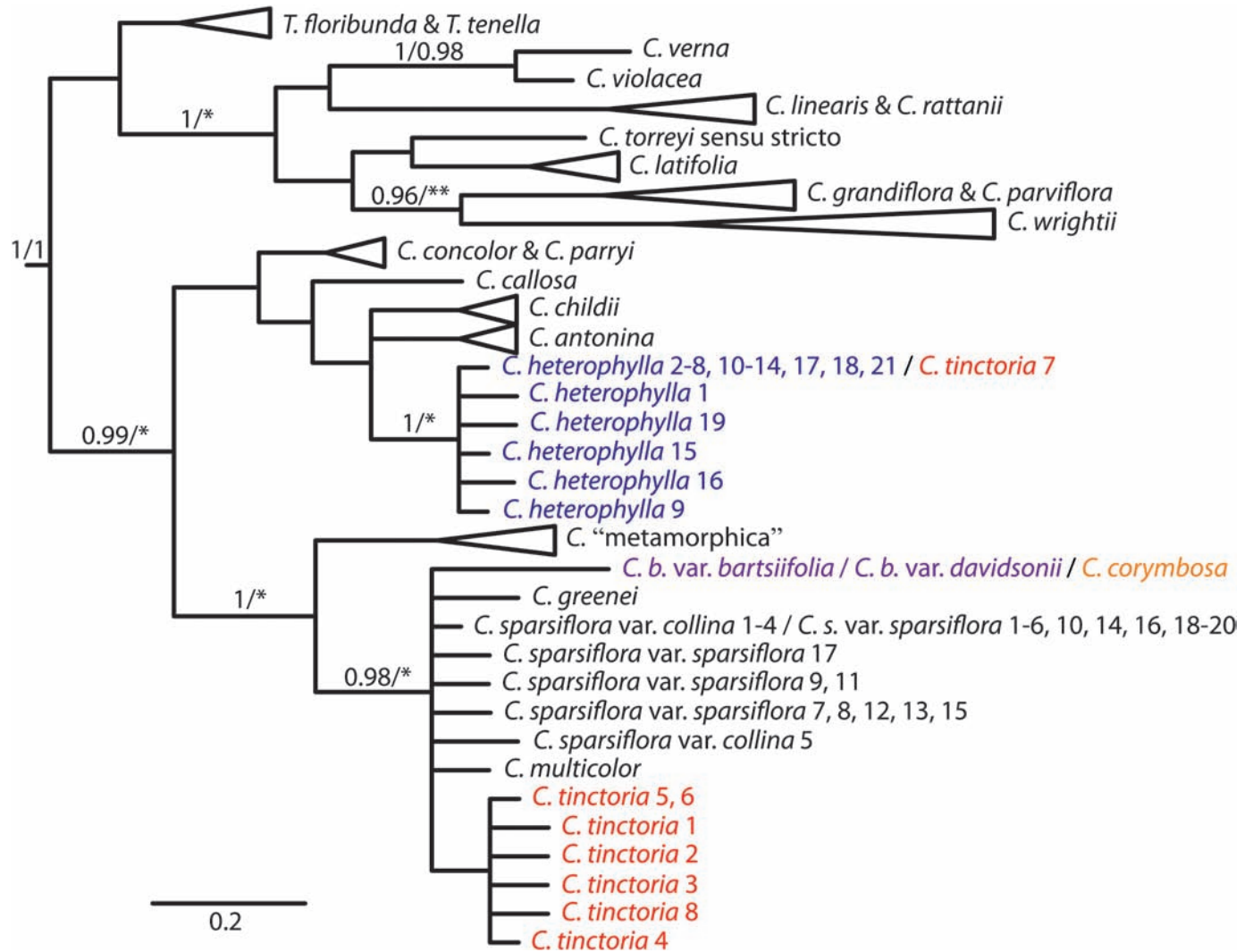


Fig. 9. Phylogram of 0.5 majority-rule consensus tree for *Collinsia* and *Tonella* based on Bayesian phylogenetic analysis of cpDNA sequences using MrBayes (scale in expected substitutions per site). Numbers following taxon names refer to population number (see Appendix 1); all sampled populations are represented for taxa without indicated numbers. Bayesian posterior probabilities (left of slash) and maximum parsimony (MP) bootstrap values (right of slash) are given for nodes supported at  $\geq 0.95$  posterior probability. One asterisk (\*) = clade with  $< 90\%$  support in 0.5 majority-rule MP bootstrap tree. Two asterisks (\*\*) = clade not resolved in 0.5 majority-rule MP bootstrap tree. Outgroup not shown. Taxa in colored font pertain to *Hybridization and evolution* section of Discussion. Abbreviations: *C.* = *Collinsia*. *s.* = *sparsiflora*. *T.* = *Tonella*.

or one reversal to pediceled flowers (represented by *C. antonina* and *C. parryi*), (2) glabrous to bearded stamens (at least twice; see Discussion), and (3) thickened seeds to flattened seeds (once; see Discussion). Flower size was mapped onto the same tree, with six unequivocal shifts from larger to smaller flowers that yielded the small-flowered lineages represented by *Tonella tenella*, *C. callosa* + *C. childii*, *C. parviflora*, *C. rattanii*, *C. sparsiflora* var. *collina*, and *C. wrightii*. Equivocal reconstructions involving an additional clade indicated either one shift to smaller flowers in the MRCA of *C. antonina* and *C. parryi* and an associated reversal to larger flowers in *C. concolor* or, instead, separate shifts to smaller flowers represented by *C. antonina* and *C. parryi* and no reversal to larger flowers. Estimates of changes in flower size in the expanded-taxon tree based on nrDNA + cpDNA data were uniformly from larger- to smaller-flowers except for reconstructions of smaller- to larger-flowers involving poorly supported

clades, i.e., within *C. sparsiflora* and within the *C. grandiflora* + *C. parviflora* clade (see Fig. 12).

Chromosomal interchanges were mapped onto the all-partitions MCC tree with only one instance of homoplasy (14 steps). Reanalysis of the all-partitions data set using MP with weighting of  $\geq 5$  for the ordered, multistate chromosomal character resulted in a shift in the position of *C. tinctoria* to the branch between *C. greenei* and *C. sparsiflora* (i.e., with *C. tinctoria* sister to *C. sparsiflora*), with no homoplasy (13 steps; Fig. 13).

Two interfertility groups in *Collinsia* were found to correspond closely to two clades (Fig. 7). One was the mostly sessile-flowered clade, based on evidence of at least partially fertile progeny from crossing data involving one or more hybrid combinations linking *C. bartsifolia*, *C. corymbosa*, *C. concolor*, *C. heterophylla*, and *C. tinctoria*. *Collinsia parryi* is part of the same clade and was included by Garber and colleagues in

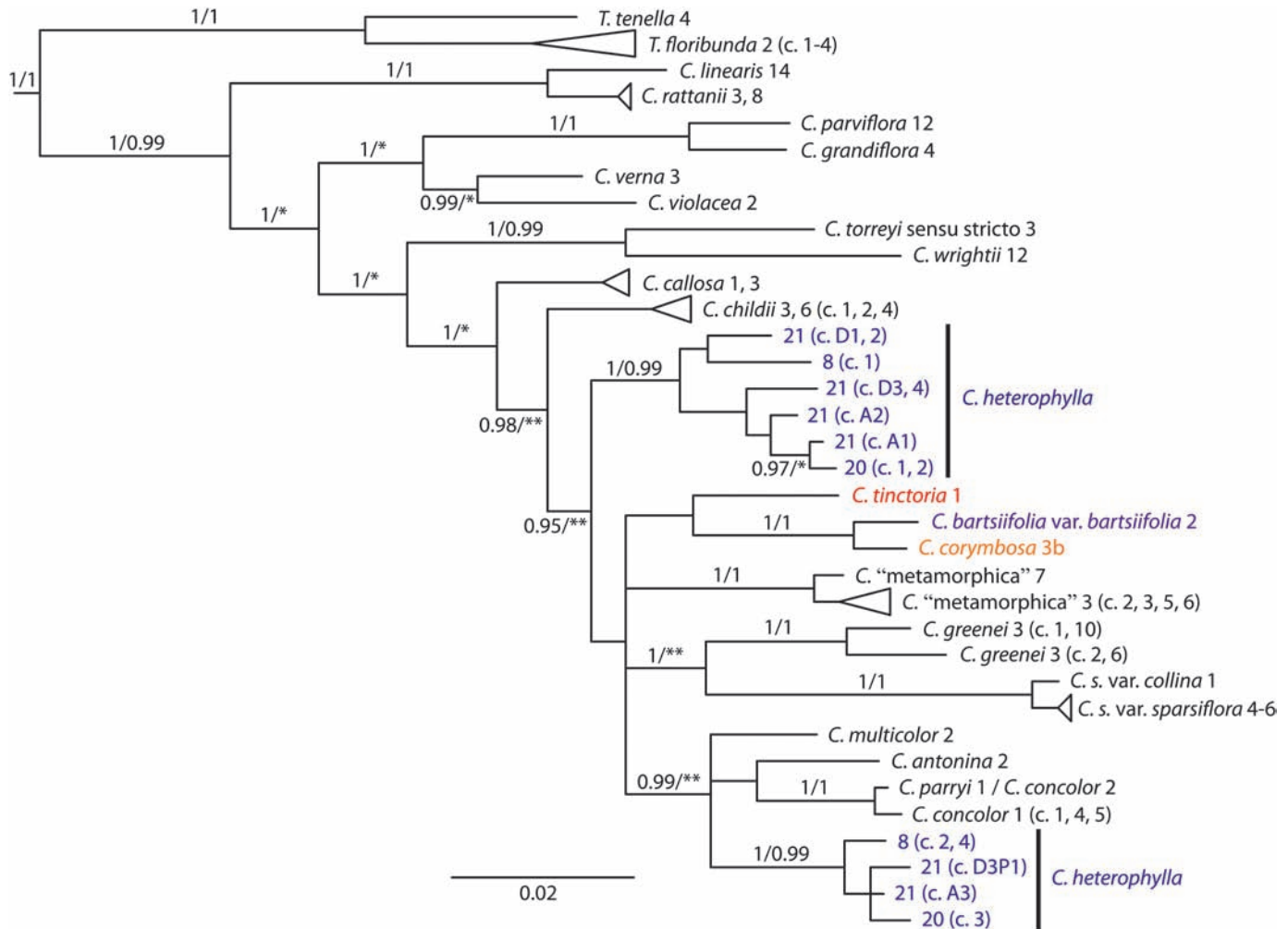


Fig. 10. Phylogram of 0.5 majority-rule consensus tree for *Collinsia* and *Tonella* based on Bayesian phylogenetic analysis of *CYCLOIDEA-1* (*CYC1*) sequences using MrBayes (scale in expected substitutions per site). Numbers following taxon names (or, for *C. heterophylla*, at branch tips) refer to population number (see Appendix 1); all sampled populations are represented for taxa without indicated numbers. Numbers in parentheses refer to different cloned sequences of *CYC1*. Bayesian posterior probabilities (left of slash) and maximum parsimony (MP) bootstrap values (right of slash) are given for nodes supported at  $\geq 0.95$  posterior probability. One asterisk (\*) = clade with  $< 90\%$  support in 0.5 majority-rule MP bootstrap tree. Two asterisks (\*\*) = clade not resolved in 0.5 majority-rule MP bootstrap tree. Outgroup not shown. Taxa in colored font pertain to Hybridization and evolution section of Discussion. Abbreviations: c. = clone(s). C. = *Collinsia*. s. = *sparsiflora*. T. = *Tonella*.

relatively few crossing attempts, which yielded one hybrid (a spontaneous amphidiploid) with *C. concolor* (Hayhome and Garber, 1968). No crossing data are available for the most recently described species of the clade, *C. antonina*. Estimated maximal timing of divergence between the most distantly related interfertile taxa in the mostly sessile-flowered clade was 4 Ma (95% HPD = 3–5.6 Ma) (Fig. 7). The other interfertility group in *Collinsia* corresponds to *C. sparsiflora* s.l., with divergence of taxa at  $\leq 0.2$  Ma (95% HPD = 0–0.6 Ma). Production of sterile hybrids by Garber and colleagues extended to a deeper node in the all-partitions MCC tree (Fig. 7); namely, to the MRCA of *C. greenei* + *C. sparsiflora* and the mostly sessile-flowered taxa, at  $\leq 4.8$  Ma (95% HPD = 3.8–6.6 Ma). All reported crosses between taxa separated by older nodes failed except one (see Discussion).

Parsimony mapping of biogeographic regions on the all-partitions MCC tree (Fig. 7), with terminal taxa coded by overall taxon distribution, yielded unequivocal estimates for nodal

states under the broad area-coding (six-area) scheme, with the CA-FP estimated for each internal node except for the MRCAs of (1) *Collinsia* + *Tonella* (Pacific Northwest), *Tonella* (Pacific Northwest), and *C. verna* + *C. violacea* (Central-Eastern North America). Mapping under the finer-scale area-coding (11-area) scheme on the all-partitions tree yielded equivocal estimates of areas for most internal nodes. With coding of OTUs by collection locality in the expanded taxon-set trees (Fig. 12), the MRCA of *Collinsia* was estimated as having occurred in the northwestern CA-FP, regardless of whether the deep structure of the tree was constrained to conform to that of the all-partitions tree or not. Most deep nodes within *Collinsia* were estimated as ancestral occurrences in the northwestern CA-FP under the nrDNA + cpDNA tree topology (Fig. 12) or in either the northwestern CA-FP or the Sierra Nevada with the all-partitions deep tree-structure imposed (results not shown). Ancestral occurrence in central and eastern North America was recovered under both tree topologies for the MRCA of *C. verna*

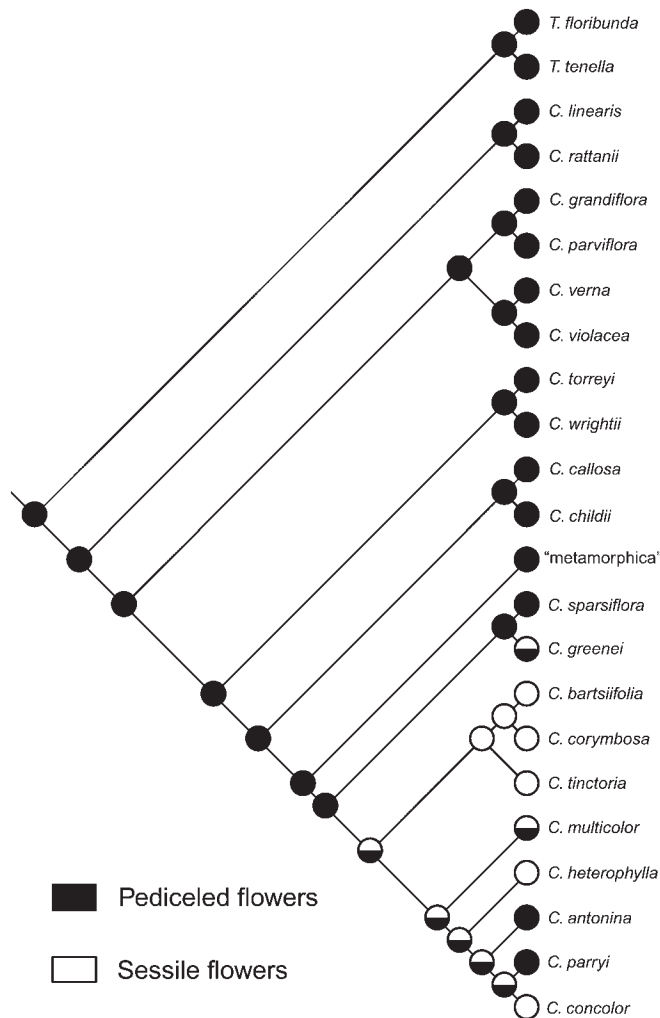


Fig. 11. Parsimony mapping of pediceled- and sessile-flowered conditions based on the topology of the combined all-partitions tree in Fig. 7. Abbreviations: C. = *Collinsia*. T. = *Tonella*. If sessile-flowers are interpreted as ancestral in the clade of *C. antonina* + (*C. concolor* + *C. parryi*) (see Fig. 12B), then sessile flowers are reconstructed as evolving once, with two reversals to pediceled flowers, in *C. antonina* and *C. parryi* (see Discussion).

and *C. violacea* and for the MRCA of the larger clade also including *C. grandiflora* and *C. parviflora* (Fig. 12). Well-supported phylogeographic structure was recovered within *T. tenella*, *C. heterophylla*, *C. latifolia*, the *C. linearis* + *C. rattanii* clade, the *C. "metamorphica"* clade, and *C. wrightii*.

