



# Population Genetic Analysis of the Threatened Plant *Leavenworthia exigua* var. *laciniata* (Brassicaceae) Reveals Virtually No Genetic Diversity and a Unique Mating System

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*Leavenworthia* (Brassicaceae) has served as a model group for investigating the evolution of mating systems in plants, yet several *Leavenworthia* species remain understudied. One such taxon is *Leavenworthia exigua* var. *laciniata*, one of three varieties of *L. exigua*, a winter-annual plant endemic to the central United States. Because *L. exigua* var. *laciniata* occupies a narrow geographic range and is experiencing major habitat loss, it was recently listed as threatened; however, little is known about its genetic diversity and implications for conservation. We conducted a range-wide population genetic study of *L. exigua* var. *laciniata* and *L. exigua* var. *exigua* to understand: (1) levels of genetic diversity within and among populations, (2) whether *L. exigua* var. *laciniata* is genetically distinct from *L. exigua* var. *exigua*, and (3) implications for conservation. *L. exigua* var. *laciniata* showed identical genotypes at all 16 microsatellite loci across most of its range, fixed heterozygosity at some loci, and significant heterozygote excesses, consistent with a lack of recombination associated with an asexual mating system, which has not been documented previously in *Leavenworthia*. Because *L. exigua* var. *laciniata* is an annual and the same genotype occurs across multiple populations, asexuality may be caused by apomixis, asexual reproduction via seed. In contrast, most populations of *L. exigua* var. *exigua* demonstrated population genetic patterns consistent with a self-compatible mating system. Because *L. exigua* var. *laciniata* is morphologically, geographically, and genetically distinct, it should be recognized as an evolutionarily significant unit for conservation. We recommend maintaining large population sizes to conserve evolutionary potential in *L. exigua* var. *laciniata*, as the likelihood that facultative sexual reproduction may occur may be greater in larger populations. Additional research in *L. exigua* var. *laciniata* is needed to confirm the occurrence of asexuality and apomixis, clarify its reproductive isolation from other taxa, and to understand whether it exhibits residual

sexual reproduction, epigenetic variation, or phenotypic plasticity to help it persist in response to environmental variation. In the future, *L. exigua* var. *laciniata* may serve as an important model in which to investigate the conservation of threatened plant species with little genetic variation in a changing climate.

**Keywords:** apomixis, asexuality, Brassicaceae, conservation genetics, genetic diversity, genetic structure, microsatellite, mating system

## INTRODUCTION

The genus *Leavenworthia* Torr. (Brassicaceae) has long served as an important model system for investigating the evolution of plant mating systems (Busch and Urban, 2011). *Leavenworthia* comprises eight species of small, North American, winter-annual plants that primarily occupy limestone glades and glade-like open habitats with rocky, shallow soils. Several early studies of the genus reported variation in mating system among species, with some species exhibiting self-compatibility [*L. aurea* Torr., *L. exigua* Rollins, *L. torulosa* A. Gray, *L. texana* Mahler, and *L. uniflora* (Michx.) Britton], some species exhibiting self-incompatibility (*L. stylosa* A. Gray), and some showing variation among populations in self-incompatibility (*L. alabamica* Rollins and *L. crassa* Rollins) (Rollins, 1963; Lloyd, 1965; Solbrig, 1972; Solbrig and Rollins, 1977). This led to a large number of subsequent studies investigating the evolution of mating system in *Leavenworthia* and its consequences for population genetic variation (Lyons and Antonovics, 1991; Charlesworth and Yang, 1998; Liu et al., 1998; Busch, 2005a,b, 2006; Anderson and Busch, 2006; Busch et al., 2010, 2011; Busch and Urban, 2011; Koelling et al., 2011; Busch and Werner, 2012; Chantha et al., 2013; Baldwin and Schoen, 2017, 2019).

Despite the large number of studies focusing on *Leavenworthia*, the vast majority of research has focused on just a few well-studied species, whereas other species remain understudied and very poorly known. For example, because both *L. alabamica* and *L. crassa* show variation among populations in whether they are selfing or self-incompatible, a large number of studies have focused on these species (Busch et al., 2010, 2011; Koelling et al., 2011; Chantha et al., 2013; Baldwin and Schoen, 2017). Indeed, the molecular basis of self-incompatibility (Busch et al., 2008, 2011; Joly and Schoen, 2011) and a novel genetic locus controlling self-incompatibility unique to *Leavenworthia* has now been characterized in *L. alabamica* (Chantha et al., 2013; Baldwin and Schoen, 2017, 2019). Because other species exhibit contrasting mating systems, such as the self-incompatible *L. stylosa* and the predominantly self-fertilizing *L. torulosa* and *L. uniflora*, they have also been used extensively to compare how mating systems affect genetic diversity and other aspects of species biology (Charlesworth and Yang, 1998; Filatov and Charlesworth, 1999; Liu et al., 1999; Busch and Werner, 2012). In contrast, apart from descriptions of their taxonomy, natural history, or ecology (Rollins, 1956; Baskin and Baskin, 1972, 1981; Busch et al., 2008; Frings et al., 2019), comparatively little previous research has focused on *L. exigua*, *L. aurea*, and *L. texana*, all of which

are listed as endangered or threatened in at least a portion of their ranges.

*Leavenworthia exigua* is a comparatively poorly known species distributed in Kentucky, Tennessee, Alabama, and northern Georgia. The species was divided into the following three intraspecific varieties based on morphology and geography: (1) *Leavenworthia exigua* var. *lutea* Rollins, which has yellow petals and is found only in Alabama, (2) *Leavenworthia exigua* var. *exigua* Rollins, which has white petals, lavender sepals, and is found in Tennessee and north Georgia, and (3) *Leavenworthia exigua* var. *laciniata* Rollins, which has white petals, green sepals, and is endemic to Kentucky (Rollins, 1963). All varieties were reported to be diploid, with a consistent chromosome number of  $n = 11$ , the lowest chromosome number in the genus. Chromosome numbers vary widely in *Leavenworthia*, with *L. exigua*, *L. crassa*, and *L. alabamica* exhibiting a chromosome count of  $n = 11$ , *L. stylosa*, *L. torulosa*, and *L. uniflora* exhibiting  $n = 15$ , and *L. texana* and *L. aurea* exhibiting  $n = 24$ . Based on self-pollination experiments conducted in *L. exigua* var. *exigua* and *L. exigua* var. *lutea*, *L. exigua* was reported to be self-compatible (Rollins, 1963), although to our knowledge, self-incompatibility in *L. exigua* var. *laciniata* has not been tested.

One of the three varieties of *L. exigua* is Kentucky glade cress (*L. exigua* var. *laciniata*), which has a naturally small distribution restricted to a small portion of Bullitt and Jefferson Counties in Kentucky. Because the geographic range of *L. exigua* var. *laciniata* is located just south of the Louisville, KY metropolitan area, which is rapidly expanding southward, its habitat is progressively being lost or degraded through residential and commercial development. Over half of the populations of *L. exigua* var. *laciniata* have already experienced declines, and many populations have been extirpated (USFWS, 2014). Because of these declines in both habitat and population size, *L. exigua* var. *laciniata* was recently listed as threatened under the U.S. endangered species act (USFWS, 2014).

Despite the fact that *L. exigua* var. *laciniata* is threatened, many aspects of its basic biology, mating system, and genetic diversity are unknown; additional research is needed to understand more about the species to facilitate conservation efforts. No previous population genetic study has focused on *L. exigua* var. *laciniata*, even though this information is critical for devising practical conservation strategies for threatened species (Frankham, 1995; Rossetto et al., 2021). For example, the amount of genetic diversity contained in populations, patterns of genetic structure, and the best strategy for protecting the genetic variation in *L. exigua* var. *laciniata* are currently unknown. Also unknown are whether the varieties of *L. exigua* are

genetically distinct and whether they deserve recognition as distinct evolutionary significant units (ESU; Moritz, 1994).

