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GENETIC DIVERSITY WITHIN AND AMONG POPULATIONS OF *TRIFOLIUM LEMMONII* (FABACEAE)

SHANNON SOMMER SATKO
Porterville College
100 E. College Avenue
Porterville, California 93257
shannon.satko@portervillecollege.edu

MICHAEL A. VINCENT
W.S. Turrell Herbarium (MU)
Biology Department
Miami University
Oxford, Ohio 4056
vincenma@miamioh.edu

ABSTRACT

Trifolium lemmonii S. Wats. is restricted in range to Plumas, Sierra, and Nevada cos., California, and Washoe Co., Nevada. Allozyme analysis was used to estimate genetic diversity of clover populations. Starch gel electrophoresis was used to determine genotypes of nine enzymes at eleven loci for 23 populations of the species. Moderate genetic variation was found. Nine of eleven loci observed were polymorphic. Average number of alleles per locus was 1.61, and 38.4% of loci were polymorphic. The mean F_{ST} value for all loci was 0.213, indicating high levels of partitioning among populations, but most of this partitioning separates *T. lemmonii* into two clusters of genetic similarity, corresponding with geography. Modified Rogers distance estimated average distance between the two geographic clusters as 0.226. No corresponding morphological differences were identified. The species appears surprisingly genetically robust given its limited range.

Trifolium lemmonii S. Wats is a lovely and little-known perennial clover that exists within a highly restricted range in the eastern Sierra Nevada mountains. The species is limited to pockets of suitable habitat within a small geographic area of Plumas, Sierra, and Nevada counties in California, and Washoe County in Nevada (Skinner & Pavlik 1994). Most populations of *T. lemmonii* are found on the Plumas National Forest in Plumas County. The only known occurrence in Nevada County is part of a Sierra County population that crosses the county line. In Washoe Co., Nevada, *T. lemmonii* has been collected from two locations: Peavine Mountain and Dogskin Mountain. Because of its highly restricted range, *T. lemmonii* has a California state listing of S3 ("vulnerable") and a ranking of 4.2 ("plants of limited distribution") in the California Rare Plant Program (CNPS 2021). A review of the nomenclature, morphology, and genetic diversity of *T. lemmonii* was conducted to contribute to a better understanding of the species.

Taxonomic treatments of *Trifolium lemmonii* have varied somewhat since its discovery. *Trifolium lemmonii* was first described and published by Sereno Watson (1876, as *Trifolium lemmoni*), with the type specimen *Lemmon 978* [GH!] (Taylor 1981). The original location given by Watson was "Lassen's Peak," but the author published a correction of the type locality as "Flat in Sierra Valley" in 1880 (Watson 1880; Martin 1943). McDermott (1910) treated *T. lemmonii* as *T. gymnocarpon* var. *lemmoni* (S. Wats.) McDerm., but subsequent authors have treated it as having species rank (Martin 1943; Gillett 1972; Vincent & Isley 2012). Martin (1943) distinguished *T. lemmonii* from *T. gymnocarpon* by its longer peduncles, thinner calyx teeth, greater number of flowers per inflorescence, awned wing petals, and enlarged rachis. Gillett (1972) noted that the calyx lobes of *T. lemmonii* are nearly the length of the tube, and that its flowers are larger than those of *T.*

gymnocarpon. Mozingo and Williams (1980) noted that *T. lemmonii* has coarsely dentate leaflet margins, in contrast to the finely-toothed leaflet margins of *T. gymnocarpon*. Latschaschvili (1976) made the combination *Lupinaster lemmonii* (S. Wats.) Latsch., but subsequent authors (Zohary & Heller 1984; Vincent & Isley 2012) have not adopted this classification.

Zohary and Heller (1984) included *Trifolium lemmonii* in sect. *Lotoidea* subsect. *Lupinaster* as part of the only group within the genus having representatives with more than three leaflets. Other authors have chosen to treat the species differently. Gillett (1972), for example, included *T. lemmonii* in sect. *Lupinaster* (Adanson) Seringe and cautioned that the New World representatives of *Lupinaster* do not seem to fall into clean, natural series, and he downplayed the importance of the presence of more than three leaflets in some species. Most recently, Ellison et al. (2006) placed *T. lemmonii* in a monophyletic sect. *Involucrarium*, in a clade with *T. bolanderi* A. Gray and *T. kingii* S. Wats.