## DISCUSSION

**Monophyly of *Collinsia* and *Tonella***—Robust support for a sister-group relationship between *Collinsia* and *Tonella* from phylogenetic analyses of combined data (Fig. 7) and of separate nrDNA (Fig. 8) and *CYC1* (Fig. 10) data sets reinforces the previous hypothesis that the two genera are closest relatives (e.g., Gray, 1886; Newsom, 1929; Schrock and Palsler, 1967; Garber, 1975; Raven and Axelrod, 1978; Armbruster et al., 2002; Wolfe et al., 2002). Based on the tree topology, enfolding of the stamens and pistil by conduplication of the central, lower

corolla lobe evolved only once, at the base of *Collinsia*, and is possibly a key innovation associated with the evolution of ~10-fold greater species diversity than has evolved in *Tonella*, which has a planar, central, lower corolla lobe and exposed stamens and pistil (Fig. 1). (Note, however, that a key innovation cannot be defensibly concluded from only one comparison [see Sanderson and Donoghue, 1994; Hodges and Arnold 1995].) The keeled, central, lower corolla lobe in flowers of *Collinsia* (see Figs. 2–6) enforces sternotribic pollination (Rust and Clement, 1977) and protects pollen from theft by syrphid flies, small pollen-collecting bees, and other pollen-feeding insects (W. S. Armbruster, unpublished observations) and is part of a complex of floral characters that can affect reproductive success in *Collinsia* (Kalisz et al., 1999, 2004; Armbruster et al., 2002; Elle and Carney, 2003; Kalisz and Vogler, 2003; Parachnowitsch and Elle, 2004).

Greene's (1887) placement of both species of *Tonella* within *Collinsia* was based on his discovery of wild plants of *C. heterophylla* (= *C. bicolor* Benth.) with aberrant flowers that resembled those of *Tonella*; each flower had a planar, as opposed to keeled, central, lower corolla lobe. Greene (1887, p. 55) concluded that "... since a collinsia of the bilabiate type can sportively array itself partly in tonella blossoms ... there seems to be no support left for *Tonella* ... They are perfect collinsias in all but what is now shown to be the mere accident, of a plane rather than folded lower corolla-lobe." Spontaneous open-keeled mutants also have been observed in several species of *Collinsia* grown under greenhouse conditions from field-collected seed (S. Kalisz, unpublished data). Our data show that despite the occurrence of such mutant floral forms in *Collinsia*, evolutionary shifts from keeled to nonkeeled floral morphology have not occurred in the ingroup clade; continued recognition of *Tonella* is justified.

**Sessile- and pediceled-flowered groups in *Collinsia***—The long-standing, informal, infrageneric classification of *Collinsia* into two groups or "sections" (Gray, 1886; Jepson, 1925; Newsom, 1929; Pennell, 1951; Munz, 1968; Garber, 1975; Neese, 1993) distinguished by pedicel length relative to calyx length—that is, "sessile-flowered," with pedicels absent or generally shorter than calyces, or "pediceled-flowered," with pedicels generally longer than calyces—does not precisely reflect phylogeny, based on our findings. Pediceled flowers are unequivocally ancestral in *Collinsia* (and *Tonella*) and probably evolved secondarily from a sessile-flowered state in *C. antonina* and *C. parryi*, which are nested within the otherwise mostly sessile-flowered clade and are resolved here as the two closest relatives of the sessile-flowered *C. concolor*. Although parsimony mapping of sessile- and pediceled-flowered states based on the small taxon-set (all-partitions) MCC tree (Fig. 7) did not resolve unequivocally the evolution of pediceled flowers in *C. antonina* and *C. parryi* (Fig. 11), the well-supported, nested position of *C. parryi* in a grade of *C. concolor* lineages in the expanded-taxon tree (Fig. 12B) is consistent with separate origins of pediceled flowers in *C. antonina* and *C. parryi*. Under that scenario, presence of sessile-flowers in *C. concolor* and other sessile-flowered taxa of *Collinsia* would be strictly homologous.

Of the two pediceled-flowered taxa of *Collinsia* that are nested in the otherwise mostly sessile-flowered clade, only one, *C. parryi*, was included in previous biosystematic studies (Hayhome and Garber, 1968; see Garber, 1975). *Collinsia parryi* successfully crossed only with *C. concolor*, its closest relative

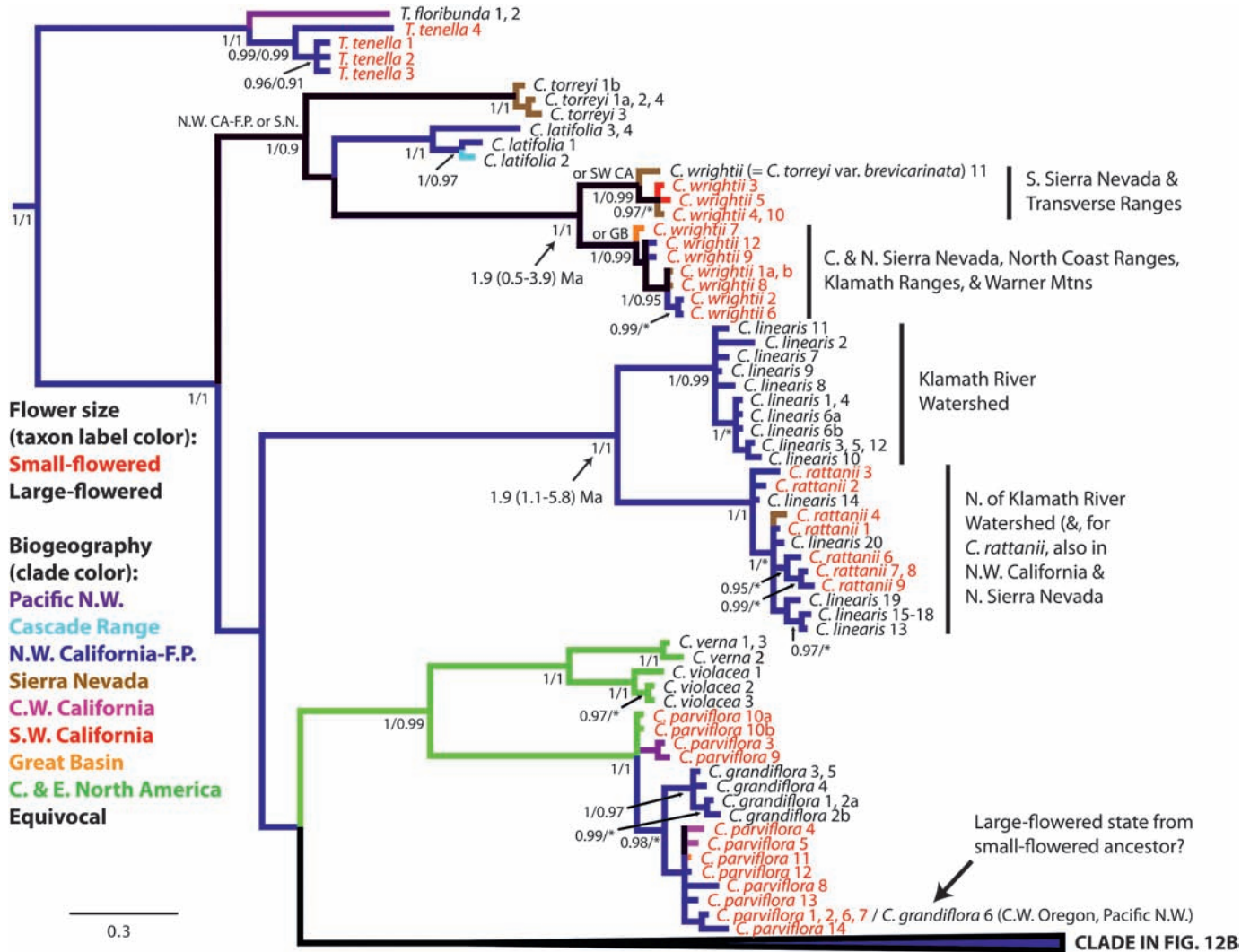


Fig. 12. Phylogram of 0.5 majority-rule consensus tree for *Collinsia* and *Tonella* based on Bayesian phylogenetic analysis of combined nrDNA and cpDNA sequence data using MrBayes (scale in expected substitutions per site). Geographic areas assigned to branches are based on results from parsimony mapping on the maximum-clade-credibility (MCC) tree, found using TreeAnnotator in the BEAST package (Drummond and Rambaut, 2007). Topology of the MCC tree (not shown) is congruent with resolved structure in the consensus tree except for only slightly differing positions of two samples (*C. tinctoria* 6 and *C. wrightii* 7). For each branch of unequivocal area assignment, the estimate shown is also the predominant unequivocal estimate found throughout the posterior distribution of trees, based on results using Trace Character Over Trees in Mesquite (Maddison and Maddison, 2009). Areas indicated above biogeographically equivocal nodes indicate equally parsimonious estimates for that node (and for more apical, equivocal nodes on contiguous branches). Estimated maximal divergence times, with 95% highest probability density in parentheses, indicated for some nodes are from a nrDNA + cpDNA chronogram (using BEAST, not shown) of a subset of samples from well-supported clades resolved here. Numbers following taxon names refer to population number (see Appendix 1). Numbers in parentheses following population number refer to different cloned sequences of nuclear ribosomal DNA (all of the ITS region except for *C. tinctoria* sample 1 clones, of ETS). Bayesian posterior probabilities (left of slash) and maximum parsimony (MP) bootstrap values (right of slash) are given for nodes supported at  $\geq 0.95$  posterior probability. One asterisk following a slash (\*) = clade with  $< 90\%$  support in 0.5 majority-rule MP bootstrap tree. Two asterisks (\*\*\*) = clade not resolved in 0.5 majority-rule MP bootstrap tree. Clade support values in parentheses indicate (higher) support for nodes if the putatively hybrid taxon *C. tinctoria* was removed from the analyses. Outgroup not shown. Abbreviations: c. = clone(s). *C.* = *Collinsia*. *s.* = *sparsiflora*. *T.* = *Tonella*.

based on our molecular trees. The resulting single hybrid obtained was reported to be a spontaneous allotetraploid (Hayhome and Garber, 1968). Newsom (1929, p. 287) regarded *C. parryi* as "... probably a segregate of the *sparsiflora* group" and to our knowledge *C. parryi* was not suggested to be a close relative of any of the sessile-flowered taxa prior to availability of molecular data (Baldwin and Armbruster, 2002). Lack of detected molecular divergence between some samples of the large-flowered *C. concolor* and short-flowered *C. parryi* in nrDNA, cpDNA,

and *CYCI* sequences indicates a striking example of rapid morphological evolution in *Collinsia*.

Two species of *Collinsia* (*C. greenei* and *C. multicolor*) have pedicels that can be longer or shorter than calyces on the same plant and therefore have been of uncertain placement. *Collinsia greenei*, treated by Gray (1886) and Jepson (1925) in the sessile-flowered group, by Pennell (1951) in the pediceled-flowered group, and by Newsom (1929), Munz (1968), and Neese (1993) in both groups, was resolved as sister to *C. sparsiflora* in





with a possible separate origin of bearded filaments in the ancestry of *C. sparsiflora* (*C. greenii* lacks filament bearding).

**Seed shape**—Newsom (1929) also regarded seed shape (thickened and bean-shaped as opposed to flattened and often winged or cup-shaped) as a taxonomically important character. Gray (1886) noted earlier that sessile-flowers in *Collinsia* are associated with flattened (“meniscoidal”) seeds. Notwithstanding seed-shape intermediacy or variation in some species, thickened seeds were mapped as ancestral in *Collinsia*, with a single shift to flattened seeds in the MRCA of the undisputed sessile-flowered species plus *C. greenii*, the *C. “metamorphica”* clade, and *C. sparsiflora*.

**Evolutionary divergence and interfertility**—On the basis of the all-partitions chronogram (Fig. 7), members of *Collinsia* with sessile flowers descended from a much more recent common ancestor ( $\leq 4$  [3–5.6] Ma) than those with pediceled flowers ( $\leq 11.7$  [9–12.2] Ma), in accord with biosystematic and cytogenetic findings of Garber and colleagues (see Garber, 1975) and Greenlee and Rai (1986). In those earlier studies, *Collinsia* hybrids of at least low fertility were generally obtained from crosses between sessile-flowered taxa. In contrast, crosses between most pediceled-flowered taxa or between sessile- and pediceled-flowered taxa either failed or yielded sterile hybrids. The major exception to those patterns was the recovery of moderately to highly fertile hybrids from crosses in various combinations between the pediceled-flowered taxa *C. sparsiflora* var. *arvensis*, *C. bruceae*, and *C. solitaria* (Ahloowalia and Garber, 1961), which all have been subsequently treated within the circumscription of *C. sparsiflora* (e.g., Neese, 1993) and are estimated here to have diverged from a common ancestor since the Pleistocene ( $\leq 0.21$  [0–0.6] Ma).