In this study, we sampled multiple populations of two of the three varieties of *L. exigua* (*L. exigua* var. *laciniata* and *L. exigua* var. *exigua*), genotyped them at 16 polymorphic microsatellite loci, and analyzed the resulting genetic data to understand: (1) genetic diversity and structure within and among populations of *L. exigua* var. *laciniata*, (2) whether *L. exigua* var. *laciniata* is distinct genetically from *L. exigua* var. *exigua*, and (3) the implications for prioritization of *in-situ* and *ex-situ* conservation efforts. Based on the assumption that *L. exigua* var. *laciniata* is self-compatible (as this mating system was found in the other two varieties of *L. exigua*), we expected *L. exigua* var. *laciniata* to show patterns of population genetic diversity and structure similar to those found previously in other self-compatible species (e.g., Liu et al., 1998; Koelling et al., 2011; Swift et al., 2016; Edwards et al., 2019), namely, low within-population genetic diversity and high among-population diversity, low observed heterozygosity, and large positive inbreeding coefficients. Based on their allopatric geographic distributions and distinctive morphological features, we also expected that the varieties of *L. exigua* would be

genetically distinct from one another and worthy of recognition as ESUs for conservation purposes.

## METHODS

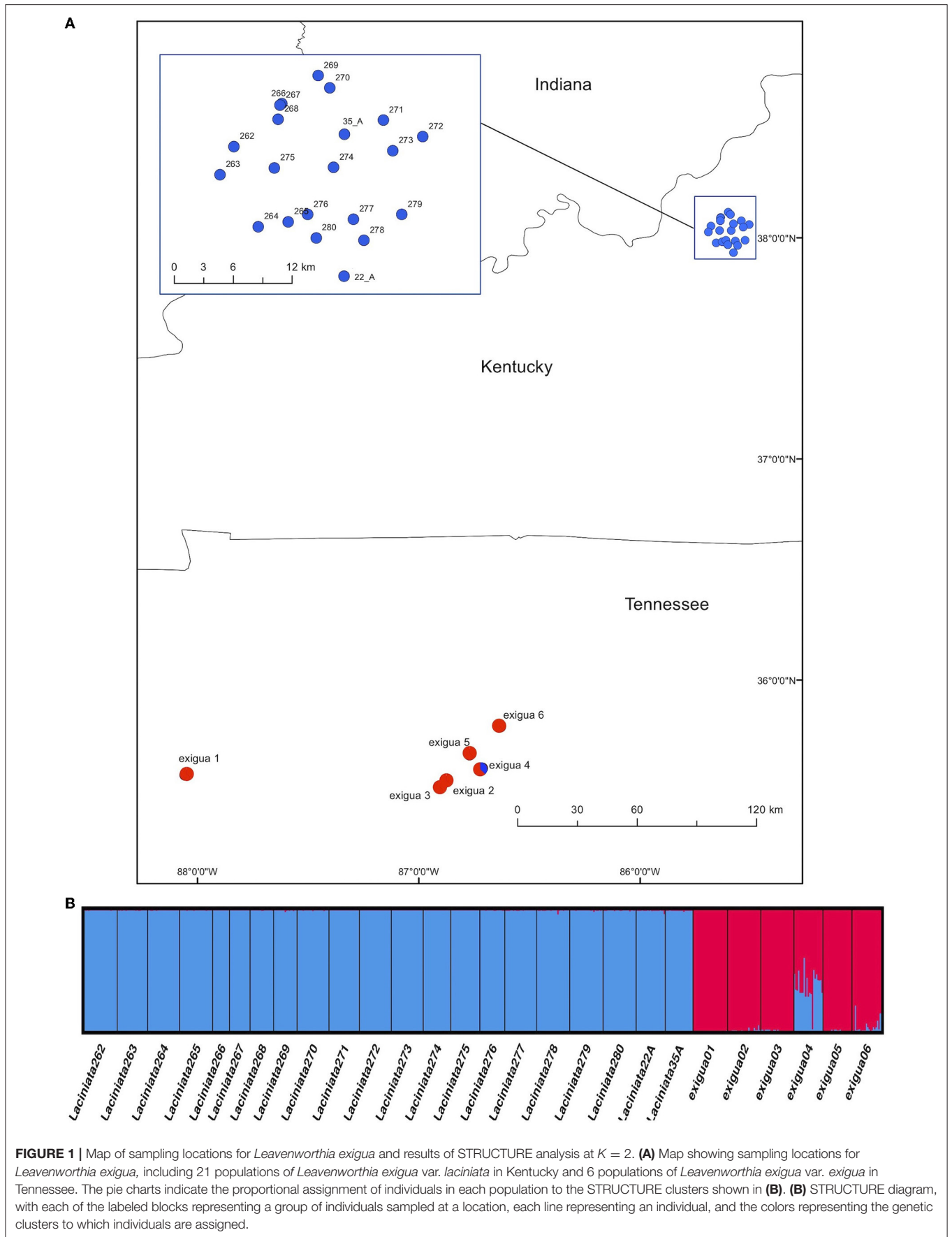
### Sample Collection

We conducted field work to sample populations of *Leavenworthia exigua* in April of 2016. We collected leaf tissue of between 16 and 25 individuals per population from 21 populations from throughout the geographic range of *L. exigua* var. *laciniata* and six populations of *L. exigua* var. *exigua* (Table 1; Figure 1A), resulting in 440 individuals of *L. exigua* var. *laciniata* and 136 individuals of *L. exigua* var. *exigua*, or 576 individuals in total. Individuals were collected as evenly as possible from throughout each site to capture the entire geographic extent of the population in each sampling location. Although *L. exigua* is not known to be clonal, we sampled from plants at least 1 meter apart to ensure that we sampled distinct individuals. Samples were dried in silica gel and brought back to the Conservation Genetics laboratory at the Missouri Botanical Garden for subsequent DNA analysis. We collected a voucher specimen in each population, which was deposited in the herbarium at the Missouri Botanical Garden