Trifolium lemmonii generally occurs in *Artemisia arbuscula* subsp. *arbuscula* (dwarf sagebrush) flats, on clayey, rocky soils 1580-2075m in elevation. Populations differ considerably in size from about 28 individuals scattered over about $28m^2$, to thousands of individuals spread over an area of roughly $244km^2$.

Disturbances to *Trifolium lemmonii* include grazing, fire, and recreational vehicles (Taylor 1981). Most of the known populations are found on National Forest land, and are or have been grazed by cattle or sheep, and presumably deer.

Trifolium lemmonii was largely ignored for many years, with only one U.S. Forest Service collection being made between 1907 and 1972. When Gordon True (1974) collected *T. lemmonii* in 1972, it was hailed as a rediscovery of the species and drew attention to the clover. Since then, the conservation status of *T. lemmonii* has been a matter of debate. State and federal agencies began paying attention to *T. lemmonii*, and as more and more occurrences of the clover were documented, immediate concern for the species decreased (Taylor 1981). Currently, *Trifolium lemmonii* is considered a watch list species by the Plumas National Forest, the California Native Plant Society (California Native Plant Society, Rare Plant Program 2021) and the Nevada Natural Heritage Program (Nevada Natural Heritage Program 2021), indicating that it merits further monitoring. Because *T. lemmonii* has a restricted range, has historically been considered at risk, and is a species of interest to the National Forest that controls the majority of its habitat, an assessment of the status of the species was in order.

One way to observe the fitness of a plant species is to look at its genetic diversity and how that diversity is partitioned. A method for observing genetic variation is allozyme analysis using starch gel electrophoresis. Similar studies of both rare and common clovers that have used this technique allow for comparisons between species (Hickey et al. 1991; Linscott 1994), which may provide a point of reference in estimating the genetic robustness of the species. Another way to estimate plant diversity is through an analysis of morphological observations. When vegetative and floral character differences are slight, phenetic methods can be a valuable tool for objectively delimiting subgroups.

The objectives of this study were 1) to estimate genetic diversity within and among populations of *Trifolium lemmonii* using allozyme analysis; 2) to test for subspecific morphological variation using phenetic analysis, and to determine whether or not morphological differences correspond with any observed genetic differences; and 3) to gather information based on genetic diversity that may assist in determining an appropriate conservation status of *T. lemmonii*.

METHODS

Field work was conducted in Plumas, Sierra, and Nevada counties in California and Washoe County in Nevada, May-July 1998. Location data from the U.S. Forest Service and from herbarium specimens (from herbaria AHUC, CAS, F, MO, MU, NESH, NY, POM, RENO, US (acronyms from Thiers 2021) were used to locate known populations of *Trifolium lemmonii*. Fifty-seven populations of the clover were located: 54 from California, and 3 from Nevada (Fig. 1).

Genetic Diversity

For the purpose of this study, a population was considered to be a spatially continuous or nearly continuous grouping of individuals. Voucher specimens were collected from the *Trifolium lemmonii* locations and deposited in the Willard Sherman Turrell Herbarium (MU) at Miami University. In populations selected for allozyme analysis, two leaves were collected from each of 30 individuals per population, spaced throughout the population. Laboratory analysis was conducted on fresh, refrigerated material when possible, and frozen material when more than two weeks elapsed between collection time and laboratory analysis.

Twenty-three populations of *Trifolium lemmonii* were sampled for allozyme analysis; 3 from Washoe County, 3 from Sierra County, 1 from Nevada County, and 16 from Plumas County. These populations were selected to cover the species range as evenly as possible (Figure 2). Each population used in the allozyme analysis was assigned a 4-character code. For each population, 20 individuals of the 30 sampled were used for analysis. The 20 individuals were selected from usable material that was roughly evenly spread along the original collection transect line(s). An exception was the population designated L07C that only had a sample size of 10.