On the basis of the all-partitions chronogram (Fig. 7), no pair of *Collinsia* taxa estimated to have diverged from a common ancestor more than 4 (3–5.6) Ma yielded fertile hybrids and only one species pair estimated to have diverged from a common ancestor more than 4.8 (3.8–6.6) Ma yielded any hybrids in studies by Garber and colleagues (see Garber, 1975) or Greenlee and Rai (1986). The exceptionally wide, successful hybrid combination was between *C. heterophylla* and *C. verna*, with complete sterility and lack of chromosomal pairing at meiosis I reported (Greenlee and Rai, 1986).

Although divergence in chromosomal arrangements can have major effects on interfertility and most examined species of *Collinsia* are differentiated by chromosomal interchanges (see Garber, 1975), recency of common ancestry is evidently roughly correlated with crossability and interfertility of taxa in the genus, as expected if sterility barriers have arisen largely as a by-product of evolutionary divergence rather than as a driver of diversification (see also Randle, 2009). A frequent bias toward alternate segregation at meiosis I resulted in higher than expected fertility in interspecific hybrids produced by Garber and colleagues (see Garber, 1960, 1975) and may limit the effect of chromosomal rearrangements on postzygotic reproductive isolation in *Collinsia*. Garber (1960, p. 243) nonetheless regarded such rearrangements to be “a major factor in speciation” in the genus, primarily in limiting recombination between rearranged chromosomal segments of divergent lineages (Grant, 1956).

**Hybridization and evolution**—The strongest evidence from molecular data for a hybrid constitution of any taxon in *Collinsia* was found in *C. tinctoria* (Fig. 5), a diploid sessile-flowered

species generally of rocky habitats. *Collinsia tinctoria* has been placed in taxonomic proximity to two species of sandy soils, *C. bartsifolia* and *C. corymbosa* (Fig. 4), in some treatments of *Collinsia* (Newsom, 1929; Pennell, 1951; Munz, 1968), based in part on their sharing of sessile flowers, flattened seeds, and a short upper corolla lip. Neese (1993) suggested morphological intergradation among those three species and *C. heterophylla* (Fig. 6), which includes populations with a short upper corolla lip in southwestern California (sometimes recognized as var. *austromontana*), well outside the geographic distribution of *C. tinctoria*. We are unaware of any verified natural hybrids between any of the four species, although Neese (1993, p. 1026) suggested that *C. heterophylla* “... may hybridize with *C. bartsifolia*, *C. multicolor*, and *C. tinctoria* (more study needed)”.

Sequences of *C. tinctoria* were placed in distinct clades in both nrDNA and cpDNA trees, with a subset of *C. tinctoria* sequences in a clade with *C. bartsifolia* and *C. corymbosa* in the cpDNA trees (Fig. 9) and in the nrDNA MP trees (see Fig. 8) and a different subset of sequences in a clade with *C. heterophylla* (Figs. 8, 9). In the cpDNA trees, sequences from seven of the eight sampled populations of *C. tinctoria* constituted a clade in an unresolved polytomy with *C. bartsifolia* + *C. corymbosa*, *C. greenii*, *C. multicolor*, and *C. sparsiflora*; the eighth population of *C. tinctoria* yielded a cpDNA sequence that is identical to the sequence found in most sampled populations of *C. heterophylla*. On the basis of parsimony reconstruction of character evolution, the cpDNA sequence shared by the one *C. tinctoria* sample and most samples of *C. heterophylla* is ancestral for the *C. heterophylla* lineage and is widespread across modern populations of *C. heterophylla*; either recent chloroplast capture or ancient hybridization could explain occurrence of that chloroplast haplotype in *C. tinctoria*. In nrDNA trees, some sequences of *C. tinctoria* were resolved within a grade of lineages diverging just basal to a lineage containing all sequences of *C. heterophylla*; other sequences of *C. tinctoria* were resolved either in a clade with *C. bartsifolia* and *C. corymbosa* (in MP trees) or as a lineage diverging along a branch connecting *C. bartsifolia* and *C. corymbosa* with *C. heterophylla* and other sequences of *C. tinctoria* (BI trees; Fig. 8). Cloned ITS or ETS sequences from the same individual plants of *C. tinctoria* were placed in each of those contrasting positions. Association of *C. tinctoria* sequences with the same two clades (i.e., with *C. bartsifolia* + *C. corymbosa* and with *C. heterophylla*) in both cpDNA and nrDNA trees and lack of comparable detected variation in other species lead us to conclude that hybridization, rather than lineage sorting or slow concerted evolution, caused the observed molecular patterns. Concerns that inclusion of a hybrid taxon might disrupt phylogenetic analyses were mostly unrealized and affected only weakly supported clades; exclusion of *C. tinctoria* from analyses resulted in nrDNA BI trees conforming to the nrDNA MP topology (with *C. heterophylla* in a clade with *C. antonina*, *C. concolor*, and *C. parryi* rather than with *C. bartsifolia* and *C. corymbosa*) and resulted in an all-partition MCC tree with *C. childii* sister to *C. “metamorphica”* rather than sister to *C. callosa*, and with *C. multicolor* sister to *C. bartsifolia* and *C. corymbosa* rather than to *C. antonina*, *C. concolor*, *C. heterophylla*, and *C. parryi* (results not shown).

Molecular evidence for a hybrid constitution of populations of *C. tinctoria* that are widely separated geographically—in the San Francisco Bay Area, North Coast Ranges (results not shown, for plant from Chiles Valley, Napa County; both ITS and ETS clonal diversity is extensive), and central and southern

Sierra Nevada—is consistent with the hypothesis that *C. tinctoria* represents a species of homoploid hybrid origin rather than a recent participant in introgressive hybridization with other species. Presence of cpDNA haplotypes of each putative parental lineage across populations of *C. tinctoria* could reflect genetic contribution by reciprocal hybrids in the common ancestry of *C. tinctoria* or conceivably polyphyly of *C. tinctoria*, with separate hybrid speciation events based on similar parent lineages, as suggested for *Helianthus anomalus* (Schwarzbach and Rieseberg, 2002). Lack of nrDNA mutations diagnostic for resolved sublineages of *C. heterophylla* within sequences of *C. tinctoria* indicates that the variation and polymorphism observed in *C. tinctoria* are best explained by hybridization that predated the divergence of sampled members of *C. heterophylla* from a common ancestor ( $\leq 1.4$  [0.4–2.3] Ma; see Fig. 12B). Origin of *C. tinctoria* also apparently predated divergence of *C. bartsifolia* and *C. corymbosa* from a common ancestor, at  $\leq 1.4$  (0.3–1.5) Ma (Fig. 7), similar to the divergence time for *C. heterophylla* lineages.

The likelihood of recent introgression involving *C. tinctoria* appears low based on Garber and colleagues' finding that *C. tinctoria* was exceptionally difficult to hybridize to other sessile-flowered species of *Collinsia* except to the allopatric, coastal-dune endemic *C. corymbosa* (Garber, 1960, 1975; Ahloowalia and Garber, 1961; Bell and Garber, 1961). All crosses of *C. tinctoria* to *C. bartsifolia* failed and crosses to *C. heterophylla* yielded only shriveled, inviable seeds except for one that was germinated to produce a hybrid with abortive anthers and some fertile ovules (Bell and Garber, 1961).

Other evidence that may reflect a deep history of evolutionarily significant hybridization in *Collinsia* comes from conflicts in clade composition between cpDNA and nuclear trees. Although results from the partition homogeneity test indicated significant heterogeneity among cpDNA, nrDNA, and *CYCI*, clade support from trees based on analysis of individual regions (Figs. 8–10) indicate conflicts of only weak to moderate support based on (conservative) MP bootstrap values, primarily between cpDNA and nuclear trees. In particular, cpDNA trees (Fig. 9) indicate that the mostly sessile-flowered *C. bartsifolia*, *C. corymbosa*, *C. multicolor*, and *C. tinctoria* are more closely related to *C. greenei* and *C. sparsiflora* than to *C. antonina*, *C. concolor*, *C. heterophylla*, and *C. parryi*, in contrast to the nrDNA trees (Fig. 8) and to patterns of interfertility in *Collinsia* (assuming gradual decay of interfertility through lineages). In addition, cpDNA trees indicate that the pediceled-flowered species *C. linearis*, *C. rattanii*, and *C. torreyi* s.l. constitute a clade with *C. grandiflora*, *C. parviflora*, *C. verna*, and *C. violacea* rather than a basal grade of lineages in *Collinsia*, as in the *CYCI* trees (Fig. 10). Delayed lineage/allelic sorting or slow concerted evolution could contribute to the observed incongruities in clade composition. We also cannot rule out homoplasy as the sole cause of the deep-clade incongruities between trees based on each of the different gene regions; MP bootstrap support for one or both clades is below 90% in the cpDNA–nuclear DNA “conflicts” and below 80% in nrDNA–*CYCI* “conflicts”.

**Chromosome evolution**—Analysis of chromosome associations at meiosis I in a wide diversity of hybrid combinations involving taxa resolved here as a clade—the mostly sessile-flowered clade plus *C. greenei* and *C. sparsiflora*—allowed Garber and colleagues to propose distinct chromosome arrangements resulting from one or more reciprocal translocations (Garber, 1960; Ahloowalia and Garber, 1961; Bell and Garber,

1961; see Garber, 1975). Each taxon studied was proposed to differ from others by at least one chromosomal-arm interchange except for *C. concolor* and *C. heterophylla* s.s., which evidently shared the same arm arrangement. Recoding and mapping of the chromosome-arm arrangements as an ordered character (with number of interchanges between arrangements treated as steps) onto the all-partitions MCC tree (Fig. 7) optimized character-state changes to place the arm arrangement shared by *C. concolor* and *C. heterophylla* s.s. as unequivocally ancestral for the mostly sessile-flowered clade, with independent evolution of only one arm arrangement—for chromosomes 3 and 4—in *C. sparsiflora* and in *C. tinctoria*. Inclusion of the ordered chromosomal character in the combined-data phylogenetic analysis and assignment of a weight of  $\geq 5$  to that character resulted in placement of the *C. tinctoria* lineage between *C. sparsiflora* and *C. greenei* (Fig. 13), thereby eliminating the redundant interchange involving chromosomes 3 and 4; the overall genomic arrangement in *C. tinctoria* is one interchange removed from the *C. sparsiflora* var. *sparsiflora* arrangement and a different single interchange removed from the arrangement in *C. heterophylla* s.s.

The potential for convergent evolution of chromosome arrangements via hybridization may explain the presence of a chromosome arrangement in *C. tinctoria* that was not predicted based on the molecular trees and morphological considerations. Although *C. tinctoria* evidently descended from hybridization between ancestors of *C. heterophylla* and *C. bartsifolia* + *C. corymbosa* (see above), the arm-arrangements of chromosomes 3 and 4 in (sessile-flowered) *C. tinctoria* are like those in (pediceled-flowered) *C. sparsiflora* var. *sparsiflora*, as noted above, and unlike those of any of the other sessile-flowered taxa, including *C. bartsifolia*, *C. corymbosa*, and *C. heterophylla*. Dhillon and Garber (1962) reported the presence of “new” chromosome-arm arrangements in  $F_3$  hybrid lines involving sessile-flowered taxa, including *C. corymbosa* and *C. tinctoria*. A chromosome-arm arrangement otherwise known only from *C. multicolor* was recovered from a hybrid line from *C. corymbosa*  $\times$  *C. tinctoria*; a different hybrid line from *C. corymbosa*  $\times$  *C. multicolor* yielded plants with a chromosome-arm arrangement otherwise known only from *C. heterophylla* s.s. and *C. concolor* (see also Garber, 1975). Evolution of new chromosome arrangements associated with homoploid hybrid speciation and recurrent evolution of similar arrangements in artificial hybrid lines has been studied in great detail in *Helianthus* (Rieseberg et al., 1995, 1996) and warrants further investigation in *Collinsia*.

With or without weighting of the ordered chromosomal character in molecular phylogenetic analyses, one genomic arrangement was unequivocally reconstructed as evolving directly from a documented arrangement other than the one shared by *C. concolor* and *C. heterophylla* s.s., namely, the arrangement proposed for *C. sparsiflora* var. *collina*, which evidently evolved by two interchanges from the arrangement in *C. sparsiflora* var. *sparsiflora* (Fig. 13). Assuming that the arrangements proposed for taxa by Garber and colleagues are uniform throughout those taxa and were associated with initial evolutionary divergence, the presence of a chromosome-arm arrangement in var. *collina* that is evolutionarily derived relative to the arm arrangement in *C. sparsiflora* var. *sparsiflora* is evidence against descent of var. *sparsiflora* from an ancestor referable to var. *collina*. In other words, the chromosomal data are consistent with a large- to small-flowered evolutionary shift in *C. sparsiflora*, in contrast to the small- to large-flowered pattern

weakly resolved for *C. sparsiflora* in the expanded-taxon nrDNA + cpDNA tree (see Fig. 12B). Examples of taxa other than *C. sparsiflora* var. *collina* that are distinguished from the most chromosomally similar taxa by multiple interchanges are *C. greenei* (three interchanges), *C. heterophylla* “austromontana” (two interchanges), and *C. multicolor* (two interchanges) (Fig. 13).

Garber and colleagues also detected up to two paracentric inversions in hybrids between taxa of *Collinsia* (heterozygosity for each inversion was resolved by a dicentric bridge and acentric fragment at anaphase I), with no such inversion found to distinguish species now treated within the circumscription of *C. sparsiflora* (Garber, 1960; Ahloowalia and Garber, 1961). Absence of detected inversions in hybrids between *C. heterophylla* and *C. concolor* (Garber, 1960; Bell and Garber, 1961) is consistent with the interchange data and the close relationship resolved between those two species in the molecular trees. Overall, the inversion data are too preliminary to allow for firm phylogenetic conclusions.

**Cryptic diversity and phylogeography**—Garber and Unni’s (1965) suggestion that ecologically or intrinsically isolated “cryptic species” may occur within *Collinsia* gains support from our results. Although sequences of most traditionally recognized species or varieties either constituted clades or were unresolved within more inclusive clades, some taxa as recently treated appear to be nonmonophyletic or to contain internal phylogenetic structure corresponding to geographic or (minor) morphological variation. The most conspicuous examples of nonmonophyly involve widely disjunct, ecogeographically distinct populations that have been misassigned to the same species.