**TABLE 1** | Population information for *Leavenworthia exigua* populations sampled in the study.

Voucher no.	Collector	Variety	State	County	Locality	Latitude	Longitude
262	Edwards	var. <i>laciniata</i>	KY	Bullitt	West side of SR61	38.05	-85.68
263	Edwards	var. <i>laciniata</i>	KY	Bullitt	Jct. of SR61 and Becnel Ln	38.03	-85.69
264	Edwards	var. <i>laciniata</i>	KY	Bullitt	Jct. KY480 and Ridge Road	37.98	-85.66
265	Edwards	var. <i>laciniata</i>	KY	Bullitt	Pine Creek Barrens Nature Preserve	37.98	-85.63
266	Edwards	var. <i>laciniata</i>	KY	Jefferson	McNeeley Lake park-NW glade	38.09	-85.64
267	Edwards	var. <i>laciniata</i>	KY	Jefferson	McNeeley Lake park-W glade	38.09	-85.64
268	Edwards	var. <i>laciniata</i>	KY	Jefferson	Pennsylvania Run	38.08	-85.64
269	Edwards	var. <i>laciniata</i>	KY	Jefferson	Cedar Creek Rd. just north of long rifle lane	38.12	-85.60
270	Edwards	var. <i>laciniata</i>	KY	Jefferson	Meghan Ln, near jct with Independence school Rd.	38.11	-85.59
271	Edwards	var. <i>laciniata</i>	KY	Bullitt	Jefferson County sportsman's club	38.08	-85.54
272	Edwards	var. <i>laciniata</i>	KY	Bullitt	KY44 between King's church Rd and Sanders Lane	38.06	-85.51
273	Edwards	var. <i>laciniata</i>	KY	Bullitt	Twelve Oaks subdivision	38.05	-85.53
274	Edwards	var. <i>laciniata</i>	KY	Bullitt	In lawn at 7839 44E	38.03	-85.59
275	Edwards	var. <i>laciniata</i>	KY	Bullitt	Shady Lane, just south of Bell's Mill Rd.	38.03	-85.64
276	Edwards	var. <i>laciniata</i>	KY	Bullitt	Apple Valley Glade	37.99	-85.61
277	Edwards	var. <i>laciniata</i>	KY	Bullitt	Jackson Hills general Baptist Church lawn	37.99	-85.57
278	Edwards	var. <i>laciniata</i>	KY	Bullitt	Rocky Run Glade	37.97	-85.56
279	Edwards	var. <i>laciniata</i>	KY	Bullitt	Vaughn Rd. North of KY480	37.99	-85.53
280	Edwards	var. <i>laciniata</i>	KY	Bullitt	Jct. of 480 and Log Cabin Ln.	37.97	-85.60
35A	Littlefield	var. <i>laciniata</i>	KY	Bullitt	North side of Wells Run, near Flatlick Rd	38.06	-85.58
22A	Littlefield	var. <i>laciniata</i>	KY	Bullitt	North side of Woodsdale Road, near KY 1604	37.93	-85.58
01	Lyman	var. <i>exigua</i>	TN	Decatur	Mt. Lebanon, TN	35.57	-88.05
02	Lyman	var. <i>exigua</i>	TN	Maury	NE of Lewisburg TN	35.54	-86.88
03	Lyman	var. <i>exigua</i>	TN	Maury	NE of Lewisburg TN	35.52	-86.90
04	Lyman	var. <i>exigua</i>	TN	Marshall	North of Henry Horton State Park	35.60	-86.71
05	Lyman	var. <i>exigua</i>	TN	Marshall	NE of Chapel Hill TN	35.66	-86.77
06	Lyman	var. <i>exigua</i>	TN	Williamson	SE of Kirkland TN	35.79	-86.63

Includes voucher information, collection localities, and GPS coordinates for each population. GPS coordinates were truncated to two digits to protect populations.





(MO; **Table 1**). Although we made two attempts to locate and collect plants of *L. exigua* var. *lutea*, which at the time was known only from eight glades in six counties in Alabama, we were unable to locate these populations and therefore did not include *L. exigua* var. *lutea* in the study. A recent study reported that most historical populations of *L. exigua* var. *lutea* were extirpated, but also reported the discovery of several new populations (Frings et al., 2019).

## Genetic Analysis

We extracted DNA from each individual using a CTAB approach (Doyle and Doyle, 1987). To identify microsatellites, we followed the protocol described in Swift et al. (2016). We carried out shotgun sequencing of genomic DNA extracted from two DNA samples of *L. exigua* var. *laciniata*. The source material for the DNA samples was bulked seed from the MBG seedbank (accession 2015-1048, a population that occurs within the range of *L. exigua* var. *laciniata* in Bullitt county, KY that was identified morphologically as *L. exigua* var. *laciniata*). Each sample contained several seeds of *L. exigua* var. *laciniata* derived from multiple maternal individuals. Library preparation was conducted using NEBnext Ultra DNA sample prep kits and NEBnext multiplex oligos (NEB). The indexed samples of *L. exigua* var. *laciniata* were pooled with samples from other studies and sequenced on an Illumina Miseq using  $2 \times 150$  bp paired-end reads. We trimmed low-quality bases of the resulting reads and assembled the reads *de novo* into contigs using the Medium sensitivity/fast setting in Geneious (Biomatters Inc., Auckland, New Zealand). We searched contig consensus sequences for di-, tri-, and tetra-nucleotide microsatellite repeats using MSATCOMMANDER (Faircloth, 2008). Polymerase chain reaction (PCR) primers were designed from a subset of these sequences using the default settings in PRIMER3 (Rozen and Skaletsky, 2000; Faircloth, 2008).

We designed 144 primers and conducted an initial test for amplification using genomic DNA from two samples of *L. exigua* var. *laciniata* and one individual of *L. exigua* var. *exigua*. Microsatellite genotyping employed a universal dye-labeling approach, which involves adding an M13 tail (CACGACGTTGTTAAAACGAC) to the 5' end of forward primers and use of a corresponding, fluorescently-labeled universal primer (Boutin-Ganache et al., 2001). PCR amplifications were performed in 10  $\mu$ L reactions containing 0.5 U of GoTaq Flexi DNA polymerase (Promega corporation, Madison, Wisconsin, USA), 1 $\times$  Promega Colorless GoTaq Flexi Buffer, 1.5 mM MgCl<sub>2</sub>, 4.5 pmol each of the reverse primer and one of four fluorescently labeled M13 primers (6-FAM, VIC, NED, or PET; Applied Biosystems), 0.18 pmol of the M13-tagged forward primer, and 0.5 mM of each dNTP. PCR temperature cycling conditions were as follows: (I) 3 min at 94°C, (II) denaturation for 30 s at 94°C, (III) annealing for 30 s at 52°C, (IV) extension for 45 s at 72°C, (V) 35 repetitions of steps 2–4, and (VI) a final elongation at 72°C for 20 min. We diluted PCR products 1:10 and sent them for genotyping on an ABI3730xl DNA Analyzer, with all genotyping carried out by loading 1  $\mu$ L of the diluted PCR product, 9.0  $\mu$ L of formamide, and 0.5  $\mu$ L LIZ 500 size standard (Applied Biosystems, Waltham,

Massachusetts, USA). All genotyping was conducted at the DNA analysis facility at Science Hill at Yale University.

In the initial screening for amplification, we identified 67 loci that amplified in the two samples of *L. exigua* var. *laciniata*, of which 63 also amplified in *L. exigua* var. *exigua*. We selected 44 markers that amplified in all three samples for additional testing for amplification and polymorphism in 19 individuals of *L. exigua*, including 13 individuals of *L. exigua* var. *laciniata* and 6 individuals of *L. exigua* var. *exigua*. Although the 13 samples of *L. exigua* var. *laciniata* were sampled from throughout its geographic range, most loci exhibited little to no variation among these individuals. In contrast, we found that 21 of these loci were variable in the six individuals of *L. exigua* var. *exigua*. We therefore selected any marker that exhibited two or more alleles in *L. exigua* var. *laciniata* and those that were the most variable in *L. exigua* var. *exigua*. In total, we employed 16 markers to genotype all 576 individuals in this study (**Table 2**). For genotyping of all individuals, we used one of four different fluorescently labeled M13 primers to label each locus, and then we pooled the PCR products of the four uniquely labeled loci and ran them as described above. Fragment analysis and scoring were carried out using automated fragment scoring panels developed for each locus in GeneMarker version 1.6 (Soft Genetics LLC, State College, Pennsylvania, USA), and then the data were checked manually.

## Data Analysis

We tested for deviations from Hardy-Weinberg equilibrium (HWE) at each locus/population combination using Fisher's exact tests in Genepop (Rousset, 2008). Population genetic diversity indices and summary statistics were estimated using Genodive (Meirmans, 2006) and GenAlEx (Peakall and Smouse, 2012); diversity indices included the average number of alleles per population, number of private alleles ( $A_P$ ), observed and expected heterozygosity ( $H_O$  and  $H_E$ , respectively), and the inbreeding coefficient ( $F_{IS}$ ). Because we observed large numbers of identical genotypes, we used Genodive to calculate Nei's clonal diversity ( $D$ ) and to assign individuals to clones, with missing data not counted and a threshold of 0 differences to assign individuals to the same clone. We also analyzed the relatedness among individuals in the entire dataset using the software COANCESTRY v 1.0. Relatedness estimates were obtained using the triadic estimator (Wang, 2007) with 100 bootstraps while accounting for inbreeding. For relatedness estimates, values when individuals are unrelated, half sibs, full sibs, and parent-offspring are expected to be around 0, 0.25, 0.5, and 0.5, respectively (Wang, 2007). Values approaching 1 would indicate identical genotypes.