Leaflets were ground with sand in 2-3 drops of microbuffer grinding buffer (Linscott 1994) with added Polyvinylpyrrolidone (PVP-40) and ß–mercaptoethanol. Whatman #3 filter paper wicks were used to absorb the resulting liquid. The wicks were placed sequentially on a gel made of 13% hydrolyzed potato starch and subjected to electrophoresis using standard electrophoretic techniques with four buffer systems: 5, 7, and 11 from Soltis et. al (1983), and 8H, modified from Soltis et. al (1983). Each completed gel was sliced and stained for nine enzyme systems (Table 1).

Table 1. Nine enzyme systems observed and buffer systems used in starch gel electrophoresis. Systems 5, 7, 11,and a modified system8 (8H) from Soltis et al. (1983).

Enzyme	Buffer system
Alcohol dehydrogenase (ADH)	8H
Glucose-6-phosphate isomerase (GPI)	5
Isocitrate dehydrogenase (IDH)	11
Leucine amino peptidase (LAP)	7
Malate dehydrogenase (MDH)	11
Mannose-6-phosphate isomerase (MPI)	8H
6-Phosphogluconate dehydrogenase (6-PGDH)	5
Phosphoglucomutase (PGM)	5
Triose-phosphate isomerase (TPI)	8H

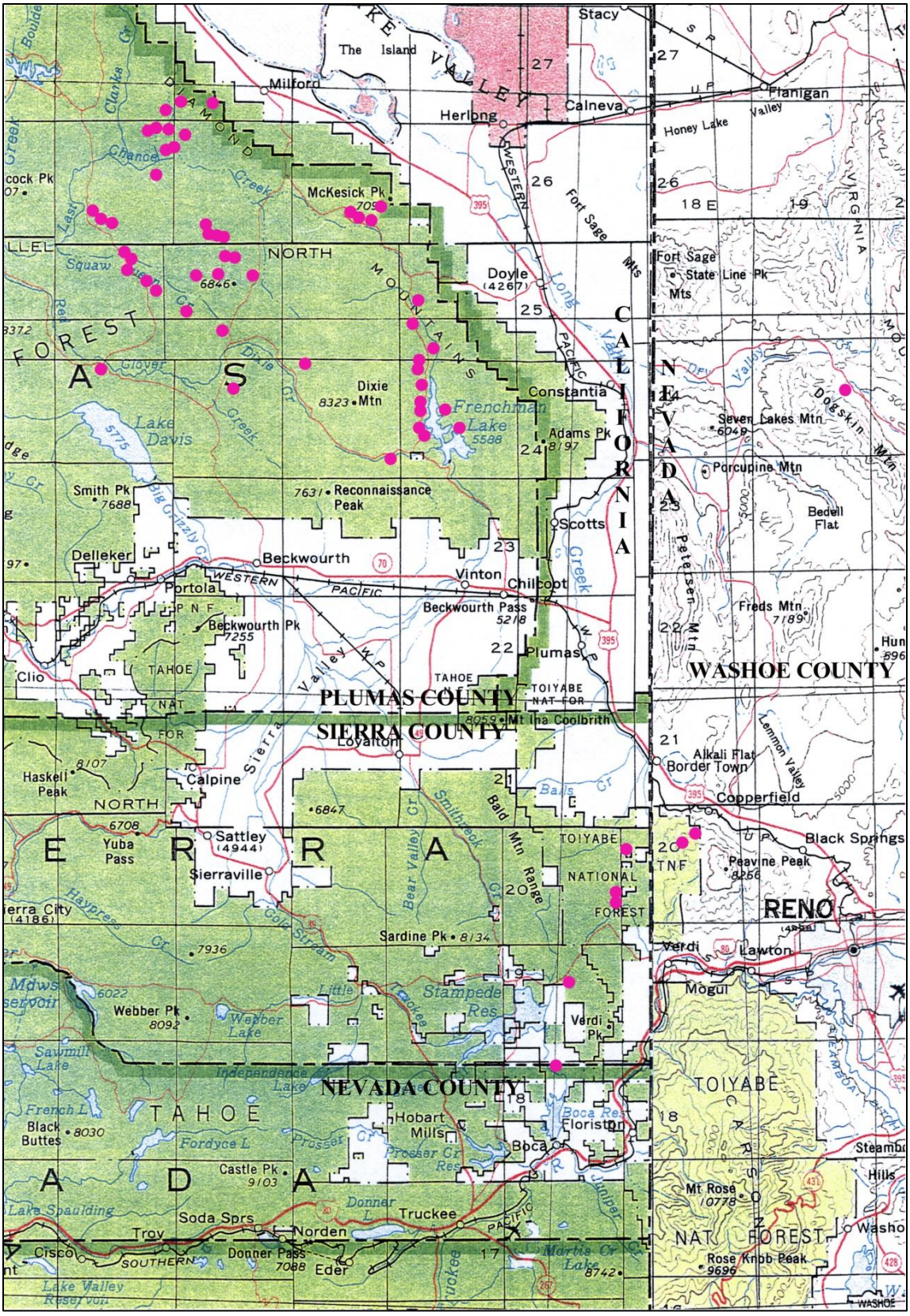

Figure 1. *Trifolium lemmonii* populations located during this study (indicated by pink dots). Map modified from U.S. Dept. of the Interior Geological Survey, 1981 (CA) and 1984 (NV).