**A new Sierran clade**—Central Sierra Nevada *Collinsia* populations in the Merced River drainage assigned previously to *C. linearis*, a taxon otherwise known from the Klamath Ranges of the northwestern CA-FP, constitute an ancient lineage that is only distantly related to *C. linearis* based on all of the molecular data (Figs. 7–10, 12). The Sierran *Collinsia* appears to be an edaphic endemic, known from isolated populations on schist exposures (thus the informal name *C. “metamorphica”*), and contains morphologically, ecologically, and molecularly divergent lineages that warrant taxonomic recognition (to be described elsewhere). Noise-free ITS + ETS structure within the *C. “metamorphica”* clade and congruent cpDNA signal indicate bluish-purple- or magenta-flowered, mostly higher-elevation lineages and a white-flowered, lower-elevation lineage. The Sierran plants, which initially appeared to be a possible example of a recently diverged peripheral isolate comparable to the famous Merced drainage species *Clarkia lingulata* (Lewis and Roberts, 1956; Gottlieb, 1974), instead represent an ancient lineage that has undergone diversification within the Yosemite region. Confusion of the Sierran plants with *C. linearis* was evidently the result of convergent evolution in floral characters and the difficulty of interpreting subtle floral variation from (pressed) herbarium specimens.

**Klamath lineages of *Collinsia linearis***—Within *C. linearis* s.s., from the Klamath Ranges, two major lineages resolved with strong support from nrDNA and nrDNA + cpDNA analyses (Figs. 8 and 12) are geographically distinct and include another long-recognized taxon, *C. rattanii*. One lineage includes all samples of *C. linearis* from the Klamath River drainage,

where the type was collected; the other lineage includes all samples from more northerly populations (in Oregon) in addition to all sampled populations (from California and Oregon) referable to the smaller-flowered *C. rattanii*, which Newsom (1929) treated as conspecific with *C. linearis* (with recognition of *C. linearis* as a variety of *C. rattanii*). Most (but not all) of the sampled populations of *C. linearis* in the Klamath drainage have much paler corollas than those of populations of *C. linearis* sampled in the sister group. Although the large-flowered members of the two clades that constitute *C. linearis* appear to have remained geographically distinct since diverging  $\leq 1.9$  (0.5–3.9) Ma (see Fig. 12A), smaller-flowered populations referable to *C. rattanii* have evidently evolved and dispersed throughout and beyond the range of *C. linearis* during that time, in keeping with the general distributional patterns of closely related large- and small-flowered taxa in *Collinsia* (Randle et al., 2009).

A phylogeographic break in *C. linearis* near the northern limit of the Klamath River drainage approximates the pattern seen in some other plant taxa, such as the sword fern, *Polystichum munitum* (Kaulf.) C. Presl, and western white pine, *Pinus monticola* Douglas ex D. Don, each with northern and southern groups that break near the California–Oregon state borders (Steinhoff et al., 1983; Soltis et al., 1997). These and other north–south phylogeographic breaks in plants involving northwestern CA-FP and the Pacific Northwest have been attributed to Pleistocene vicariance (see Soltis et al., 1997), in accord with estimated timing of the divergence in *C. linearis* and other evidence for the Klamath Ranges serving as a glacial refugium (see Raven and Axelrod, 1978).

***Collinsia antonina***—*Collinsia antonina* is an example of a species that was described before our study (Hardham, 1964) but was of uncertain taxonomic status prior to molecular phylogenetic analysis. Neese (1993) treated *C. antonina* as a synonym of a long-recognized species, *C. parryi*, which is morphologically similar and geographically disjunct. Data from ETS, cpDNA, and *CYC1* sequences corroborate the conclusion of Baldwin and Armbruster (2002), based in part on the ITS results presented here, that the small-flowered, southern Californian *C. parryi*, previously treated within the “pediceled-flowered” group, is more closely related to the large- and “sessile-flowered,” southern Californian *C. concolor* than to the small- and pediceled-flowered, central California *C. antonina* (Figs. 7–10, 12B). As with *C. “metamorphica,” C. antonina* is evidently an edaphic endemic, known only from isolated populations on semibarren exposures (of silicious-shale talus).

**Transverse Range break in *Collinsia heterophylla***—The widespread, large-flowered *Collinsia heterophylla* contains phylogenetic structure that corresponds to a previously recognized phylogeographic boundary in California. Populations sampled north of the Transverse Ranges—in the Sierra Nevada, North Coast Ranges, and South Coast Ranges—constitute a robust nrDNA and nrDNA + cpDNA lineage; populations from southwestern California, in the Transverse and Peninsular ranges, constitute a set of basally unresolved lineages within *C. heterophylla* (Figs. 12B). Although the Transverse Ranges appear to contain an important phylogeographic boundary in a diversity of Californian animals (see Calsbeek, et al., 2003; Chatzimanolis and Caterino, 2007), examples of plants showing such phylogeographic structure are lacking.

Our results for *Collinsia heterophylla* do not appear to conform to a finer-scale phylogeographic boundary within the Transverse Ranges between the Sierra Pelona and San Gabriel Mountains, as seen in various animal groups (Chatzimanolis and Caterino, 2007). We did not resolve the short-bannered var. *austromontana*, described from the Eastern Transverse Ranges (i.e., San Gabriel and San Bernardino mountains) by Newsom (1929), as a monophyletic group, and one population referable to var. *austromontana*, from the San Bernardino Mountains (14 in Appendix 1), was identical in nrDNA and cpDNA sequences to a population referable to var. *heterophylla* from the western edge of the Central Transverse Ranges, at Wheeler Gorge (19 in Appendix 1), west of the Sierra Pelona. Our results instead indicate a phylogeographic break between the Transverse Ranges and South Coast Ranges. The northern population sampled closest to the Transverse Ranges, from the Sierra Madre in Santa Barbara County (population 18 in Appendix 1), was polymorphic for ITS copy types with either all diagnostic mutations of the northern clade or only a subset of those mutations (clone 6).

Estimated age of the Transverse–South Coast ranges split in *C. heterophylla* ( $\leq 1.2$  [0.2–1.5] Ma based on nrDNA + cpDNA data; see Fig. 12B)—and in *C. antonina* vs. *C. concolor* and *C. parryi* ( $\leq 2.1$  [1.2–3.1] Ma in the all-partitions chronogram; Fig. 7), discussed above—is in keeping with geological evidence for major Late Pliocene through Pleistocene uplift in these ranges (e.g., Page et al., 1998; Blythe et al., 2000; Duca et al., 2003; see Jacobs et al., 2004). Phylogeographic resolution in *C. heterophylla* in general does not correlate well with previously noted morphological variation, including striking variation in corolla coloration across populations.

**Southern Sierra Nevada break in *Collinsia wrightii***—Phylogeography of the small-flowered, montane taxon *Collinsia wrightii* [= *C. torreyi* var. *wrightii* (S. Watson) I. M. Johnston.] (Fig. 2) indicates another pattern relevant to a Transverse Range break. Both cpDNA and nrDNA trees resolve a basal split within *C. wrightii* between a lineage of the Western and Eastern Transverse Ranges plus the southernmost Sierra Nevada (Tulare and Kern counties) and a lineage of more northerly montane regions (central and northern Sierra Nevada, Klamath Ranges, High North Coast Ranges, and Warner Mountains). The main topological difference between cpDNA and nrDNA trees is the position of the only sample of *C. wrightii* referable to *C. torreyi* var. *brevicarinata*, from the type locality in the southern High Sierra Nevada, which is weakly placed within the northerly lineage in nrDNA trees and strongly placed in the southerly lineage in cpDNA trees; combined nrDNA + cpDNA data resolve var. *brevicarinata* as sister to southerly members of *C. wrightii*, from the Western and Eastern Transverse Ranges and the southern Sierra Nevada (Fig. 12A). The results from *C. wrightii* (including *C. torreyi* var. *brevicarinata*) again appear to reflect a somewhat different phylogeographic pattern than the bulk of animal phylogeographic studies, wherein a major break is often estimated to lie between the Central and Eastern Transverse Ranges (Chatzimanolis and Caterino, 2007), as noted above. As for *C. heterophylla*, timing of the phylogeographic break in *C. wrightii* ( $\leq 1.9$  [0.5–3.9] Ma; see Fig. 12) is in line with geological evidence for major uplift in the Sierra Nevada and Transverse Ranges during the Late Pliocene and Pleistocene (Blythe et al., 2000; Wakabayashi and Sawyer, 2001; see Jacobs et al., 2004).

**Other fine-scale phylogenetic structure**—*Collinsia grandiflora* + *C. parviflora* and the small-flowered *Tonella tenella* also warrant further exploration for cryptic diversity and phylogeographic boundaries. Studies of the *C. grandiflora* + *parviflora* clade may help to reveal cryptic diversity associated with polyploidy (E. Elle, Simon Fraser University, personal communication), which in *Collinsia* is otherwise known only from *C. torreyi*. Ganders and Krause (1986) found that populations of the *C. grandiflora* + *C. parviflora* clade in British Columbia, Canada (treated here in *C. parviflora*), are tetraploid, unlike previously studied populations (see Garber, 1975). We found that British Columbian populations of the clade are identical in nrDNA and cpDNA sequences to one another and to populations of robust *C. parviflora* from the Outer North Coast Ranges of California and to a population from central-western Oregon (Lane County) with flowers as large as those of *C. grandiflora* but falling outside the well-supported *C. grandiflora* clade (Fig. 12A) and reportedly tetraploid (E. Elle, personal communication). *Tonella tenella* also contained phylogenetic structure, with a population from southern Oregon (Rogue River Valley) placed sister to a well-supported lineage including populations from California's North Coast Ranges and the San Francisco Bay Area (Fig. 12A), which may be comparable to phylogeographic patterns resolved for *C. linearis* and *C. rattanii* (see above).

**Edaphic factors**—Taxa endemic to a limited range of harsh substrates are widely dispersed across lineages of *Collinsia* and have evidently evolved repeatedly in California. Three such edaphic endemics are the most narrowly distributed species or terminal clades in *Collinsia* (*C. antonina*, *C. corymbosa*, and the *C.* “metamorphica” complex) and therefore might be suspected to be of relatively recent origin compared to more widespread taxa. Based on the molecular trees, however, none of these narrow endemics is nested phylogenetically within another taxon and each belongs to a stem lineage that diverged prior to closely related taxa that are not as geographically or edaphically restricted. *Collinsia antonina*, endemic to silicious-shale talus in the Outer South Coast Ranges of California, and *C. corymbosa*, endemic to coastal dunes in northern California, each have sister lineages that underwent divergence into large- and small-flowered taxa after splitting from a MRCA with the edaphic endemics: *C. concolor* and *C. parryi* (in the sister group of *C. antonina*) and *C. bartsifolia* var. *bartsifolia* and *C. bartsifolia* var. *davidsonii* (in the sister group of *C. corymbosa*). The *C.* “metamorphica” complex, endemic to quartzite-rich schist in the Central High Sierra Nevada, appears to be older than *C. antonina* or *C. corymbosa*, both in timing of divergence from a MRCA with other taxa and in crown age (1.1 [0.3–1.3] Ma; see Fig. 12B), in keeping with resolution of morphologically and ecologically distinct lineages within that Sierran complex (unlike in *C. antonina* or *C. corymbosa*).

*Collinsia greenei* and *C. sparsiflora* are members of the principal clade in *Collinsia* that has invaded seasonally xeric, low-elevation habitats in California (see below) and represent different stages of serpentine adaptation. *Collinsia greenei*, a serpentine endemic of California's North Coast Ranges, is estimated to have diverged from a common ancestor with living relatives since the Late Miocene or Pliocene (Fig. 7), in accord with the age of serpentine exposures in the region (Harrison et al., 2004) and with intersterility of *C. greenei* and other members of *Collinsia* (see Garber, 1975). *Collinsia sparsiflora* occurs mostly on nonserpentine soils across much of the central

and northern CA-FP, with genetically and phenotypically distinct serpentine and nonserpentine populations in the Knoxville region of the Inner North Coast Ranges (Wright et al., 2006a, b; Wright and Stanton, 2007). The presumed recent timeframe for initial, ongoing divergence between serpentine and nonserpentine ecotypes of *C. sparsiflora* var. *sparsiflora* is reflected by a lack of lineage structure across samples of these populations in our trees based on nrDNA and cpDNA mutations.

**Broad-scale biogeographic and ecological history**—Parsimony-based mapping of distributional history onto the all-partitions MCC tree (Fig. 7) indicates a general pattern of north-to-south and west-to-east dispersal in the ingroup clade, with the MRCA of *Collinsia* and *Tonella* in either the Pacific Northwest or northwestern CA-FP and the MRCA of *Collinsia* in the northwestern CA-FP. Wolfe et al. (2002) inferred the Klamath Ranges of the northwestern CA-FP as a probable place of origin for the MRCA of the tribe that includes *Collinsia* (Cheloneae), as well, based on molecular phylogenetic and distributional considerations.

The longest dispersal event in *Collinsia* that led to diversification of modern species evidently occurred from the northwestern CA-FP to central-eastern North America (Fig. 12A), where the sister-species *C. verna* and *C. violacea* are endemic and diverged since the Pliocene ( $\leq 3.6$  [1.6–5] Ma; Fig. 7). Dispersal to the eastern half of North America from the northwestern CA-FP was inferred by Wolfe et al. (2002) for the common ancestor of the closely related *Chelone* as well and is consistent with evidence for long-term occurrence of mesic conditions (including some summer rain) in the Klamath Ranges of the northwestern CA-FP (Raven and Axelrod, 1978). The possibility that the sister clade of *C. verna* and *C. violacea* (namely, the *C. grandiflora* + *C. parviflora* clade) may have originated in central or eastern North America and dispersed west is consistent with the basally divergent position of central-eastern samples of *C. parviflora* in the expanded taxon nrDNA + cpDNA trees (Fig. 12A) and warrants further study, with additional sampling of the *C. grandiflora* + *C. parviflora* clade throughout its wide range.

An ecological shift since the Late Miocene or Pliocene ( $\leq 4.8$  [3.8–6.6] Ma; Fig. 7) from montane to low-elevation, seasonally xeric habitats was mapped to the base of the clade that contains all of the sessile-flowered taxa of *Collinsia* plus *C. antonina*, *C. greenii*, *C. parryi*, and *C. sparsiflora* (results not shown). On the basis of parsimony mapping, the primarily low-elevation clade descended either from a MRCA in the northwestern CA-FP (Fig. 12B) or Sierra Nevada, with subsequent colonization of more xeric central-western and southwestern California. All taxa of the low-elevation clade have occurrences well below 500 m in elevation and only the evidently hybrid species *C. tinctoria* in that clade reaches altitudes above 2000 m a.s.l. Other members of *Collinsia* that occur within a similar elevational range are found in more mesic environments of the northwestern CA-FP. The estimated timing of origin of the low-elevation clade is consistent with the time frame for development of Mediterranean-climatic conditions in the CA-FP (Axelrod, 1973; Graham, 1999).