To understand the hierarchical partitioning of genetic variation within and among populations and individuals, we used analyses of molecular variance (AMOVA; Excoffier et al., 1992). Because patterns of genetic diversity were strikingly different between *L. exigua* var. *exigua* and *L. exigua* var. *laciniata* (see results), AMOVA was analyzed separately for the two taxa. We conducted AMOVA as implemented in Arlequin, using a locus-by-locus AMOVA with 1,000 permutations (Excoffier and Lischer, 2010).

**TABLE 2** | *Leavenworthia exigua* microsatellite primer information.

Locus name	Forward primer sequence	Reverse primer sequence	Repeat motif	Size range in var. <i>laciniata</i>	Number of alleles in var. <i>laciniata</i>	Size range in var. <i>exigua</i>	Number of alleles in var. <i>exigua</i>
Leex 131	*CATCTAGGCGCTGATGTGATC	AGCCACAATTAGATCTCTGACC	ATATC (12)	125–130	2	105–130	5
Leex 101	*TTTCTCTCGGCTGGTCAAC	TGCTGATCTAATCGTCCACAAC	AT (11)	160–178	4	156–186	8
Leex 062	*CCGCGTTGACCTTAAGCTTC	CTTCTCTCGTTGCGCATTG	AG (15)	173–181	3	169–189	4
Leex 072	*TCGTAAACAGACCCACCGTAC	ACACCTCCACCATCATCTTTC	AAC (8)	271	1	259–271	3
Leex 077	*TCACGACCCGCATTACATTAC	GGAGAAACCCTGGAGACTCTG	AG (9)	118	1	106–118	3
Leex 123	*AGGCATCTTCTGGACCAAATC	TCCAGAGCACGGCAGAATC	AG (9)	154	1	154–182	4
Leex 027	*ATTTCTCTCTGCACGTGAC	AGTCTCTTTGACAAGCCTC	AT (11)	183–187	2	161–199	9
Leex 086	*AGCTTTGCTCCAACACAGC	TCCAAGTACCCTTTCAGCTTC	AAAT (6)	271–282	2	276–283	5
Leex 128	*TGCTGGCTTTAGTGATTACGC	CTCCAACCATCAACACTCCAG	ACT (8)	117	1	117–128	3
Leex 071	*ATGGTCTCTGGTAGTGTCTG	TGGTGGATCTGGAATGGAC	AAG (12)	167–188	3	160–207	8
Leex 048	*AGAACTTGGAAAGATGGCTGC	CTCATCGCTGCGGATTCTTC	AT (9)	225–264	2	225–278	3
Leex 026	*TGGAGGAGGAAGTTGACTACG	GAGTGCCACATCAGAACCG	AAG (8)	344	1	338–347	3
Leex 129	*CTGCAGCTTCGTCATCTTCG	ACACCTTCACAGTCTCACCTC	AT (9)	124	1	121–131	5
Leex 031	*ATAGATGGGCTGGCACTCTC	GAGAGAGAATCATTGATCCGCG	ATC (9)	172	1	127–175	5
Leex 042	*CATGGCTGAGCAACCAAGTC	AGAAAGAGCAGAGTCCACCG	AG (9)	247	1	245–255	5
Leex 001	*GAACCAACTGTCTCCCTC	GGTATACCTAACGGCCCAAG	AT (10)	463–467	2	463–467	2

Primer sequences (forward and reverse) and characteristics of 16 novel microsatellite loci developed for *L. exigua*, including repeat motif, allelic size range in base pairs and number of alleles as determined in 440 individuals from 21 populations of *Leavenworthia exigua* var. *laciniata* and 136 individuals from six populations of *Leavenworthia exigua* var. *exigua*. \*An M13 tag (CACGACGTTGTAAACGAC) added to 5' end of primer for amplification with a universal, fluorescently labeled M13 primer.

Patterns of genetic structure were analyzed by calculating pairwise  $F_{ST}$  between all possible pairs of populations in Genodive. Because  $F_{ST}$  does not scale linearly with changes in diversity, we also calculated a standardized measure of pairwise population differentiation, Jost's D (Jost, 2008) in Genodive. We also used GenAlEx to calculate Nei's pairwise genetic distance among populations (Nei, 1972), which was visualized using a neighbor-joining phylogram using PAUP\* (Swofford, 2002).

To investigate patterns of genetic structure in *Leavenworthia exigua* without *a priori* grouping of individuals into populations, we also analyzed our data using the Bayesian program STRUCTURE (Pritchard et al., 2000). We analyzed patterns of genetic structure in the entire data set using 10 independent runs at each  $K$  from 1 to 15, allowing for admixture, correlated allele frequencies, with population information used only to organize individuals in figures to understand how population designations corresponded to patterns of genetic structure. After preliminary analyses to determine the adequate burn-in and number of iterations, we used a burn-in of 250,000 iterations and a run length of 500,000 iterations for each chain. To ensure convergence and repeatability, we examined the groupings across all runs at each  $K$  in CLUMPAK (Kopelman et al., 2015) to ensure that the results of analyses were fully repeatable (Gilbert et al., 2012). To determine the optimal value of  $K$ , we used Structure Harvester (Earl and Vonholdt, 2012) to visualize  $\Delta K$  (Evanno et al., 2005) and to plot the  $-\ln$  likelihood values at each value of  $K$ . As recommended in the STRUCTURE documentation (Pritchard et al., 2000), we selected the  $K$ -value at which the  $-\ln$  likelihood

values began to plateau and that showed clear patterns of genetic structure.

## RESULTS

### Patterns of Genetic Diversity in *Leavenworthia exigua*

In *L. exigua* var. *laciniata*, we found almost no genetic variation within and among populations. Eight of the 16 loci showed only a single invariant allele across all 440 individuals (Table 2), five loci showed only two alleles across all individuals of *L. exigua* var. *laciniata*, and only three loci had >2 alleles (Table 2). Of the loci that showed >1 allele, two loci demonstrated fixed heterozygosity with the two alleles invariant across all individuals of *L. exigua* var. *laciniata*, one locus showed fixed heterozygosity in all individuals of *L. exigua* var. *laciniata* except one alternative allele present in one population, and five loci showed a single invariant allele that was fixed across nearly all individuals of *L. exigua* var. *laciniata* except for one or two populations, where an alternative allele was present. Thus, the vast majority of individuals in *L. exigua* var. *laciniata* were all genetically identical with the exception of five populations that each differed from the dominant genotype by 1–2 private alleles (Table 3). We found a single genotype in each population and clonal diversity values were zero for all populations (Table 3). Further, assignment of individuals to clones identified only one single clone across all 440 individuals of *L. exigua* var. *laciniata*. Mean relatedness between individuals in each population was also extremely high, ranging from 0.83 to 1.0 (Table 3), and results largely showed high relatedness among individuals of *L. exigua* var. *laciniata*

**TABLE 3** | Levels of genetic diversity in sampled populations of *Leavenworthia exigua*, including number of sampled individuals per population, number of genotypes found in each population, clonal diversity (D), mean relatedness among individuals within a population (R), average number of alleles (A), private alleles ( $A_P$ ) observed, and expected heterozygosity ( $H_O$  and  $H_E$ , respectively), the inbreeding coefficient ( $F_{IS}$ ).