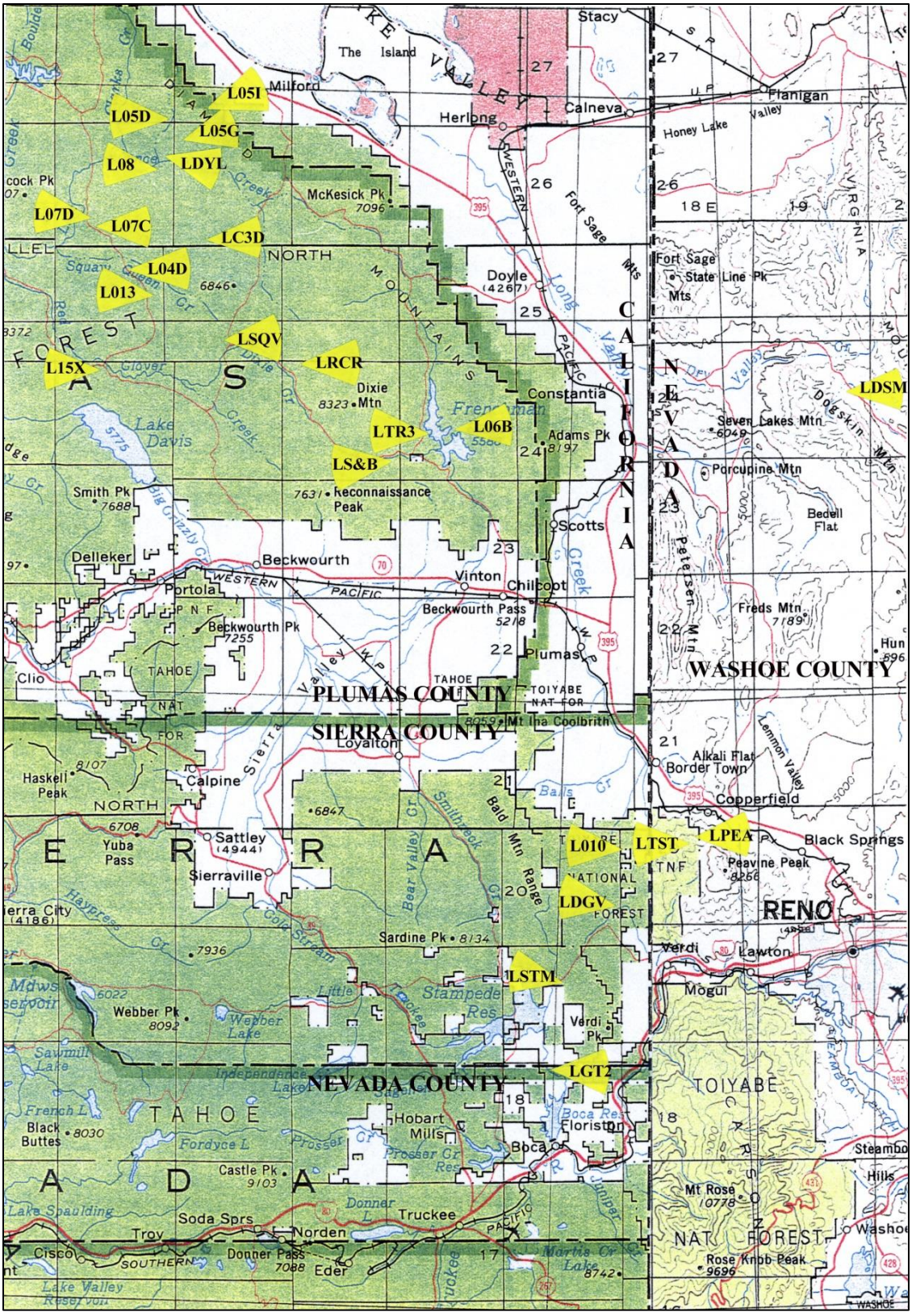

Figure 2. Locations of *Trifolium lemmonii* populations sampled for allozyme analysis. The yellow arrows point to each population location and are labeled with the code used in the study. Map modified from U.S. Dept. of the Interior Geological Survey, 1981 (CA) and 1984 (NV).

The gels were scored for homozygosity, heterozygosity and allele position for the observed loci. Alleles were designated alphabetically, with A being the fastest allele at each locus. An internal control of *Trifolium medium* was used to facilitate comparison of alleles, and line-up gels were run to confirm allele position. Analyses of allozyme data were conducted using BIOSYS-1 (Swofford and Selander 1981). For each population, BIOSYS-1 calculated mean number of alleles per locus, mean direct count heterozygosity, and percentage of polymorphic loci (using the 95% standard for polymorphic loci). Polymorphic loci were tested for agreement with Hardy-Weinberg equilibrium expectations. A Bonferroni correction was applied to avoid false conclusions of significance (Lessios 1992). BIOSYS-1 was used to determine genetic identity using Nei's (1972) procedure, and to measure genetic distance using Nei's (1978) unbiased minimum distance measure, as well as the method described by Wright (1978) and called "Modified Rogers distance" by BIOSYS-1 (Swofford and Selander 1981). Dendrograms for the measures of distance and identity were produced using the unweighted pair group method of cluster analysis. Wright's (1978) F statistics F_{IS}, F_{IT}, and F_{ST} were also calculated. After the data were analyzed as a whole, populations were divided into two regions (eastern populations: L010, LDGV, LDSM, LGT2, LPEA, LSTM, LTST; and western populations: L04D, L05D, L05G, L05I, L06B, L07C, L07D, L08, L013, L15X, LC3D, LDYL, LRCR, LSQV, LS&B, LTR3), and the statistics mentioned above were calculated separately for each of the two regions. Two sample t-tests compared the average number of alleles per locus, average heterozygosity, and percentage of polymorphic loci between the eastern and western groups of *T. lemmonii* populations.