**Taxonomic implications**—Our results indicate the need for some taxonomic changes in *Collinsia* to address problems in taxon circumscription, rank, and position. Minimally, members of the *C.* “metamorphica” clade must be treated outside *C. linearis*, and no names are currently available to do so (M. S. Park, B. G. Baldwin, and W. S. Armbruster, unpublished manuscript). *Collinsia linearis* may be best treated in an even narrower sense to exclude

populations north of the Klamath River drainage that are evidently more closely related to *C. rattanii* than to typical *C. linearis*.

*Collinsia sparsiflora*, as broadly circumscribed here, has been treated variously as a complex of several species or varieties that vary in flower size, flower shape, and inflorescence architecture, and additional variation has been found to be associated with recent evolution of serpentine ecotypes (Wright et al., 2006a, b). Tentatively, we retain recognition of var. *collina* and var. *sparsiflora* for small- and large-flowered populations that pass the test of sympatry and justifiably could be regarded as separate species, although phylogenetic resolution is lacking. Difficulty in resolving relationships within *C. sparsiflora* leaves open the question of whether floral characters associated with each of the two taxa have evolved independently in different lineages within the complex.

Our sampling to date of the *C. grandiflora* + *C. parviflora* clade suggests problems with the current taxonomy that may reflect lability in evolution of flower size, which has been a key character for distinguishing the two taxa. More sampling across the exceptionally broad geographic distribution of the *C. grandiflora* + *C. parviflora* clade, including ongoing studies of ploidy (E. Elle, Simon Fraser University, personal communication), should help to refine classification of taxa in the group.

In *C. torreyi* s.l., three taxa usually treated as varieties (var. *latifolia*, var. *torreyi*, and var. *wrightii*) represent lineages that evidently diverged prior to diversification of the largely sessile-flowered clade in *Collinsia* (Fig. 7). Sympatry between *C. torreyi* var. *latifolia* and *C. wrightii* (= *C. torreyi* var. *wrightii*) and between *C. torreyi* s.s. (= *C. torreyi* var. *torreyi*) and *C. wrightii* is widespread, and hybrids are unknown. Recognition of *C. torreyi* var. *latifolia*, *C. torreyi* s.s., and *C. wrightii* (now including *C. torreyi* var. *brevicarinata*) as different species is therefore warranted, and a new combination at species rank is needed for var. *latifolia*:

***Collinsia latifolia*** (Newsom) B. G. Baldwin, Kalisz & Armbr., comb. et stat. nov.—*Collinsia torreyi* A. Gray var. *latifolia* Newsom, Botanical Gazette 87: 299. 1929. TYPE: USA, Oregon, Ashland Butte, *Cusick* 2893 (holotype: POM (no. 42879)!; isotype: GH).

**Conclusions**—Diversity in *Collinsia* is greater than previously documented and includes narrowly endemic lineages that appear vulnerable to habitat loss and warrant conservation attention. Recently diverged lineages in *Collinsia* are often associated with distinct habitats, including harsh substrates (e.g., serpentine), and/or geographic boundaries that have been implicated in evolutionary divergence in other organisms as consequences of Plio-Pleistocene geoclimatic factors, such as mountain building and glaciation. Repeated shifts in flower size, mostly in the direction from larger to smaller flowers, have also characterized evolution of *Collinsia* (and *Tonella*), as has chromosome evolution by structural rearrangements. Rapid evolution of strong intersterility barriers evidently has not been a dominant theme in *Collinsia* evolution and has left open the potential for homoploid hybrid speciation, which appears to have occurred in the evolution of *C. tinctoria*.

#### LITERATURE CITED

- AHLOOWALIA, B. S., AND E. D. GARBER. 1961. The genus *Collinsia*. XIII. Cytogenetic studies of interspecific hybrids involving species with pediceled flowers. *Botanical Gazette* 122: 219–228.
- AKAIKE, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.

- ARMBRUSTER, W. S., C. P. H. MULDER, B. G. BALDWIN, S. KALISZ, B. WESSA, AND H. NUTE. 2002. Comparative analysis of late floral development and mating-system evolution in tribe Collinsieae (Scrophulariaceae s.l.). *American Journal of Botany* 89: 37–49.
- AXELROD, D. I. 1973. History of the Mediterranean ecosystem in California. In F. di Castri and H. A. Mooney [eds.], *Mediterranean type ecosystems: Origin and structure*, 225–277. Springer, Berlin, Germany.
- AXELROD, D. I. 1992. Miocene floristic change at 15 Ma, Nevada to Washington, U.S.A. *Palaeobotanist* 41: 234–239.
- BALDWIN, B. G., AND W. S. ARMBRUSTER. 2002. *Collinsia antonina* is evolutionarily distinct from *C. parryi* (Scrophulariaceae sensu lato). *Madroño* 49: 295–297.
- BALDWIN, B. G., AND S. MARKOS. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: Congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* 10: 449–463.
- BALDWIN, B. G., AND M. J. SANDERSON. 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proceedings of the National Academy of Sciences, USA* 95: 9402–9406.
- BALDWIN, B. G., AND B. L. WESSA. 2000. Origin and relationships of the tarweed–silversword lineage (Compositae–Madiinae). *American Journal of Botany* 87: 1890–1908.
- BELL, S. L., AND E. D. GARBER. 1961. The genus *Collinsia*. XII. Cytogenetic studies of interspecific hybrids involving species with sessile flowers. *Botanical Gazette* 122: 210–218.
- BLYTHE, A. E., D. W. BURBANK, K. A. FARLEY, AND E. J. FIELDING. 2000. Structural and topographic evolution of the central Transverse Ranges, California, from apatite fission-track, (U-Th)/He and digital elevation model analyses. *Basin Research* 12: 97–114.
- CALSBECK, R., J. N. THOMPSON, AND J. E. RICHARDSON. 2003. Patterns of molecular evolution and diversification in a biodiversity hotspot: The California Floristic Province. *Molecular Ecology* 12: 1021–1029.
- CHARLESWORTH, D., AND S. MAYER. 1995. Genetic variability of plant characters in the partial inbreeder *Collinsia heterophylla* (Scrophulariaceae). *American Journal of Botany* 82: 112–120.
- CHATZIMANOLIS, S., AND M. S. CATERINO. 2007. Toward a better understanding of the “Transverse Range Break”: Lineage diversification in southern California. *Evolution* 61: 2127–2141.
- DHILLON, T. S. 1975. The genus *Collinsia*. XXXI. Cytogenetics of a spontaneous reciprocal translocation in *C. parryi*. *Botanical Gazette* 136: 27–29.
- DHILLON, T. S., AND E. D. GARBER. 1962. The genus *Collinsia*. XIX. “New” genomes from two fertile interspecific hybrids. *Cytologia* 27: 189–203.
- DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- DRUMMOND, A. J., S. Y. W. HO, M. J. PHILLIPS, AND A. RAMBAUT. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biology* 4: e88.
- DRUMMOND, A. J., AND A. RAMBAUT. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214.
- DUCEA, M., M. A. HOUSE, AND S. KIDDER. 2003. Late Cenozoic denudation and uplift rates in the Santa Lucia Mountains. *California Geology* 31: 139–142.
- ELLE, E. 2004. Floral adaptations and biotic and abiotic selection pressures. In Q. C. B. Cronk, J. Whitton, R. H. Ree, and I. E. P. Taylor [eds.], *Plant adaptation: Molecular genetics and ecology*, 111–118. Proceedings of an international workshop held 11–13 December 2002, in Vancouver, British Columbia, Canada. NRC Research Press, Ottawa, Ontario, Canada.
- ELLE, E., AND R. CARNEY. 2003. Reproductive assurance varies with flower size in *Collinsia parviflora* (Scrophulariaceae). *American Journal of Botany* 90: 888–896.
- FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1995. Constructing a significance test for incongruence. *Systematic Biology* 44: 570–572.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791.
- FLOWER, B. P., AND J. P. KENNETT. 1994. The middle Miocene climatic transition: East Antarctic ice sheet development, deep ocean circulation and global carbon cycling. *Palaeogeography, Palaeoclimatology, Palaeoecology* 108: 537–555.
- GANDERS, F. R., AND G. R. KRAUSE. 1986. Systematics of *Collinsia parviflora* and *C. grandiflora* (Scrophulariaceae). *Madroño* 33: 63–70.
- GARBER, E. D. 1956. The genus *Collinsia*. I. Chromosome number and chiasma frequency of species in the two sections. *Botanical Gazette* 118: 71–73.
- GARBER, E. D. 1958. The genus *Collinsia*. VII. Additional chromosome numbers and chiasmata frequencies. *Botanical Gazette* 120: 55–56.
- GARBER, E. D. 1960. The genus *Collinsia*. IX. Speciation and chromosome repatterning. *Cytologia* 25: 233–243.
- GARBER, E. D. 1975. *Collinsia*. In R. C. King [ed.], *Handbook of genetics*, vol. 2. Plants, plant viruses, and protists, 333–359. Plenum, New York, New York, USA.
- GARBER, E. D., AND S. L. BELL. 1962. The genus *Collinsia*. XV. A cytogenetic study of three fertile interspecific hybrids with an interchange complex of six chromosomes. *Botanical Gazette* 123: 190–197.
- GARBER, E. D., AND T. S. DHILLON. 1962. The genus *Collinsia*. XX. Cytogenetic studies of interspecific hybrids. *Botanical Gazette* 123: 291–298.
- GARBER, E. D., AND J. GORSIC. 1956. The genus *Collinsia*. II. Interspecific hybrids involving *C. heterophylla*, *C. concolor*, and *C. sparsiflora*. *Botanical Gazette* 118: 73–77.
- GARBER, E. D., AND M. K. UNNI. 1965. The genus *Collinsia*. XXIV. Taxonomic status of *C. stricta* Greene. *Botanical Gazette* 126: 133–136.
- GOTTLIEB, L. D. 1974. Genetic confirmation of the origin of *Clarkia linguata*. *Evolution* 28: 244–250.
- GRAHAM, A. 1999. Late Cretaceous and Cenozoic history of North American vegetation north of Mexico. Oxford University Press, Oxford, UK.
- GRANT, V. 1956. Chromosome repatterning and adaptation. *Advances in Genetics* 8: 89–107.
- GRAY, A. 1880. *Collinsia*. In J. D. Whitney, *Botany*, vol. 1, 2nd ed., Geological Survey of California, 552–555. Little, Brown, and Co., Boston, Massachusetts, USA.
- GRAY, A. 1886. Synoptical flora of North America, vol. 2, part 1, 2nd ed. Ivison, Blakeman, Taylor, and Co., New York, New York, USA.
- GREENE, E. L. 1887. A curious *Collinsia*. *Pittonia* 1: 52–55.
- GREENLEE, J. K., AND K. S. RAI. 1986. Interspecific hybridization and karyotype analysis in two species in the genus *Collinsia*. *Cytologia* 51: 631–636.
- HARDHAM, C. B. 1964. A new *Collinsia* from Monterey County, California. *Leaflets of Western Botany* 10: 133–135.
- HARRISON, S., H. D. SAFFORD, AND J. WAKABAYASHI. 2004. Does the age of exposure of serpentine explain variation in endemic plant diversity in California? *International Geology Review* 46: 235–242.
- HAYHOME, B. A., AND E. D. GARBER. 1968. The genus *Collinsia*. XXIX. Preferential pairing in diploid, triploid, and tetraploid interspecific hybrids involving *C. stricta* × *C. concolor* and related species. *Cytologia* 33: 246–255.
- HICKMAN, J. C. [ed.] 1993. *The Jepson manual: Higher plants of California*. University of California Press, Berkeley, California, USA.
- HILEMAN, L. C., AND D. A. BAUM. 2003. Why do paralogs persist? Molecular evolution of *CYCLOIDEA* and related floral symmetry genes in Antirrhineae (Veronicaceae). *Molecular Biology and Evolution* 20: 591–600.
- HILEMAN, L. C., E. M. KRAMER, AND D. A. BAUM. 2003. Differential regulation of symmetry genes and the evolution of floral morphologies. *Proceedings of the National Academy of Sciences, USA* 100: 12814–12819.
- HIORTH, G. 1933. Genetische versuche mit *Collinsia*. IV. Die analyse eines nahezu sterilen art-bastardes. 1. Die diploiden bastarde zwischen *Collinsia bicolor* und *C. bartsiaefolia*. 2. Die polyploiden bastarde zwischen *Collinsia bicolor* und *C. bartsiaefolia*. *Zeitschr Indukt Abstamm U Vererbungslehre* 66: 106–157, 245–274.