Population name	Variety	Sample size	# genotypes	D	R	A	$A_P$	$H_O$	$H_E$	$F_{IS}$
Lac 262	var. <i>laciniata</i>	24	1	0	0.94	1.19	0	0.172	0.093	-0.855*
Lac 263	var. <i>laciniata</i>	22	1	0	0.97	1.19	0	0.182	0.094	-0.942*
Lac 264	var. <i>laciniata</i>	23	1	0	0.91	1.19	0	0.163	0.091	-0.786*
Lac 265	var. <i>laciniata</i>	24	1	0	0.96	1.19	0	0.177	0.093	-0.903*
Lac 266	var. <i>laciniata</i>	12	1	0	1.00	1.19	0	0.188	0.094	-1.000*
Lac 267	var. <i>laciniata</i>	15	1	0	0.98	1.19	0	0.183	0.094	-0.956*
Lac 268	var. <i>laciniata</i>	17	1	0	0.91	1.25	0	0.176	0.100	-0.766*
Lac 269	var. <i>laciniata</i>	17	1	0	0.88	1.38	2	0.183	0.115	-0.598
Lac 270	var. <i>laciniata</i>	23	1	0	0.99	1.19	0	0.185	0.094	-0.971*
Lac 271	var. <i>laciniata</i>	22	1	0	0.89	1.19	0	0.156	0.091	-0.702*
Lac 272	var. <i>laciniata</i>	23	1	0	0.95	1.13	0	0.106	0.061	-0.747*
Lac 273	var. <i>laciniata</i>	23	1	0	0.97	1.13	0	0.114	0.062	-0.837*
Lac 274	var. <i>laciniata</i>	20	1	0	0.85	1.19	0	0.097	0.073	-0.330
Lac 275	var. <i>laciniata</i>	21	1	0	0.95	1.13	0	0.110	0.062	-0.762*
Lac 276	var. <i>laciniata</i>	18	1	0	0.85	1.25	0	0.156	0.107	-0.464*
Lac 277	var. <i>laciniata</i>	23	1	0	0.90	1.25	0	0.181	0.108	-0.671*
Lac 278	var. <i>laciniata</i>	24	1	0	0.79	1.38	1	0.180	0.123	-0.471*
Lac 279	var. <i>laciniata</i>	24	1	0	0.91	1.25	1	0.137	0.083	-0.645*
Lac 280	var. <i>laciniata</i>	24	1	0	0.88	1.25	1	0.166	0.098	-0.690*
Lac 35A	var. <i>laciniata</i>	21	1	0	0.83	1.19	1	0.107	0.069	-0.546*
Lac 22A	var. <i>laciniata</i>	20	1	0	0.94	1.31	0	0.148	0.104	-0.421
Exigua 01	var. <i>exigua</i>	25	7	0.677	0.94	1.50	13	0.189	0.118	-0.598*
Exigua 02	var. <i>exigua</i>	24	18	0.964	0.42	2.88	6	0.160	0.424	0.623*
Exigua 03	var. <i>exigua</i>	24	11	0.862	0.56	2.00	0	0.161	0.336	0.520*
Exigua 04	var. <i>exigua</i>	21	2	0.095	0.51	2.19	3	0.146	0.268	0.456*
Exigua 05	var. <i>exigua</i>	21	4	0.662	0.81	1.56	3	0.131	0.194	0.325*
Exigua 06	var. <i>exigua</i>	21	13	0.829	0.55	1.88	3	0.180	0.365	0.508*

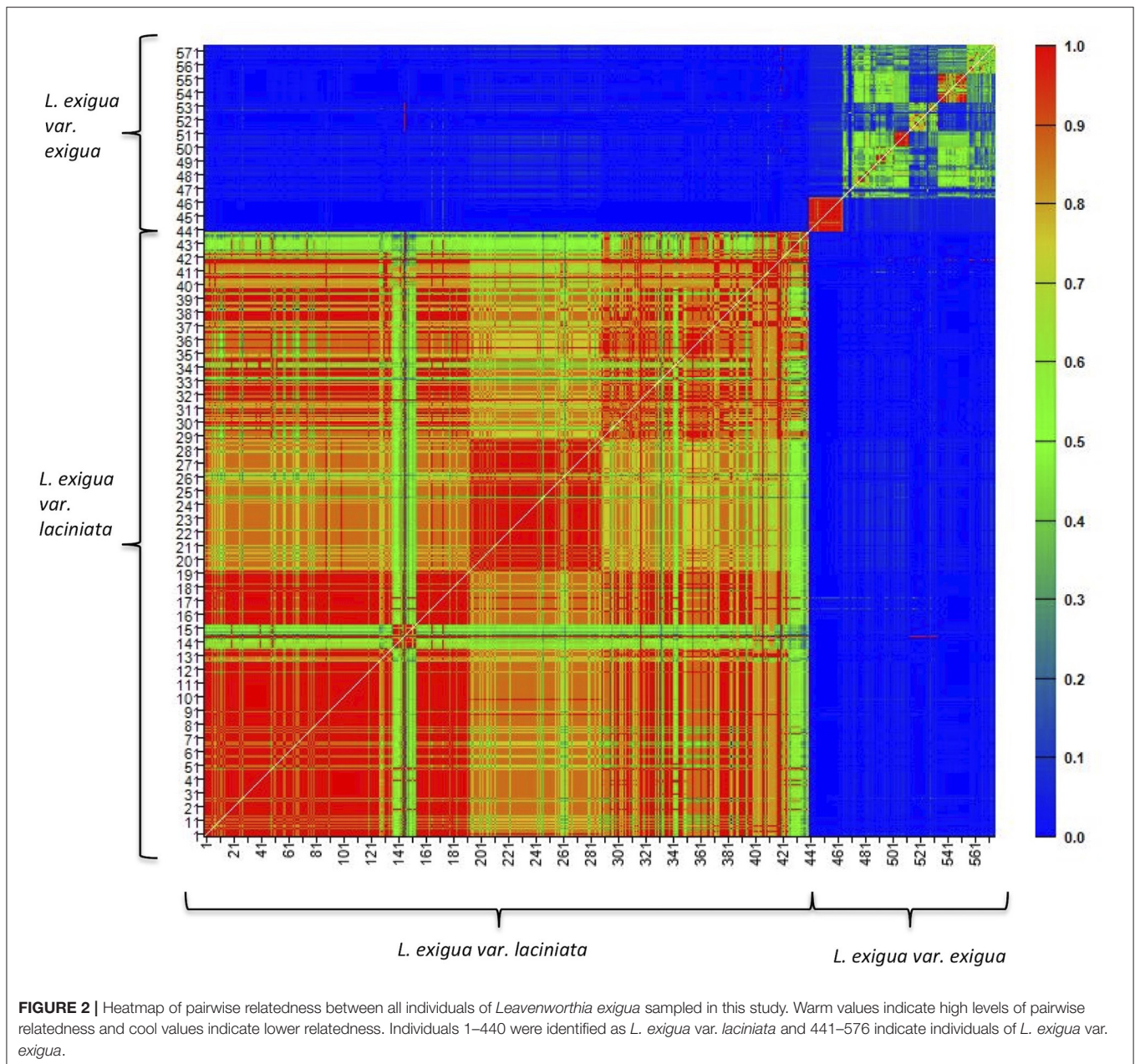
Asterisks by the inbreeding coefficient values indicate populations that exhibited significant deviations from Hardy-Weinberg equilibrium ( $P > 0.05$ ), with asterisks by negative  $F_{IS}$  values indicating significant heterozygote excesses and asterisks by the positive  $F_{IS}$  values indicating significant heterozygote deficiencies.

regardless of where the individuals were sampled (Figure 2). One exception to this is population 269, which showed two private alleles (Table 3) and showed lower relatedness values in comparison to individuals from different populations. Because several loci demonstrated fixed heterozygosity, the average number of alleles per locus was slightly greater than one for all populations of *L. exigua* var. *laciniata* (range 1.188–1.375; Table 3) and observed heterozygosity (range 0.106–0.188; Table 3) was greater than expected heterozygosity (range 0.061–0.115) in all populations, resulting in negative values of  $F_{IS}$  in every population (range -0.464 to -1; Table 3). All but one population of *L. exigua* var. *laciniata* demonstrated significant deviations from Hardy-Weinberg equilibrium (i.e., significant heterozygote excess; Table 3) due to some loci exhibiting fixed heterozygosity across all individuals in a population.