Morphology and Morphological Data Analysis

Principal coordinate analysis (PCOORDA) involving 40 vegetative and floral characters measured from 63 *Trifolium lemmonii* specimens was performed using NTSYSpc (Numerical Taxonomic System for the IBM, Rohlf 1997). Each specimen was designated with a code to distinguish whether the specimens came from eastern or western populations. After the initial PCOORDA was performed, less informative characters were removed from analysis in order to better resolve any existing groups. Groups of characters were culled out several times, and PCOORDA was conducted for each new assemblage of characters in an attempt to identify subgroups. Details and results for the morphological analyses can be found in Sommer (1999).

Ecological observations, including associated species, slope, aspect, and evidences of disturbance were also recorded for *Trifolium lemmonii* populations.

RESULTS

Genetic Diversity

Nine enzyme systems with 11 loci were found to be consistently scoreable for the 23 populations examined. Of the 11 loci, nine (81.8%) were polymorphic. GPI-1 and TPI-2 were monomorphic across all populations. MDH-1 was monomorphic for the eastern populations, and polymorphic in 25% of the western populations. Allozyme analysis shows that *Trifolium lemmonii* is separated into two clusters of genetic similarity. These clusters follow a geographic pattern: an eastern cluster in Sierra (CA), Nevada (CA), and Washoe (NV) Counties, and a western cluster in Plumas County (CA) (Figure 3). With the exception of LPEA (an eastern population that only showed an average of 1.18 alleles per locus and 18.2% of polymorphic loci), the populations show considerable genetic diversity for a species of such restricted range. Average number of alleles per locus for all populations was 1.61, and the average percent of polymorphic loci for all populations was 38.4%.