- HODGES, S. A., AND M. L. ARNOLD. 1995. Spurring plant diversification: Are floral nectar spurs a key innovation? *Proceedings of the Royal Society of London. Series B. Biological Sciences* 262: 343–348.
- JACOBS, D. K., T. A. HANEY, AND K. D. LOUIE. 2004. Genes, diversity, and geologic process on the Pacific Coast. *Annual Review of Earth and Planetary Sciences* 32: 601–652.
- JEPSON, W. L. 1925. A manual of the flowering plants of California. Associated Students Store, Berkeley, California, USA.
- JOHNSON, L. A., AND D. E. SOLTIS. 1994. *marK* DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. *Systematic Botany* 19: 143–156.
- KALISZ, S. 1989. Fitness consequences of mating system, seed weight, and emergence date in a winter annual, *Collinsia verna*. *Evolution* 43: 1263–1272.
- KALISZ, S., AND D. W. VOGLER. 2003. Benefits of autonomous selfing under unpredictable pollinator environments. *Ecology* 84: 2928–2942.
- KALISZ, S., D. VOGLER, B. FAILS, M. FINER, E. SHEPARD, T. HERMAN, AND R. GONZALES. 1999. The mechanism of delayed selfing in *Collinsia verna* (Scrophulariaceae). *American Journal of Botany* 86: 1239–1247.
- KALISZ, S., D. W. VOGLER, AND K. M. HANLEY. 2004. Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature* 430: 884–887.
- KAY, K. M., J. B. WHITTALL, AND S. A. HODGES. 2006. A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: An approximate molecular clock with life history effects. *BMC Evolutionary Biology* 6: 36.
- KENNEDY, B. F., AND E. ELLE. 2008a. The inbreeding depression cost of selfing: Importance of flower size and population size in *Collinsia parviflora* (Veronicaceae). *American Journal of Botany* 95: 1596–1605.
- KENNEDY, B. F., AND E. ELLE. 2008b. The reproductive assurance benefit of selfing: Importance of flower size and population size. *Oecologia* 155: 469–477.
- LANKINEN, Å. 2009. Upper petal lip colour polymorphism in *Collinsia heterophylla* (Plantaginaceae): Genetic basis within a population and its use as a genetic marker. *Journal of Genetics* 88: 205–215.
- LANKINEN, Å., AND W. S. ARMBRUSTER. 2007. Pollen competition reduces inbreeding depression in *Collinsia heterophylla* (Plantaginaceae). *Journal of Evolutionary Biology* 20: 737–749.
- LANKINEN, Å., W. S. ARMBRUSTER, AND L. ANTONSEN. 2007. Delayed stigma receptivity in *Collinsia heterophylla* (Plantaginaceae): Genetic variation and possible adaptive significance in relation to pollen competition, delayed self-pollination, and mating-system evolution. *American Journal of Botany* 94: 1183–1192.
- LANKINEN, Å., AND S. KIBOI. 2007. Pollen donor identity affects timing of stigma receptivity in *Collinsia heterophylla* (Plantaginaceae): A sexual conflict during pollen competition? *American Naturalist* 170: 854–863.
- LANKINEN, Å., J. MAAD, AND W. S. ARMBRUSTER. 2009. Pollen-tube growth rates in *Collinsia heterophylla* (Plantaginaceae): One-donor crosses reveal heritability but no effect on sporophytic-offspring fitness. *Annals of Botany* 103: 941–950.
- LAU, J. A., A. C. MCCALL, K. F. DAVIES, J. K. MCKAY, AND J. W. WRIGHT. 2008. Herbivores and edaphic factors constrain the realized niche of a native plant. *Ecology* 89: 754–762.
- LEWIS, H., AND M. R. ROBERTS. 1956. The origin of *Clarkia lingulata*. *Evolution* 10: 126–138.
- MADDISON, W. P., AND D. R. MADDISON. 2009. Mesquite: A modular system for evolutionary analysis, version 2.6. Website <http://mesquiteproject.org>.
- MADJIDIAN, J. A., AND A. LANKINEN. 2009. Sexual conflict and sexually antagonistic coevolution in an annual plant. *PLoS ONE* 4: e5477.
- MAYER, S. S., D. CHARLESWORTH, AND B. MEYERS. 1996. Inbreeding depression in four populations of *Collinsia heterophylla* Nutt. (Scrophulariaceae). *Evolution* 50: 879–891.
- MUNZ, P. 1968. A California flora, with supplement. University of California Press, Berkeley, California, USA.
- NEESE, E. C. 1993. *Collinsia*. In J. C. Hickman [ed.], *The Jepson manual: Higher plants of California*, 1024–1027. University of California Press, Berkeley, California, USA.
- NEWSOM, V. M. 1929. A revision of the genus *Collinsia* (Scrophulariaceae). *Botanical Gazette* 87: 260–301.
- NYLANDER, J. A. A. 2004. MrModeltest 2.3. Computer program and documentation distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.
- PAGE, B. M., G. A. THOMPSON, AND R. G. COLEMAN. 1998. Late Cenozoic tectonics of the central and southern coast ranges of California. *Geological Society of America Bulletin* 110: 846.
- PARACHNOWITSCH, A. L., AND E. ELLE. 2004. Variation in sex allocation and male–female trade-offs in *Collinsia parviflora* (Scrophulariaceae s.l.). *American Journal of Botany* 91: 1200–1207.
- PENNELL, F. W. 1951. *Collinsia*. In L. Abrams, *Illustrated flora of the Pacific states: Washington, Oregon, and California*, vol. 3, 774–782. Stanford University Press, Stanford, California, USA.
- POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: Testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
- RAMBAUT, A., AND A. J. DRUMMOND. 2009. Tracer v1.5. Computer program and documentation distributed by the authors. Website <http://tree.bio.ed.ac.uk/software/tracer/> [accessed 22 December 2009].
- RANDLE, A. M. 2009. Ecological and evolutionary factors that influence species boundaries in *Collinsia*. Ph.D. dissertation, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.
- RANDLE, A. M., J. B. SLYDER, AND S. KALISZ. 2009. Can differences in autonomous selfing ability explain range size among sister-taxa pairs of *Collinsia* (Plantaginaceae)? An extension of Baker's Law. *New Phytologist* 183: 618–629.
- RAVEN, P. H., AND D. I. AXELROD. 1978. Origin and relationships of the California flora. *University of California Publications in Botany* 72: 1–134.
- RIESEBERG, L. H., B. SINERVO, C. R. LINDER, M. UNGERER, AND D. M. ARIAS. 1996. Role of gene interactions in hybrid speciation: Evidence from ancient and experimental hybrids. *Science* 272: 741–745.
- RIESEBERG, L. H., C. VAN FOSSEN, AND A. M. DESROCHERS. 1995. Hybrid speciation accompanied by genomic reorganization in wild sunflowers. *Nature* 375: 313–316.
- RONQUIST, F., AND J. P. HUELSENBECK. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- RUST, R. W., AND S. L. CLEMENT. 1977. Entomophilous pollination of the self-compatible species *Collinsia sparsiflora* Fisher and Meyer. *Journal of the Kansas Entomological Society* 50: 37–48.
- SANDERSON, M. J., AND M. J. DONOGHUE. 1994. Shifts in diversification rate with the origin of angiosperms. *Science* 264: 1590–1593.
- SCHECHTER, S. P., AND T. D. BRUNS. 2008. Serpentine and non-serpentine ecotypes of *Collinsia sparsiflora* associate with distinct arbuscular mycorrhizal fungal assemblages. *Molecular Ecology* 17: 3198–3210.
- SCHROCK, G. F., AND B. F. PALSER. 1967. Floral development, anatomy, and embryology of *Collinsia heterophylla* with some notes on ten other species of *Collinsia* and on *Tonella tenella*. *Botanical Gazette* 128: 83–104.
- SCHWARZBACH, A. E., AND L. H. RIESEBERG. 2002. Likely multiple origins of a diploid hybrid sunflower species. *Molecular Ecology* 11: 1703–1715.
- SEGRAVES, K. A., AND J. N. THOMPSON. 1999. Plant polyploidy and pollination: Floral traits and insect visits to diploid and tetraploid *Heuchera grossulariifolia*. *Evolution* 53: 1114–1127.
- SIMMONS, M. P. 2004. Independence of alignment and tree search. *Molecular Phylogenetics and Evolution* 31: 874–879.
- SOLTIS, D. E., M. A. GITZENDANNER, D. D. STRENGE, AND P. S. SOLTIS. 1997. Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Plant Systematics and Evolution* 206: 353–373.
- STANLEY, S. M. 1979. *Macroevolution—Pattern and process*. W. H. Freeman, San Francisco, California, USA.
- STEBBINS, G. L. 1957. Self fertilization and population variability in higher plants. *American Naturalist* 91: 337–354.
- STEELE, K. P., AND R. VILGALYS. 1994. Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene *matK*. *Systematic Botany* 19: 126–142.

- STEINHOFF, R. J., D. G. JOYCE, AND L. FINS. 1983. Isozyme variation in *Pinus monticola*. *Canadian Journal of Forest Research* 13: 1122–1132.
- SWOFFORD, D. L. 2002. PAUP\*: Phylogenetic analysis using parsimony (\*and other methods), version 4.0b10. Sinauer, Sunderland, Massachusetts, USA.
- VIEIRA, C. P., J. VIEIRA, AND D. CHARLESWORTH. 1999. Evolution of the cycloidea gene family in *Antirrhinum* and *Misopates*. *Molecular Biology and Evolution* 16: 1474–1483.
- WAKABAYASHI, J., AND T. L. SAWYER. 2001. Stream incision, tectonics, uplift, and evolution of topography of the Sierra Nevada, California. *Journal of Geology* 109: 539–562.
- WEIL, J., AND R. W. ALLARD. 1964. The mating system and genetic variability in natural populations of *Collinsia heterophylla*. *Evolution* 18: 515–525.
- WOLFE, A. D., S. L. DATWYLER, AND C. P. RANDLE. 2002. A phylogenetic and biogeographic analysis of the Cheloneae (Scrophulariaceae) based on ITS and *matK* sequence data. *Systematic Botany* 27: 138–148.
- WRIGHT, J. W., K. F. DAVIES, J. A. LAU, A. C. MCCALL, AND J. K. MCKAY. 2006a. Experimental verification of ecological niche modeling in a heterogeneous environment. *Ecology* 87: 2433–2439.
- WRIGHT, J. W., AND M. L. STANTON. 2007. *Collinsia sparsiflora* in serpentine and nonserpentine habitats: Using F2 hybrids to detect the potential role of selection in ecotypic differentiation. *New Phytologist* 173: 354–366.
- WRIGHT, J. W., M. L. STANTON, AND R. SCHERSON. 2006b. Local adaptation to serpentine and non-serpentine soils in *Collinsia sparsiflora*. *Evolutionary Ecology Research* 8: 1–21.
- YANG, Z., AND B. RANNALA. 1997. Bayesian phylogenetic inference using DNA sequences: A Markov chain Monte Carlo method. *Molecular Biology and Evolution* 14: 717–724.

APPENDIX 1. Voucher information and GenBank accession numbers for *Collinsia*, *Tonella*, and outgroup taxa sampled in this study. Californian collections are deposited at JEPS unless otherwise specified; non-Californian collections are at UC. Voucher information is presented in alphabetical order by taxon and within taxa, by state and county occurrences. Sequential numbering of voucher entries corresponds to numbers following taxon names in figures. GenBank accession numbers for DNA sequences are given after voucher information in the following order: ITS (internal transcribed spacer region=ITS-1, 5.8S subunit, ITS-2), ETS (3' end of external transcribed spacer upstream of 18S gene), 3' *matK*/3' *trnK* intron, and *CYC1* (*CYCLOIDEA-1*). Abbreviations: AMR = April M. Randle, BGB = Bruce G. Baldwin, Co. = County, CR = Christian Richey, ECN = Elizabeth C. Neese, MSP = Michael S. Park, SK = Susan Kalisz, WSA = W. Scott Armbruster.

**Taxon:** Collection location (voucher entry), *voucher*, GenBank accessions: ITS, ETS, 3' *matK*/3' *trnK* intron, *CYC1*.

- Collinsia antonina*** Hardham: USA, California, *Monterey Co.*, Outer South Coast Ranges, San Antonio Hills (1) *ECN 21500*, HQ653130, HQ653326, HQ653529, —, (2) Fort Hunter Liggett Military Reservation, E side of County Road G14, ca. 0.2 km N of southern Reservation boundary, *MSP*, *WSA*, *BGB*, & *CR 1063*, HQ653131, HQ653327, HQ653530, HQ653394, (3) Fort Hunter Liggett, Sulphur Springs Road, 3.9 road km N from Infantry Road junction, *MSP* & *BGB 930*, HQ653132, HQ653328, HQ653531, —.
- C. bartsiiifolia*** Benth. **var. *bartsiiifolia***: USA, California, (1) *Lake Co.*, Inner North Coast Ranges, Clear Lake vicinity, S of Lakeport, *BGB* & *WSA 858*, AF385344, HQ653267, HQ653479, —; *Santa Cruz Co.*, Outer South Coast Ranges, Santa Cruz Mountains, Sandhills, Ben Lomond vicinity, (2) Quail Hollow Ranch, *J. McGraw s.n.*, HQ653072, HQ653271, HQ653483, HQ653384, (3) Olympia Wellfield, *J. McGraw s.n.*, HQ653073, HQ653272, HQ653484, —.
- C. bartsiiifolia*** Benth. **var. *davidsonii*** (Parish) Newsom: USA, California, (1) *Madera Co.*, = *C. stricta* Greene, Central Sierra Nevada Foothills, Wahlberg Ranch, *B. Brock 401* (UC), HQ653074, HQ653273, HQ653485, —; *Monterey Co.*, Outer South Coast Ranges, (2) Fort Hunter Liggett, 3.2 km W of Cosio Knob, *E. Painter* & *ECN HL2546*, HQ653069, HQ653268, HQ653480, —, (3) Fort Hunter Liggett, Mission Road, Training Area 6, *MSP 1212*, HQ653070, HQ653269, HQ653481, —; (4) *San Benito Co.*, Inner South Coast Ranges, Coalinga Road, *BGB*, *WSA*, & *MSP 1378*, HQ653071, HQ653270, HQ653482, —; (5) *Santa Barbara Co.*, Outer South Coast Ranges, below Santa Barbara Canyon, *BGB* & *S. J. Bainbridge 1033*, HQ653075, HQ653274, HQ653486, —.
- C. callosa*** Parish: USA, California, (1) *Kern Co.*, Western Transverse Ranges, NW side of Cerro Noroeste, *BGB* & *WSA 947*, AF385354, HQ653250, HQ653462, HQ653375; (2) High Southern Sierra Nevada, Piute Mountain Road, ~4.8 km from Bodfish Road, *D. Gowen 356*, HQ653055, HQ653252, HQ653464, —; (3) *San Bernardino Co.*, San Bernardino Mountains, near Hesperia, *A. Lankinen 03-01*, HQ653054, HQ653251, HQ653463, HQ653374.
- C. childii*** Parry ex A. Gray: USA, California, (1) *Fresno Co.*, Southern High Sierra Nevada, E edge of Pinehurst, *ECN 21501*, AF385355, HQ653253, HQ653465, —; (2) *Mariposa Co.*, Central High Sierra Nevada, Iron Creek, *MSP 1608*, HQ653057, HQ653255, HQ653467, —; (3) *Monterey Co.*, Outer South Coast Ranges, Santa Lucia Range, Chew's Ridge, *BGB* & *WSA 1201a*, HQ653060, HQ653258, HQ653470, —; (4) *San Bernardino Co.*, San Bernardino Mountains, along State Route 138, 0.3 road km S of Old Mill Road (near town of Crestline), *MSP 1840*, HQ653059, HQ653257, HQ653469, —; (5) *San Diego Co.*, Peninsular Ranges, Palomar Mountain, Fry Creek Campground, *MSP 1825*, HQ653058, HQ653256, HQ653468, —; (6) *Santa Barbara Co.*, Outer South Coast Ranges, Sierra Madre Ridge, Bates Canyon, *BGB*, *WSA*, & *SK 1375*, HQ653056, HQ653254, HQ653466, HQ653377–HQ653379 (*CYC1* clones 1, 2, 4).
- C. concolor*** Greene: USA, California, *Riverside Co.*, Peninsular Ranges (1) W edge San Jacinto Mountains, S-SE of Hemet (Sage Road/Red Mountain Road intersection), *ECN 21539A*, individual 1, AF385350, HQ653331, HQ653534, —, individual 2, HQ653137, HQ653335, HQ653538, HQ653397 (*CYC1* clones 1, 4, 5), (2) San Jacinto Mountains, near Valle Vista, *A. Lankinen 03-03*, HQ653134, HQ653332, HQ653535, HQ653396, (3) Little Thomas Mountain, *MSP 2030*, HQ653138, HQ653336, HQ653539, —; *San Diego Co.*, Peninsular Ranges, (4) S of Mesa Grande, Black Canyon, *MSP 1817*, HQ653135, HQ653333, HQ653536, —, (5) Walker Canyon Ecological Preserve (near Boulevard), *WSA s.n.* (19 Apr 2009), HQ653136, HQ653334, HQ653537, —.
- C. corymbosa*** Herder: USA, California, *Mendocino Co.*, North Coast, (1) S end of Ten Mile Dunes, *ECN 21140*, AF385345, HQ653275, HQ653487, —, (2) near Virgin Creek, *AMR* & *K. M. Hanley MD-A*, HQ653076, HQ653276, HQ653488, —, (3) north of Mill Creek, *AMR* & *K. M. Hanley MD-B*, HQ653077, HQ653277, HQ653489, —, *BGB* & *WSA 859*, HQ653078, HQ653278, HQ653490, HQ653385.
- C. grandiflora*** Douglas ex Lindl.: USA, California, (1) *Humboldt Co.*, Outer North Coast Ranges, NW side of Horse Mountain, *ECN 21359*, AF385341, HQ653238, HQ653450, —; *Trinity Co.*, Klamath Ranges, (2) Canyon Creek (5.1 road km N of Junction City), *WSA* & *CR 06-101*, HQ653045, HQ653239, HQ653451, —, *CR s.n.* (24 May 2007), HQ653049, HQ653243, HQ653455, —; Oregon, *Jackson Co.*, Rogue River Valley, (3) Upper Table Rock, *BGB* & *WSA 1191*, HQ653046, HQ653240, HQ653452, —, (4) Lower Table Rock, *BGB* & *WSA 1194*, HQ653048, HQ653242, HQ653454, HQ653371, (5) Crowfoot Road, *AMR s.n.* (1 Jun 2005), HQ653047, HQ653241, HQ653453, —; (6) *Lane Co.*, Trout Creek, *E. Elle s.n.* (Jun 2004), HQ653036, HQ653229, HQ653441, —.
- C. greenei*** A. Gray ex Greene: USA, California, (1) *Colusa Co./Lake Co. border*, High North Coast Ranges, Snow Mountain vicinity (0.3 trail km