In comparison to *L. exigua* var. *laciniata*, *L. exigua* var. *exigua* showed much greater genetic variation. Although a subset of individuals was genetically identical within each population, we found between 2 and 18 distinct genotypes in each population of *L. exigua* var. *exigua* and clonal diversity ranged from 0.095

to 0.964 (Table 3). For all populations of *L. exigua* var. *laciniata* except Exigua 01, mean relatedness values were lower than those found in *L. exigua* var. *laciniata*, ranging from 0.42 to 0.81 (Table 3), although they were still generally high, likely due to inbreeding in most populations (see below). Mean relatedness in population Exigua 01 was 0.95, similar to values found in *L. exigua* var. *laciniata*. Mean relatedness was also higher within populations than between populations (Figure 2). We found between 3 and 9 alleles at each locus across all 136 individuals of *L. exigua* var. *exigua* (Table 2). Within populations, the average number of alleles per locus ranged from 1.5 to 2.875 (Table 3). Populations of *L. exigua* var. *exigua* contained unique genetic variation, ranging from 3 to 13 private alleles in every population except population Exigua 3 (Table 3). For all populations except Exigua 01, expected heterozygosity ranged from 0.118 to 0.424, observed heterozygosity ranged from 0.131 to 0.189 (Table 3), and expected heterozygosity was greater than observed heterozygosity. Correspondingly, these populations demonstrated significant heterozygote deficiencies and  $F_{IS}$  values that were <0.325 (Table 3). In contrast, population Exigua





01 exhibited greater observed heterozygosity than expected heterozygosity, a negative  $F_{IS}$  value of  $-0.598$ , and a significant heterozygote deficiency, all due to three loci that exhibited fixed heterozygosity across the majority of individuals in the population.

## Genetic Structure

AMOVA analyses to understand the hierarchical partitioning of genetic variation within and among populations and individuals revealed large differences in how genetic variation was partitioned in the two varieties of *L. exigua*. In *L. exigua* var. *laciniata*, populations were highly genetically similar to each other and individuals within populations were all genetically

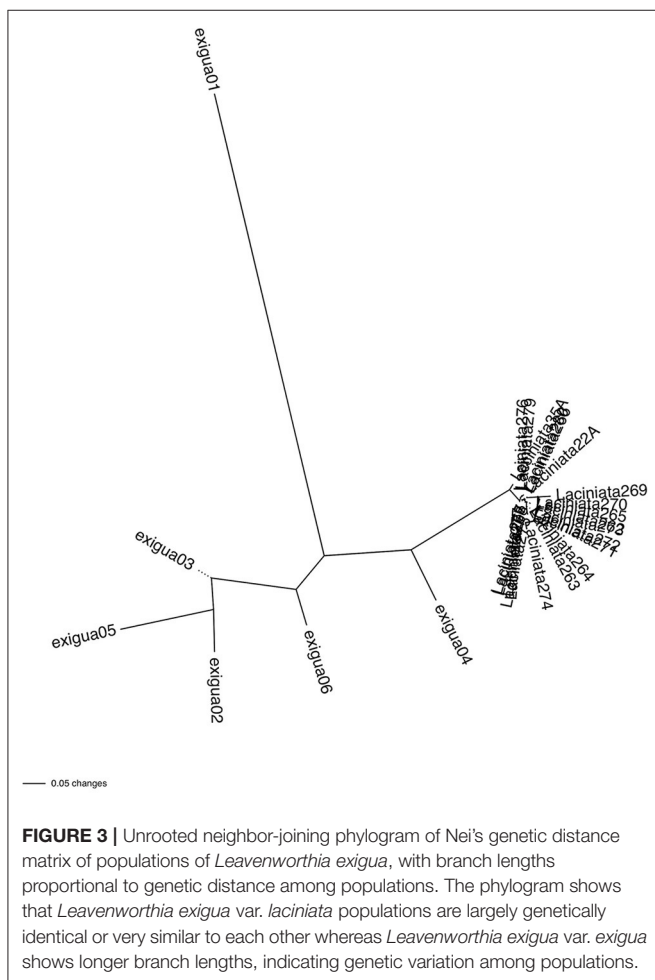
identical, such that 20% of the genetic variation was partitioned among populations, 0% was partitioned among individuals within populations, and 80% was found within individuals (Table 4). In *L. exigua* var. *exigua*, populations were genetically divergent from one another and at least some individuals within populations differed genetically, such that 56% of the variation was partitioned among populations, 13% of the variation was partitioned among individuals within populations, and 31% of the variation was found within individuals (Table 4).

Results of analyses of genetic structure as estimated by pairwise  $F_{ST}$  and Jost's  $D$  were similar to each other, with lower pairwise  $F_{ST}$  values between populations of *L. exigua* var. *laciniata* and greater values found both between populations



**TABLE 4** | Results of AMOVA analyses for *Leavenworthia exigua*, with *L. exigua* var. *laciniata* and *L. exigua* var. *exigua* analyzed separately.

	d.f.	Sum of squares	Variance components	Percent variation explained
<b><i>L. exigua</i> var. <i>laciniata</i></b>				
Among populations	20	276.73	0.30	20.62%
Among individuals within populations	419	484.35	-0.01	-0.72%
Within individuals	440	518.0	1.18	80.11%
Total	879	1279.08	1.47	
<b><i>L. exigua</i> var. <i>exigua</i></b>				
Among populations	5	387.25	1.67	55.71%
Among individuals within populations	130	224.76	0.40	13.31%
Within individuals	136	126.50	0.93	30.99%
Total	271	738.52	3.00	



of *L. exigua* var. *exigua* and between populations of *L. exigua* var. *laciniata* and *L. exigua* var. *exigua* (Supplementary Table 1). Most pairwise  $F_{ST}$  values between populations of *L. exigua* var. *laciniata* ranged from 0 to 0.15, but comparisons involving two specific populations (Lac269 and Lac35A) were all much greater, ranging between 0.06 and 0.49. Pairwise  $F_{ST}$  values ranged from

0.12 to 0.81 between populations of *L. exigua* var. *exigua* and from 0.50 to 0.89 between populations of *L. exigua* var. *laciniata* and *L. exigua* var. *exigua* (Supplementary Table 1). Jost's  $D$ , which is scaled according to the diversity present in a population, ranged from 0.00 to 0.08 between populations of *L. exigua* var. *laciniata*, from 0.08 to 0.81 between populations of *L. exigua* var. *exigua*, and from 0.34 to 0.81 between populations of *L. exigua* var. *laciniata* and *L. exigua* var. *exigua* (Supplementary Table 1).

Similar to the analyses of Pairwise  $F_{ST}$  and Jost's  $D$ , the phylogram based on Nei's genetic distance showed the populations of *L. exigua* var. *laciniata* forming a tight cluster, which was separated by a long branch from populations of *L. exigua* var. *exigua*, with populations of *L. exigua* var. *exigua* also separated by longer branches (Figure 3). Of the *L. exigua* var. *exigua* populations, the Exigua 04 population was placed as the most genetically similar to *L. exigua* var. *laciniata*, and the Exigua 01 population was the most genetically divergent from all other populations (Figure 3).

For analyses with the Bayesian program STRUCTURE, the Evanno method and plots of the  $-\ln$  probability of the data both showed that the optimal number of clusters in the data was  $K = 2$  (Supplementary Figure 1). At  $K = 2$ , STRUCTURE clustered all individuals of *L. exigua* var. *laciniata* into one cluster and the populations of *L. exigua* var. *exigua* into the other (Figure 1B) except the population Exigua 04 was shown to be admixed between the clusters containing the two varieties (Figure 1B). We also inspected results of analyses at greater values of  $K$ ;  $K = 3$  divided population Exigua 01 into its own group, whereas  $K = 4$  did not show any additional clear patterns of genetic structure (results not shown).