Table 2 summarizes the measures of genetic diversity for each sampled population, as well as for all the populations taken as a whole, for the eastern cluster, and for the western cluster. In each measure of diversity (average number of alleles per locus, average direct-count heterozygosity, and

percent of polymorphic loci), the western populations were found to be more diverse than the eastern populations. Two sample t-tests revealed a significant difference between the eastern and western groups of populations for these three measures of diversity (Table 3).

The western populations have 10 alleles that were not observed in any of the eastern populations (Table 4). The eastern populations have no unique alleles. Alleles not found in the eastern populations are spread throughout the western cluster, with 14 of the 16 sampled populations containing at least one of the uncommon alleles.

No populations were found to be homozygous at all loci. When using an uncorrected critical value of α=0.05, 7.7% of the loci were found not to fit the Hardy-Weinberg equilibrium model (HWE). All loci that diverged from HWE showed a deficiency of heterozygotes. However, when the Bonferroni correction was performed, all loci conformed to the HWE model.

Table 5 summarizes Wright's (1978) F statistics for the populations taken as a whole, for the eastern cluster of populations, and for the western cluster of populations. These statistics reflect a genetic division between eastern and western populations of *T. lemmonii*. According to Wright (1978), values for F that are greater than 0.05 indicate significant genetic differentiation. Values from F=0.15 to F=0.25 suggest "moderately great differentiation," and F values greater than F=0.25 indicate "very great differentiation." When looked at separately, the mean F_{ST} values for the eastern populations (F_{ST}=0.09) and the western populations (F_{ST}=0.087) suggest modest genetic partitioning within each geographic region, with 9.6% of the variation being found among the eastern populations and 8.7% being found among the western populations. When the data from all the populations is combined, the total F_{ST} value shows a large amount of genetic partitioning (F_{ST}=0.213) that was not seen within either of the regional clusters. This suggests that much of the partitioning of genetic diversity is taking place between the two geographic regions.

F_{IS} and F_{IT} values for the nine polymorphic loci show little differentiation at some loci, and great differentiation at others. Data for all populations taken together shows three times as much differentiation among the populations (F_{ST}=0.213) as within them (F_{IS}=0.069).

Most of the partitioning of genetic diversity is found in one enzyme system (ADH). The A allele is more common in the western populations and the B allele is more common in the eastern populations (Figure 4). If ADH is removed from analysis, mean F_{ST} drops to 0.116.

ADH shows moderate genetic partitioning within the eastern populations (F_{ST}=0.119) and the western populations (F_{ST}=0.090), but when all the populations are looked at together, ADH has an F_{ST} value of 0.407; that is, 40.7% of the genetic diversity is partitioned among populations. The extreme partitioning of genetic variation (40.7%) among populations may suggest some differential selection for ADH alleles between the two regions (Wright, 1978).

The east-west division of the species is also reflected in values for genetic distance and identity. The dendrogram generated from the Modified Rogers distance values shows that genetic distance separates the eastern populations from the western populations (Figure 5). Average distance between the two clusters was 0.226, whereas average distances within the eastern (0.106) and western (0.099) clusters was less. When Modified Rogers distance is calculated without ADH, LDSM (an eastern population) is excluded from both clusters as an outlier, and five of the western populations (LS&B, L06B, L04D, LSQV, LTR3) cluster with the remaining six eastern populations.

Table 2. Summary of measures of genetic variation for sampled *Trifolium lemmonii* populations. Percentage of polymorphic loci is determined by the 0.95 criterion, and standard errors are shown in parentheses. Numbers generated by BIOSYS-1 (Swofford & Selander 1981).