N-NE of Summit Spring), *BGB* 952, HQ653102, HQ653295, HQ653498, —; *Lake Co.*, Inner North Coast Ranges, (2) NE of Middletown (along State Highway 29, 3.7 road km NE of Butts Canyon Road junction), *BGB* & *WSA* 851, AF385338, HQ653296, HQ653499, —, (3) Highland Springs Road, *AMR*, *K. M. Hanley*, & *BGB HSR-A*, HQ653104, HQ653298, HQ653501, HQ653387–HQ653388 (*CYCI* clones 1, 2, 6, 10); (4) *Napa Co.*, Inner North Coast Ranges, State Highway 128 junction with Berryessa-Knoxville Road (Turtle Rock), *CR s.n.* (May 2005), HQ653103, HQ653297, HQ653500, —.

**C. heterophylla** Buist ex Graham: USA, California, (1) *Alameda Co.*, San Francisco Bay Area/San Joaquin Valley border, Tesla Road (1 road km E of divide), *BGB* & *WSA* 856, HQ653141, HQ653340, HQ653543, —; (2) *El Dorado Co.*, Northern Sierra Nevada Foothills, South Fork American River at Chili Bar (N of Placerville, at State Highway 193 crossing), *CR s.n.* (May 2005), individual 1, HQ653152, HQ653351, HQ653554, —, individual 2, HQ653153, HQ653352, HQ653555, —; (3) *Los Angeles Co.*, Western Transverse Ranges, Warm Springs Canyon, *MSP* 1605, HQ653151, HQ653350, HQ653553, —; *Mariposa Co.*, Central Sierra Nevada Foothills, (4) W. of Mt. Bullion, *BGB* & *S. J. Bainbridge* 828, AF385337, HQ653337, HQ653540, —, (5) South Fork Merced River at Hite's Cove, *BGB*, *SK*, & *T. J. Rosatti* 1009, HQ653139, HQ653338, HQ653541, —; *Monterey Co.*, (6) Inner South Coast Ranges, Gabilan Range, 3.6 road km W of San Benito Co. line along Gloria Road, *BGB* & *S. J. Bainbridge* 1273b, HQ653143, HQ653342, HQ653545, —, (7) Outer South Coast Ranges, ca. 6.5 road km E of summit of Lockwood-San Ardo Road, *BGB* & *WSA* 1377, HQ653146, HQ653345, HQ653548, —, (8) Outer South Coast Ranges, Upper Carmel River watershed, Hastings Natural History Reservation, *AMR*, *K. M. Hanley*, & *MSP HBR-A*, HQ653157, HQ653356, HQ653559, HQ653406–HQ653407 (*CYCI* clones 1, 2, 4), (9) Outer South Coast Ranges, Santa Lucia Range, ca. 1.5 km NW of Basket Spring (ca. 2.8 km S of San Miguel Creek), *ECN*, *E. Painter*, *BGB*, *M. Wetherwax*, *T. Morosco*, *K. Downing*, & *H. Forbes HLI471*, HQ653158, HQ653357, HQ653560, —; *Napa Co.*, Inner North Coast Ranges, (10) State Highway 121, ca. 3.2 road km N of Wooden Valley Road junction, *BGB* & *WSA* 1204a, HQ653142, HQ653341, HQ653544, —, (11) Pope Valley vicinity, upper Wantrup Preserve, *WSA* & *CR s.n.* (21 Apr 2005), HQ653144, HQ653343, HQ653546, —; (12) *Riverside Co.*, Peninsular Ranges, Santa Ana Mountains, Bear Canyon Trail, *MSP* 2029, HQ653147, HQ653346, HQ653549, —; *San Bernardino Co.*, (13) San Gabriel Mountains/San Bernardino Mountains border, near Cajon Pass, *MSP* 1569 (= var. *austromontana* Newsom), HQ653148, HQ653347, HQ653550, —, (14) San Bernardino Mountains, town of Cedarpines Park, *MSP s.n.* [no voucher; same population as *A. C. Sanders* 14938 (UCR)] (= var. *austromontana* Newsom), HQ653150, HQ653349, HQ653552, —; *San Diego Co.*, Peninsular Ranges, (15) W of Ramona on State Route 78 at Indian Oaks Road, *MSP* & *C. M. Waterman* 1797, HQ653155, HQ653354, HQ653557, —, (16) S of Mesa Grande, Black Canyon, *MSP* 1808, HQ653156, HQ653355, HQ653558, —; (17) *San Luis Obispo Co.*, Outer South Coast Ranges, Santa Lucia Range, Upper Lopez Canyon Road, 3.9 road km from junction with Hi Mountain Road, *BGB* & *S. J. Bainbridge* 894, HQ653140, HQ653339, HQ653542, —; (18) *Santa Barbara Co.*, Outer South Coast Ranges, Sierra Madre, Bates Canyon, *BGB*, *SK* & *WSA* 1373, HQ653159–HQ653161 (ITS clones 2, 6, 11, 12), HQ653358, HQ653561, —; (19) *Santa Clara Co.*, San Francisco Bay Area, Mt. Hamilton Range, Kammerer Ranch, *S. J. Bainbridge s.n.* (17 May 2007), HQ653154, HQ653353, HQ653556, —; *Ventura Co.*, Western Transverse Ranges, (20) Wheeler Gorge, *WSA WG-A*, HQ653149, HQ653348, HQ653551, HQ653404–HQ653405 (*CYCI* clones 1–3), (21) Sisar Canyon, *WSA s.n.*, HQ653145, HQ653344, HQ653547, HQ653398–HQ653403 (*CYCI* clones A1–A3, D1–D4, D3P1).

**C. latifolia** (Newsom) B. G. Baldwin, Kalisz & Armbr.: USA, California, (1) *Mendocino Co.*, High North Coast Ranges, US Forest Service Route 23N60, 0.6 road km N of US Forest Service Route 23N69 junction, *BGB* 899, HQ652998, HQ653189, HQ653430, —; (2) *Shasta Co.*, High Cascade Range, Noble Pass, *G. Gillett* 618, HQ653001, HQ653192, HQ653433, —; *Siskiyou Co.*, Klamath Ranges, (3) N Fork Salmon River drainage, Taylor Lake, *CR s.n.* (9 Jun 2007), HQ652999, HQ653190, HQ653431, —, (4) Marble Mountains, Boulder Creek Trail, *CR s.n.* (20 Jun 2007), HQ653000, HQ653191, HQ653432, —.

**C. linearis** A. Gray: USA, California, *Humboldt Co.*, Outer North Coast Ranges, (1) Bair Road, halfway between Hoopa and Pine Ridge summit,

*CR s.n.* (3–4 Jun 2007), HQ653017, HQ653208, HQ653583, —, (2) Bair Road, 1.8 road km NE of Pine Ridge Road junction, *CR s.n.* (3–4 Jun 2007), HQ653023, HQ653215, HQ653590, —, (3) Bair Road, 1.3 road km NE of Pine Ridge Road junction, *CR s.n.* (3–4 Jun 2007) HQ653019, HQ653210, HQ653585, —, (4) Bair Road, 24.1 road km NE of State Highway 299 junction, *CR s.n.* (3–4 Jun 2007), HQ653018, HQ653209, HQ653584, —, (5) W side of Horse Mountain, *ECN* 21358, AF385343, HQ653211, HQ653586, —; (6) Titlow Hill Road, *MSP s.n.*, individual 1, HQ653021, HQ653213, HQ653588, —, individual 2, HQ653022, HQ653214, HQ653589, —; *Siskiyou Co.*, Klamath Ranges, (7) South Fork Salmon River, along Cecilville Road, 689 m elev., *CR s.n.* (8 Jun 2007), HQ653024, HQ653216, HQ653591, —, (8) South Fork Salmon River, along Cecilville Road, 1218 m elev., *CR s.n.* (8 Jun 2007), HQ653026, HQ653218, HQ653593, —, (9) US Forest Service road to Taylor Lake, 0.5 road km S-SE from Sawyers Bar Road junction, *CR s.n.* (9 Jun 2007), HQ653025, HQ653217, HQ653592, —, (10) Marble Mountains, along Boulder Creek Trail, 1939 m elev., *CR s.n.* (20 Jun 2007), HQ653027, HQ653219, HQ653594, —; *Trinity Co.*, Klamath Ranges, (11) near Burnt Ranch, along State Highway 299, *WSA* & *CR* 06-102 HQ653010, HQ653201, HQ653576, —, (12) Junction City, *CR s.n.* (24 May 2007), HQ653020, HQ653212, HQ653587, —; Oregon, *Jackson Co.*, (13) Rogue River Valley, Upper Table Rock, *BGB* & *WSA* 1192, HQ653029, HQ653221, HQ653596, —, (14) Rogue River Valley, Lower Table Rock, *BGB* & *WSA* 1195, HQ653028, HQ653220, HQ653595, HQ653369, (15) Rogue River Valley, Crowfoot Road, *AMR s.n.* (1 Jun 2005), HQ653011, HQ653202, HQ653577, —, (16) Rogue River Valley, Butte Falls Road, *AMR s.n.* (3 Jun 2005), HQ653012, HQ653203, HQ653578, —, (17) Applegate Valley, Trillium Mountain, *AMR s.n.* (2006), HQ653013, HQ653204, HQ653579, —, (18) Applegate Valley, Applegate 500 (Quartz fire burn), *AMR s.n.* (29 Jun 2005), HQ653014, HQ653205, HQ653580, —, (19) Applegate Valley, Little Applegate Road, *AMR s.n.* (2006), HQ653016, HQ653207, HQ653582, —, (20) Greensprings area, Grizzly Peak Road, *AMR GPR06*, HQ653015, HQ653206, HQ653581, —.

**C. "metamorphica" complex**: USA, California, *Mariposa Co.*, Central High Sierra Nevada, Merced River drainage, (1, 2) Trumbull Peak, *MSP* 1614, HQ653061, HQ653259, HQ653471, —, *MSP* 1897, HQ653062, HQ653260, HQ653472, —, (3) south end of Iron Mountain, *BGB* & *WSA* 1483, HQ653063, HQ653261, HQ653473, HQ653381–HQ653383 (*CYCI* clones 2, 3, 5, 6), (4) South Fork Merced River, *MSP* 1899, HQ653064, HQ653262, HQ653474, —, (5) Zip Creek, *MSP* 2147, HQ653068, HQ653266, HQ653478, —; (6) north end of Iron Mountain, *MSP* 1905, HQ653067, HQ653265, HQ653477, —, (7) Merced River near El Portal (Foresta Road), *BGB* & *WSA* 1484, HQ653065, HQ653263, HQ653475, HQ653380, (8) Merced River at Pigeon Gulch, *MSP* 1871, HQ653066, HQ653264, HQ653476, —.

**C. multicolor** Lindl. & Paxton: USA, California, (1) *San Mateo Co.*, San Francisco Bay Area, Crystal Springs Road, *MSP* 1195, HQ653129, HQ653324, HQ653527, —; (2) *Santa Cruz Co.*, San Francisco Bay Area, Swanton Road, *ECN* 21122, AF385353, HQ653325, HQ653528, HQ653393.

**C. parryi** A. Gray: *San Bernardino Co.*, (1) W end of San Bernardino Mountains, *ECN* 21530, AF385349, HQ653329, HQ653532, HQ653395, (2) San Gabriel Mountains, Lone Pine Canyon, *A. Lankinen* 03-02, HQ653133, HQ653330, HQ653533, —.