## DISCUSSION

### Genetic Diversity and Mating System in *Leavenworthia exigua* var. *laciniata*

Contrary to expectations, the results of this study suggest that *L. exigua* var. *laciniata* may exhibit an asexual mating system. Given that the other two varieties of *L. exigua* are self-compatible (Rollins, 1963), we expected that *L. exigua* var. *laciniata* would show population genetic patterns similar to other self-compatible *Leavenworthia* (Liu et al., 1998; Koelling et al., 2011), such as low

within-population genetic variation, greater among-population variation, low observed heterozygosity, and high positive values of  $F_{IS}$ . All of these were found in all populations of *L. exigua* var. *exigua* except the Exigua 01 population. In contrast, *L. exigua* var. *laciniata* showed virtually no within- or among-population genetic variation at all. Assignment of individuals to clones assigned all 440 individuals of *L. exigua* var. *laciniata* to a single clone, and relatedness between individuals was high regardless of the population in which it was sampled. Results also showed that all individuals in 17 out of 22 populations were genetically identical to each other, while the only variation observed in *L. exigua* var. *laciniata* was unique fixed differences at 1–2 alleles per population in each of five populations, which may have arisen due to point mutations in the dominant genotype in each population that subsequently became fixed. We also observed fixed heterozygosity at several loci, significant excesses of heterozygotes relative to HWE, and high negative inbreeding coefficients, all of which are consistent with the lack of sexual recombination characteristic of asexuality (Balloux et al., 2003). Asexuality has not been documented previously in the genus *Leavenworthia*; the possibility of the existence of a taxon with an asexual mating system in *Leavenworthia* is particularly surprising given that it is such a well-studied genus used as a model for investigating the evolution of mating systems.

One alternative explanation for the lack of genetic variation found in *L. exigua* var. *laciniata* is that it is an artifact of the particular microsatellite markers developed for this study, possibly related in some way to how we isolated the microsatellite loci. However, we employed well-established marker-development protocols that we have used successfully to identify polymorphic microsatellites in a wide diversity of plant species (Swift et al., 2016; Edwards et al., 2019, 2021; Mohn et al., 2021). Furthermore, while developing the markers, we attempted to avoid ascertainment bias by incorporating material from multiple maternal individuals of *L. exigua* var. *laciniata* in the pool of seeds used for marker development, and we screened 44 loci for polymorphism in individuals sampled from across the range of *L. exigua* var. *laciniata*. Yet despite the efforts taken to isolate variable markers, we detected only a small amount of variation at six loci employed for genotyping, while the remaining 10 loci were invariant. These same markers were also variable within and among populations of *L. exigua* var. *exigua* despite being isolated from *L. exigua* var. *laciniata*. To verify the results of microsatellite analysis, we examined the results of a small preliminary AFLP analysis of 48 individuals grown from seeds sampled from five populations of *L. exigua* var. *laciniata* (B. Williams, unpublished data). Although asexuality is difficult to assess using dominant markers, this analysis also revealed low genetic diversity and extremely low genetic distances among populations (Nei's  $D = 0.01$ – $0.06$ ; B. Williams, unpublished data). However, additional comparative genome-wide genetic analyses of *L. exigua* var. *laciniata* and other related taxa are needed to explore the extent of genomic variation in *L. exigua* var. *laciniata* to test further the hypothesis of asexuality.

Although the microsatellite evidence generated in the present study may indicate an asexual mating system in *L. exigua* var.

*laciniata*, how asexuality could be accomplished is an open question. In general, asexual reproduction can be achieved by plants in two ways: vegetative reproduction/clonality and apomixis. Vegetative reproduction/clonality is accomplished chiefly by budding from roots, rhizomes, stems, or storage organs such as tubers, resulting in the production of new ramets of the same genetic individual (Yang and Kim, 2016). Population genetic patterns for such species frequently reveal one or a few fixed heterozygous genotypes within a population, but these species usually exhibit differences in the genotypes present among populations (e.g., Gitzendanner et al., 2012; Fehlbeg, 2017). Clonality also generally has the effect of increasing an individual's lifespan, such that clonal species are typically perennials with lifespans of >5 years, with some genets living up to tens of thousands of years (de Witte and Stöcklin, 2010). *L. exigua* var. *laciniata* has a winter-annual life history strategy, individuals are not thought to persist for more than 1 year, and plants do not develop rhizomes or stolons; it is therefore unlikely that vegetative reproduction is the means by which asexual reproduction is occurring in *L. exigua* var. *laciniata*. The fact that the same genotype of *L. exigua* var. *laciniata* is found across its range indicates that it dispersed among populations. This suggests that asexuality may instead be the result of apomixis, or asexual reproduction by seed. However, apomixis is extremely uncommon in the Brassicaceae, reported from only five genera (*Boechera*, *Draba*, *Erysimum*, *Parrya*, and *Phoenicautis*) and one intergeneric hybrid (*Raphanobrassica*) (Mandáková et al., 2021) of the 349 accepted genera in the Brassicaceae (The Angiosperm Phylogeny Group et al., 2016). If asexuality in *L. exigua* var. *laciniata* is accomplished through apomixis, this would be an independent evolutionary origin of apomixis in the family. However, additional research is needed to investigate whether *L. exigua* var. *laciniata* is indeed apomictic.

Apomixis occurs through the formation of a diploid embryo without involving the fusion of haploid gametes or the act of fertilization (Hand and Koltunow, 2014). The mechanisms that cause apomixis are generally grouped into two categories: sporophytic or gametophytic (Hand and Koltunow, 2014). During sporophytic apomixis, the embryo sac develops following the typical angiosperm sexual pathway, but the embryo arises from diploid somatic ovule cells that surround the embryo sac (Hand and Koltunow, 2014). These embryo cells can develop to maturity only if the sexually derived embryo sac is fertilized, as the sexual and asexual embryos share the endosperm; a seed may therefore contain multiple embryos, although the sexual embryo does not always develop (Hand and Koltunow, 2014). In gametophytic apomixis, which is the more common type of apomixis in angiosperms, the embryo sac develops from either a diploid megaspore mother cell (diplospory) or from any diploid cell of the nucellus (apospory) (Hand and Koltunow, 2014).

Although extensive research has been conducted to understand apomixis in *Boechera*, presently, it is still unclear if *L. exigua* var. *laciniata* is apomictic and if so, whether similar mechanisms gave rise to apomixis in *Leavenworthia*. In *Boechera*, species vary in ploidy and sexuality; some species are diploid and sexual, some are diploid apomicts, and some are triploid apomicts. Although all apomixis in *Boechera* is gametophytic

(Lovell et al., 2013), it can occur both through diplospory and apospory (Carman et al., 2019). Diploid apomicts are thought to have arisen either through hybridization (Beck et al., 2006; Carman et al., 2019) or spontaneously through genetic factors that cause apomixis (Lovell et al., 2013), whereas triploid apomicts are thought to have arisen through intraspecific hybridization between a diploid apomict and another, genetically divergent diploid species (Lovell et al., 2013). Although a previous phylogenetic study in *Leavenworthia* suggested that past hybridization occurred between *L. exigua* and *L. torulosa* (Beck et al., 2006), *L. exigua* var. *laciniata* was not included in this analysis, such that it is unclear whether hybridization may have led to its formation. Chromosome counts for all varieties of *L. exigua* were  $n = 11$  (Rollins, 1963), the lowest number in the genus *Leavenworthia*, indicating that *L. exigua* var. *laciniata* is diploid. The diploid apomicts in *Boechera* produce seeds that have either with a 2C:5C or a 2C:6C embryo-to-endosperm ratio (Lovell et al., 2013). Preliminary flow cytometry of five seeds from one maternal individual of *L. exigua* var. *laciniata* showed that they may have the standard embryo-to-endosperm ratio of 2C:3C generally found in non-apomictic, sexual diploids (Tim Sharbel, pers. comm.). However, it is unclear whether this is consistent across maternal individuals and populations. Thus, flow cytometry of paired leaf and seed samples from multiple populations from all three varieties of *Leavenworthia exigua* and other *Leavenworthia* species with the same chromosome number is needed to further investigate the possibility of apomixis in *L. exigua* var. *laciniata*.

## Genetic Distinctiveness of Varieties of *L. exigua*

The next goal of the study was to address whether *L. exigua* var. *laciniata* is genetically distinct from the other two varieties of *L. exigua*. In addition to the differences in morphology and distinct geographical ranges noted by Rollins (1963), the results of the present study show that the varieties may differ in mating system. In contrast to the putative asexual mating system exhibited by *L. exigua* var. *laciniata*, most populations of *L. exigua* var. *exigua* displayed population genetic patterns consistent with those found in other *Leavenworthia* species with a sexual, self-compatible mating system, including lower observed heterozygosity than expected heterozygosity, high positive inbreeding coefficients, and significant heterozygote deficiencies relative to Hardy-Weinberg equilibrium (Liu et al., 1998; Koelling et al., 2011). *L. exigua* var. *exigua* also showed a much greater number of distinct genotypes per population and overall greater genetic variation than *L. exigua* var. *laciniata*, with the majority of genetic variation partitioned among populations, as is common in self-compatible plant species. However, population Exigua 01 exhibited population genetic patterns more similar to those exhibited by *L. exigua* var. *laciniata*, potentially indicating that one population of *L. exigua* var. *exigua* may exhibit asexuality and that the mating system may vary among populations of *L. exigua* var. *exigua*. Additional sampling of populations across the range of *L. exigua* var. *exigua* is needed to investigate this possibility further. The two varieties analyzed in the present study are also

significantly genetically differentiated, likely because gene flow, which acts to minimize differences among populations, may not be occurring between the two varieties because of geographical separation due to allopatry and potential asexuality in *L. exigua* var. *laciniata*.

Because we were unable to locate populations of *L. exigua* var. *lutea*, we were unable to analyze its mating system and its distinctiveness from the other two varieties of *L. exigua*. Previous analyses showed that *L. exigua* var. *lutea* is self-compatible, such that we expect that it would show population genetic patterns similar to those found in most populations of *L. exigua* var. *exigua*. *L. exigua* var. *lutea* is also allopatric, such that it may not have the opportunity to interbreed with the other two varieties; however, additional research is needed to test whether it is genetically distinct from the other two varieties of *L. exigua*. The extent of reproductive isolation among the three varieties of *L. exigua* is also unknown. If *L. exigua* var. *laciniata* is asexual, then it could be reproductively isolated from the other varieties of *L. exigua*, although many apomictic species retain viable pollen, serving as a mechanism facilitating sexual reproduction (Majeský et al., 2017). Additional analyses involving experimental crosses within and among the varieties paired with genetic and cytogenetic analyses of the resulting offspring are needed to further understand the extent to which the varieties exhibit reproductive isolation and whether *L. exigua* var. *laciniata* exhibits residual sexuality. If indeed *L. exigua* var. *laciniata* is asexual and reproductively isolated from the other two varieties of *L. exigua*, then it may warrant being recognized as a unique species. At the minimum, it should be considered as an evolutionary significant unit for conservation purposes.

## Conservation Implications

The final goal of this study was to evaluate the implications for prioritization of *in-situ* and *ex-situ* conservation efforts for *L. exigua* var. *laciniata*. One of the main objectives of conservation genetics is to preserve the evolutionary potential of a species (Moritz, 2002). In sexual organisms, because recombination can produce different combinations of adaptive genes that can increase fitness, protecting the maximum amount of genetic variation in a species is thought to be important for preserving its adaptive capacity. In practice, researchers may identify unique genetic clusters and devise recommendations to protect these genetic groups to ensure the conservation of all unique subsets of genetic variation present in the species (e.g., Edwards et al., 2021). In *L. exigua* var. *laciniata*, the few observed genetic differences are probably the result of single mutations occurring in an individual that were then passed asexually to its offspring. Thus, to preserve the full range of genetic variation in *L. exigua* var. *laciniata*, it would therefore be necessary to ensure the protection of the five populations that exhibit these small mutations (i.e., those containing private alleles in Table 3), along with at least one population that has the dominant genotype.

Arguably, however, because the evolutionary process is somewhat different for asexual species, the strategy for protecting their evolutionary potential should be different than for sexual

species. In asexual organisms, adaptation may occur if a mutation arises in an individual that increases its fitness relative to its competitors, in which case it will quickly spread through the population. This normally occurs infrequently (Jiang et al., 2010), as most mutations are neutral or have deleterious effects. Furthermore, in obligately asexual species, because no recombination occurs, when two beneficial mutations arise in a population, they cannot recombine and have to compete with each other (Gerrish and Lenski, 1998), such that one may be eliminated from the population. In the absence of recombination, deleterious mutations are also expected to accumulate in asexual species in a process known as Muller's ratchet (Muller, 1964). However, most apomictic plants exhibit some low levels of facultative sexual reproduction, in which case recombination can segregate multiple deleterious mutations into single linkage groups that can be eliminated through selection; this process was shown previously to reduce mutational load in an apomictic species with low rates of facultative sexual reproduction (Hodač et al., 2019). Although it is unknown whether facultative sexual reproduction occurs in *L. exigua* var. *laciniata*, because the likelihood of sexual reproduction likely increases in larger populations, we recommend protecting as many individuals as possible to maintain the evolutionary potential in *L. exigua* var. *laciniata*.

For *ex-situ* conservation, the normal strategy for seed banking for sexual organisms is to collect seed from a geographically representative set of populations to capture the full range of genetic diversity present in a species (Guerrant et al., 2014), which allows the *ex-situ* collection to serve as a backup in the case that unique genetic variation in the species is lost. Given that *L. exigua* var. *laciniata* exhibits virtually no genetic variation, this will be easily accomplished in *L. exigua* var. *laciniata*. However, even if they do not contain large amounts of genetic diversity, *ex-situ* collections could still serve as material for reintroductions to promote the persistence of the species. Reintroductions using a large number of individuals, regardless of source, would help increase population size, and concomitantly increase the evolutionary potential of the species.

## Conclusions and Future Directions

Although the genus *Leavenworthia* has been used extensively as a model for investigating the evolution of mating systems in plants, the results of this study indicate that one of the most poorly known, understudied species in the genus may have a previously unrecognized, asexual mating system that is unique to the genus. The existence of an asexual species with virtually no genetic variation in such a well-studied genus offers important opportunities for future research. Given the interest in the evolution of mating systems in *Leavenworthia*, it is likely that the discovery of a new mating system in *Leavenworthia* will spark additional research into the origins and mechanisms underlying asexuality in *Leavenworthia*. Furthermore, the lack of genetic variation in *L. exigua* var. *laciniata* provides opportunities for understanding how genetic variation affects a species' response to its environment and its

ability to persist; for example, we are currently investigating whether *Leavenworthia* species that differ in mating systems and levels of genetic variation exhibit differences in epigenomic and phenotypic plasticity in response to environmental stress. Similarly, we are also investigating whether epigenomic variation and phenotypic variation is present in the threatened *L. exigua* var. *laciniata* that may enable it to persist in response to environmental variation despite a lack of genetic variation. Indeed, *L. exigua* var. *laciniata* may be a useful model for understanding the importance (or lack thereof) of genetic variation in the persistence of rare plants and how to conserve a threatened species with virtually no genetic variation in a changing climate.

## DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the Figshare repository, <http://dx.doi.org/10.6084/m9.figshare.19116224>; and GenBank, accession number PRJNA808735.

## AUTHOR CONTRIBUTIONS

CE designed the study, acquired funding for the study, and conducted field work. BB and BW conducted lab work. BB made maps. CE and BW analyzed the data. CE wrote the manuscript. All authors revised the article and approved the final version of the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcosc.2022.831085/full#supplementary-material>



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