**C. parviflora** Douglas ex Lindl.: CANADA, British Columbia, (1) Tyhee Lake, Smithers, *WSA s.n.*, HQ653032, HQ653225, HQ653437, —, (2) Vancouver Island, Kin Beach, *E. Elle s.n.*, HQ653033, HQ653226, HQ653438, —; (3) Yukon Territory, hill across Tagish Road from Crag Lake, *B. A. Bennett s.n.*, HQ653038, HQ653231, HQ653443, —; USA, California, *Alameda Co.*, San Francisco Bay Area, Mt. Hamilton Range, (4) Tarraville Creek, *BGB* & *WSA* 854, AF385340, HQ653222, HQ653434, —, (5) head of Williams Gulch, *BGB*, *S. J. Bainbridge*, & *W. Legard* 1479, HQ653030, HQ653223, HQ653435, —; *Humboldt Co.*, Outer North Coast Ranges, (6) Titlow Hill Road, 4.3 road km NW of Friday Ridge Road junction, *CR s.n.* (3–4 Jun 2007), HQ653034, HQ653227, HQ653439, —, (7) near Grouse Mountain, along Friday Ridge Road, 3.5 road km S of Titlow Hill Road junction, *CR s.n.* (3–4 Jun 2007), HQ653035, HQ653228, HQ653440, —; (8) *Siskiyou Co.*, Klamath Ranges, E side of Taylor Lake, *CR s.n.* (9 Jun 2007), HQ653039, HQ653232, HQ653444, —; (9) Idaho, *Adams Co.*, Little Salmon River, 20.1 km N of New Meadows along US 95, *B. Ertter* 19398, HQ653042, HQ653235, HQ653447, —; (10)

- Michigan, Upper Peninsula, *Marquette Co.*, Mountain Lake (near Big Bay), *C. Heckel s.n.*, individual 1, HQ653043, HQ653236, HQ653448, —, individual 2, HQ653044, HQ653237, HQ653449, —; (11) Nevada, *Washoe Co.*, Modoc Plateau, State Highway 8A, 8 road km W of State Highway 34 junction, *BGB, M. J. Sanderson, & M. J. Wojciechowski 866*, HQ653031, HQ653224, HQ653436, —; Oregon, *Jackson Co.*, (12) Klamath Ranges, Pilot Rock, near Ashland, *AMR & K. M. Hanley s.n.* (19 May 2004), HQ653037, HQ653230, HQ653442, HQ653370, (13) Rogue River Valley, Lower Table Rock, summit, *BGB & WSA 1196*, HQ653029, HQ653234, HQ653446, —, (14) Rogue River Valley, Lower Table Rock, ca. 0.4–0.8 trail km below summit, along main trail, *BGB & WSA 1197*, HQ653028, HQ653220, HQ653445, —.
- C. rattanii** A. Gray: USA, California, *Glenn Co.*, High North Coast Ranges, (1) N of Snow Mountain, along US Forest Service Road M3, 1.1 road km S of Ivory Mill Road junction, *CR s.n.* (25 May 2007), HQ653007, HQ653198, HQ653573, —, (2) US Forest Service Road FH-7, 8.4 road km S of Plaskett Meadows Campground, *CR s.n.* (25 May 2007), HQ653008, HQ653199, HQ653574, —; (3) *Lake Co.*, Inner North Coast Ranges, Harrington Flat Road, just S of Boggs Lake, *BGB & WSA 849*, AF385342, HQ653193, HQ653567, HQ653367; *Plumas Co.*, Northern High Sierra Nevada, (4) *M. Wetherwax s.n.* (22 May 1995), HQ653002, HQ653194, HQ653568, —, (5) State Highway 70, 0.5 road km W of Squirrel Creek (11.3 air km SE of Quincy), *L. Ahart 10956*, HQ653003, —, HQ653569, —; (6) *Siskiyou Co.*, Klamath Ranges, Marble Mountains, along Boulder Creek Trail, *CN s.n.* (20 Jun 2007), HQ653009, HQ653200, HQ653575, —; Oregon, *Jackson Co.*, (7) Applegate Valley, Trillium Mountain, *AMR s.n.* (2006), HQ653004, HQ653195, HQ653570, —, (8) Applegate Valley, Applegate 500 (Quartz fire burn), *AMR s.n.* (2006), HQ653005, HQ653196, HQ653571, HQ653368, (9) Greensprings area, Keen Creek Road, *AMR s.n.* (2006), HQ653006, HQ653197, HQ653572, —.
- C. sparsiflora** Fisch. & C. A. Mey. **var. collina** (Jeps.) Newsom: USA, California, (1) *Alameda Co.*, San Francisco Bay Area, Mt. Hamilton Range, Tarraville Creek, *BGB & WSA 855*, AF385339, HQ653299, HQ653502, HQ653389; *Napa Co.*, Inner North Coast Ranges, (2) Pope Valley vicinity, Wantrup Preserve, *CR s.n.*, HQ653119, HQ653314, HQ653517, —, (3) Pope Valley, Ink Grade / Pope Valley Road junction, *WSA s.n.*, HQ653114, HQ653309, HQ653512, —; (4) *Nevada Co.*, Northern Sierra Nevada Foothills, Lime Kiln Road, W of State Route 49, *MSP 1769*, HQ653127, HQ653322, HQ653525, —; (5) *San Benito Co.*, Inner South Coast Ranges, confluence of Clear Creek and San Benito River, *MSP 2125*, HQ653128, HQ653323, HQ653526, —.
- C. sparsiflora** Fisch. & C. A. Mey. **var. sparsiflora**: USA, California, (1) *Contra Costa Co.*, San Francisco Bay Area, Marsh Creek headwaters, Morgan Territory, *MSP 1035*, HQ653105, HQ653300, HQ653503, —; *Lake Co.*, Inner North Coast Ranges, (2) Boggs Lake, *BGB, WSA, & SK 1067*, HQ653106, HQ653301, HQ653504, —, (3) State Route 29, ca. 5 road km NE of Middletown, *BGB & WSA 1190*, HQ653107, HQ653302, HQ653505, —; *Napa Co.*, Inner North Coast Ranges, (4) 3400 Wooden Valley Road (McQueeney's Ranch entrance), *BGB, WSA, & SK 1202b*, HQ653108, HQ653303, HQ653506, HQ653390, (5, 6) State Route 121, ca. 3 road km N of Wooden Valley Road junction, *BGB, WSA, & SK 1203b*, large-flowered, HQ653109, HQ653304, HQ653507, HQ653391, medium-flowered, HQ653115, HQ653310, HQ653513, HQ653392, (7) "Mercury Meadow" (serpentine at Knoxville), *J. Wright s.n.*, HQ653124, HQ653319, HQ653522, —, (8) "Mercury Woods" (non-serpentine at Knoxville), *J. Wright s.n.*, HQ653126, HQ653321, HQ653524, —, (9) "Saddle Ridge" (serpentine near Knoxville), *J. Wright s.n.*, HQ653120, HQ653315, HQ653518, —, (10) Pope Valley vicinity, Wantrup Preserve, *WSA & CR s.n.* (21 Apr 2005), HQ653110, HQ653305, HQ653508, —, (11–16) Knoxville vicinity, McLaughlin Natural Reserve, *Shannon Peters MLI-6* (30 Apr 2006), (11) on serpentine soil, HQ653121, HQ653316, HQ653519, —, (12) on non-serpentine soil, HQ653122, HQ653317, HQ653520, —, (13) on serpentine soil, HQ653125, HQ653320, HQ653523, —, (14) on serpentine soil, HQ653116, HQ653311, HQ653514, —, (15) on non-serpentine soil, HQ653123, HQ653318, HQ653521, —, (16) on non-serpentine soil, HQ653111, HQ653306, HQ653509, —, (17) Outer North Coast Ranges, Bear Canyon, *CR s.n.* (18 May 2005), HQ653118, HQ653313, HQ653516, —; (18) *Yolo Co.*, "Gold Pit" (non-serpentine ca. 1.6 km NW of Knoxville), *J. Wright s.n.*, HQ653112, HQ653307, HQ653510, —; Oregon, *Jackson Co.*, Rogue River Valley, Lower Table Rock, (19) summit (white flowers), *BGB & WSA 1193*, HQ653117, HQ653312, HQ653515, —, (20) base (violet flowers), *BGB & WSA 1199*, HQ653113, HQ653308, HQ653511, —.
- C. tinctoria** Hartw. ex Benth: USA, California, (1) *Contra Costa Co.*, San Francisco Bay Area, Mt. Diablo, *BGB & WSA 951*, HQ653082, HQ653283–HQ653287 (ETS clones 1–12), HQ653495, HQ653386; (2) *El Dorado Co.*, Northern Sierra Nevada Foothills, Placerville, Mosquito Road, *CR s.n.* (May 2005), HQ653079, HQ653280, HQ653492, —; (3) *Fresno Co.*, Southern High Sierra Nevada, Hume Lake Road, *MSP 1326*, HQ653080, HQ653281, HQ653493, —; (4) *Mariposa Co.*, Central High Sierra Nevada, Bull Creek Road, *WSA & CR 06-103*, HQ653089–HQ653093 (ITS clones 1–12), HQ653289, HQ653497, —; (5) *Napa Co.*, Inner North Coast Ranges, Chiles Valley Road, *CR s.n.* (May 2005), HQ653094–HQ653101 (ITS clones 1–12), HQ653290–HQ653294 (ETS clones 1–12), HQ675014, —; (6) *Sonoma Co.*, Outer North Coast Ranges, Bohemian Highway, 5 road km N of Graton Road junction (N of Camp Meeker), *BGB & WSA 857*, AF385346, HQ653279, HQ653491, —; *Tulare Co.*, Southern High Sierra Nevada, (7) Greenhorn Mountains, South Creek (near Johnsondale), *MSP 1930*, HQ653081, HQ653282, HQ653494, —, (8) Kaweah River drainage, *BGB 1133*, HQ653083–HQ653088 (ITS clones 1–12), HQ653288, HQ653496, —.
- C. torreyi** A. Gray: USA, California, (1) *El Dorado Co.*, Northern High Sierra Nevada, Wright's Lake vicinity, *MSP 767*, individual 1, HQ652982, HQ653171, HQ653417, —, individual 2, HQ652985, HQ653175, HQ653421, —; (2) *Mariposa Co.*, Central High Sierra Nevada, Merced River drainage, N end of Iron Mountain, *MSP 1907*, HQ652983, HQ653172, HQ653418, —; (3, 4) *Tuolumne Co.*, State Route 108, E of Pinecrest, *ECN 21345*, HQ652984, HQ653174, HQ653420, HQ653365, *ECN 21356*, AF385347, HQ653173, HQ653419, —.
- C. verna** Nutt: USA, Illinois, (1) *Will Co.*, Raccoon Grove Forest Preserve (near Monee), *SK s.n.*, AF385351, HQ653244, HQ653456, —; (2) Michigan, *Kalamazoo Co.*, Tu Avenue, *SK s.n.*, HQ653051, HQ653246, HQ653458, —; (3) Pennsylvania, Greene/Washington county line, Enlow Fork, *SK s.n.*, HQ653050, HQ653245, HQ653457, HQ653372.
- C. violacea** Nutt: USA, (1) Illinois, *Shelby Co.*, Harmon Cemetery, off Highway 128, *C. Ivey s.n.* (20 May 2005), HQ653053, HQ653249, HQ653461, —; Missouri, (2) *Gasconade Co.*, ca. 1.6 km S of Hermann, *J. Paul s.n.* (2004), HQ653052, HQ653248, HQ653460, HQ653373; (3) *St. Clair Co.*, ca. 10 km W of Osceola and Highway 13, *ECN 21533B*, AF385352, HQ653247, HQ653459, —.
- C. wrightii** S. Watson: USA, California, (1) *El Dorado Co.*, Northern High Sierra Nevada, Wright's Lake vicinity, *MSP 775*, individual 1, HQ652986, HQ653176, HQ653422, —, individual 2, HQ652987, HQ653177, HQ653423, —; (2) *Glenn Co.*, High North Coast Ranges, saddle just N of Black Butte, *CR s.n.*, HQ652989, HQ653179, HQ653425, —; *Kern Co.*, (3) Western Transverse Ranges, Mt. Pinus Road, ca. 0.8–1.6 road km above Mil Potrero Highway junction, *BGB & WSA 948*, AF385348, HQ653183, HQ653562, —; (4) High Southern Sierra Nevada, Greenhorn Range, W slope of Sunday Peak, *E. Twisselmann 5246*, HQ652994, HQ653185, HQ653564, —; (5) *Los Angeles Co.*, San Gabriel Mountains, Table Mountain, *MSP 2039*, HQ652993, HQ653184, HQ653563, —; (6) *Mendocino Co.*, High North Coast Ranges, S of Anthony Peak, Wells Cabin Campground, *MSP 1371b*, HQ652997, HQ653188, HQ653429, —; (7) *Modoc Co.*, Warner Mountains, *MSP 1945*, HQ652991, HQ653181, HQ653427, —; (8) *Mono Co.*, High Central Sierra Nevada, Silver Creek Meadows, *D. Taylor 17519*, HQ652992, HQ653182, HQ653428, —; (9) *Siskiyou Co.*, Klamath Ranges, N Fork Salmon River drainage, Taylor Lake, *CR s.n.* (9 Jun 2007), HQ652990, HQ653180, HQ653426, —; (10) *Tulare Co.*, High Southern Sierra Nevada, Kern Plateau, Deadwood Meadow, *E. Twisselmann 17923*, HQ652995, HQ653186, HQ653565, —; (11) = *C. torreyi* A. Gray var. *brevicarinata* Newsom, Southern High Sierra Nevada, Kaweah River Drainage, N of Hockett Meadow, *MSP 1933*, HQ652996, HQ653187, HQ653566, —; (12) unknown locality, *ECN 21416*, HQ652988, HQ653178, HQ653424, HQ653366.
- Tonella floribunda** A. Gray: USA, (1) Idaho, *Idaho Co.*, Rapid River Trail, ~9.7 km SW of Riggins, *B. Ertter 19390*, HQ652981, HQ653169, HQ653415, —; (2) Washington, *Asotin Co.*, Snake River drainage, 4.8 km S of Asotin, *ECN 21538B*, AF385336, HQ653170, HQ653416, HQ653363–HQ653364 (CYC1 clones a, b).

*T. tonella* (Benth.) A. Heller: USA, California, (1) *Lake Co.*, Inner North Coast Ranges, Boggs Lake, *BGB & WSA 852*, AF385335, HQ653165, HQ653411, —; (2) *Napa Co.*, Inner North Coast Ranges, Howell Mountain, Las Posadas State Forest, Metcalf Trail, *WSA & CR s.n.* (20 Apr 2005), HQ652979, HQ653167, HQ653413, —; (3) *Santa Clara Co.*, San Francisco Bay Area, Mt. Hamilton Range, Blue Oak Ranch, *S. J. Bainbridge s.n.* (16 Apr 2007), HQ652980, HQ653168, HQ653414, —; (4) Oregon, *Jackson Co.*, Rogue River Valley, Lower Table Rock, *BGB & WSA 1198*, HQ652978, HQ653166, HQ653412, HQ653362.

**Outgroup taxa**

*Chelone glabra* L: USA, Massachusetts, *Franklin Co.*, Whately, collector unknown, UC1779237, HQ652976, HQ653163, HQ653409, HQ653360.

*Keckiella cordifolia* (Benth.) Straw: USA, California, San Luis Obispo Co., Outer South Coast Ranges, Santa Lucia Range, Lopez Canyon, *BGB 892*, HQ652977, HQ653164, HQ653410, HQ653361.

*Penstemon hartwegii* Benth: Mexico, Puebla, collector unknown, UC Botanical Garden 2002.0860, HQ652975, HQ653162, HQ653408, HQ653359.

---