Population	mean sample size	mean number of alleles/locus	mean direct-count heterozygosity	percent of loci polymorphic
LS&B	19.0 (0.4)	1.36 (0.15)	0.076 (0.038)	36.4
LRCR	19.9 (0.1)	1.36 (0.15)	0.059 (0.035)	36.4
L06B	20.0 (0.0)	1.55 (0.21)	0.118 (0.049)	45.5
LDYL	19.8 (0.1)	1.91 (0.21)	0.123 (0.038)	54.5
L08	19.9 (0.1)	2.09 (0.28)	0.105 (0.037)	45.5
L051	19.9 (0.1)	1.73 (0.19)	0.100 (0.033)	45.5
LSQV	19.8 (0.1)	1.64 (0.24)	0.168 (0.075)	36.4
L15X	20.0 (0.0)	1.73 (0.19)	0.091 (0.032)	45.5
L07C	9.9 (0.1)	1.45 (0.21)	0.109 (0.055)	36.4
L07D	19.7 (0.2)	1.64 (0.24)	0.074 (0.031)	36.4
L04D	18.5 (0.8)	1.91 (0.25)	0.107 (0.044)	45.5
L05D	19.8 (0.1)	1.91 (0.31)	0.093 (0.045)	36.4
L013	19.8 (0.1)	1.82 (0.26)	0.133 (0.048)	54.5
L05G	20.0 (0.0)	1.64 (0.28)	0.068 (0.030)	45.5
LTR3	19.4 (0.5)	1.64 (0.24)	0.166 (0.078)	36.4
LC3D	19.7 (0.3)	1.73 (0.27)	0.077 (0.036)	36.4
LGT2	19.8 (0.2)	1.55 (0.21)	0.120 (0.053)	45.5
LDGV	19.6 (0.3)	1.55 (0.21)	0.064 (0.036)	27.3
L010	19.8 (0.2)	1.36 (0.15)	0.078 (0.042)	27.3
LDSM	19.8 (0.1)	1.45 (0.21)	0.128 (0.061)	36.4
LTST	19.8 (0.1)	1.27 (0.14)	0.078 (0.056)	27.3
LPEA	19.7 (0.2)	1.18 (0.12)	0.079 (0.054)	18.2
LSTM	19.7 (0.1)	1.55 (0.28)	0.074 (0.041)	27.3
average: all populations	19.3	1.61	0.099	38.4
average: eastern populations	19.7	1.42	0.089	29.9
average: western populations	17.9	1.69	0.104	42.1

Table 3. Probability values from two-tail, two-sample t-test comparing average measures of genetic diversity between the eastern and western clusters of *Trifolium lemmonii* populations (α=0.05).

Measure of genetic diversity	Probability value
Mean number of alleles/locus/population	0.027*
Mean direct-count heterozygosity	0.035*
Percent polymorphic loci (0.95 criterion)	0.013*

Table 4. Alleles found in western populations but not in eastern populations.

Locus	Designation for allele	Western populations containing the allele
6-PGDH	A	L06B
GPI-2	E	L05G, LC3D
PGM	D	LDYL, LSQV, L05D, LC3D
PGM	E	L08, L07D, L04D
LAP	A	LRCR, L08, L051, LSQV, L04D, L013, LC3D
ADH	D	L06B, LTR3
MPI	A	L013
IDH	A	L08
IDH	D	LDYL
MDH	A	LDYL, L08, L04D, LC3D

Table 5. Wright's (1978) average F statistic values for variable loci for all populations, eastern populations, and western populations of *Trifolium lemmonii.*

Populations	Locus	F_{IS}	F_{IT}	F_{ST}
All	6-PGDH	-0.068	-0.014	0.051
	GPI-2	0.090	0.168	0.085
	PGM	-0.032	0.109	0.136
	LAP	0.233	0.296	0.081
	ADH	0.085	0.457	0.407
	MPI	0.074	0.230	0.169
	TPI-3	0.323	0.384	0.091
	IDH	0.192	0.254	0.076
	MDH	0.102	0.172	0.078
	mean	0.069	0.267	0.213
Eastern	6PGDH	-0.088	-0.012	0.070
	GPI-2	0.141	0.322	0.210
	PGM	-0.007	0.026	0.033
	LAP	0.161	0.228	0.080
	ADH	0.303	0.386	0.119
	MPI	0.164	0.287	0.148
	TPI-3	-0.026	-0.004	0.022
	IDH	0.506	0.560	0.110
	mean	0.115	0.200	0.096
Western	6-PGDH	-0.063	-0.015	0.044
	GPI-2	0.074	0.105	0.033
	PGM	-0.047	0.071	0.113
	LAP	0.247	0.304	0.076
	ADH	0.032	0.120	0.090
	MPI	-0.059	0.075	0.127
	TPI-3	0.336	0.391	0.082
	IDH	-0.055	-0.009	0.043
	MDH	0.102	0.168	0.073
	mean	0.050	0.132	0.087

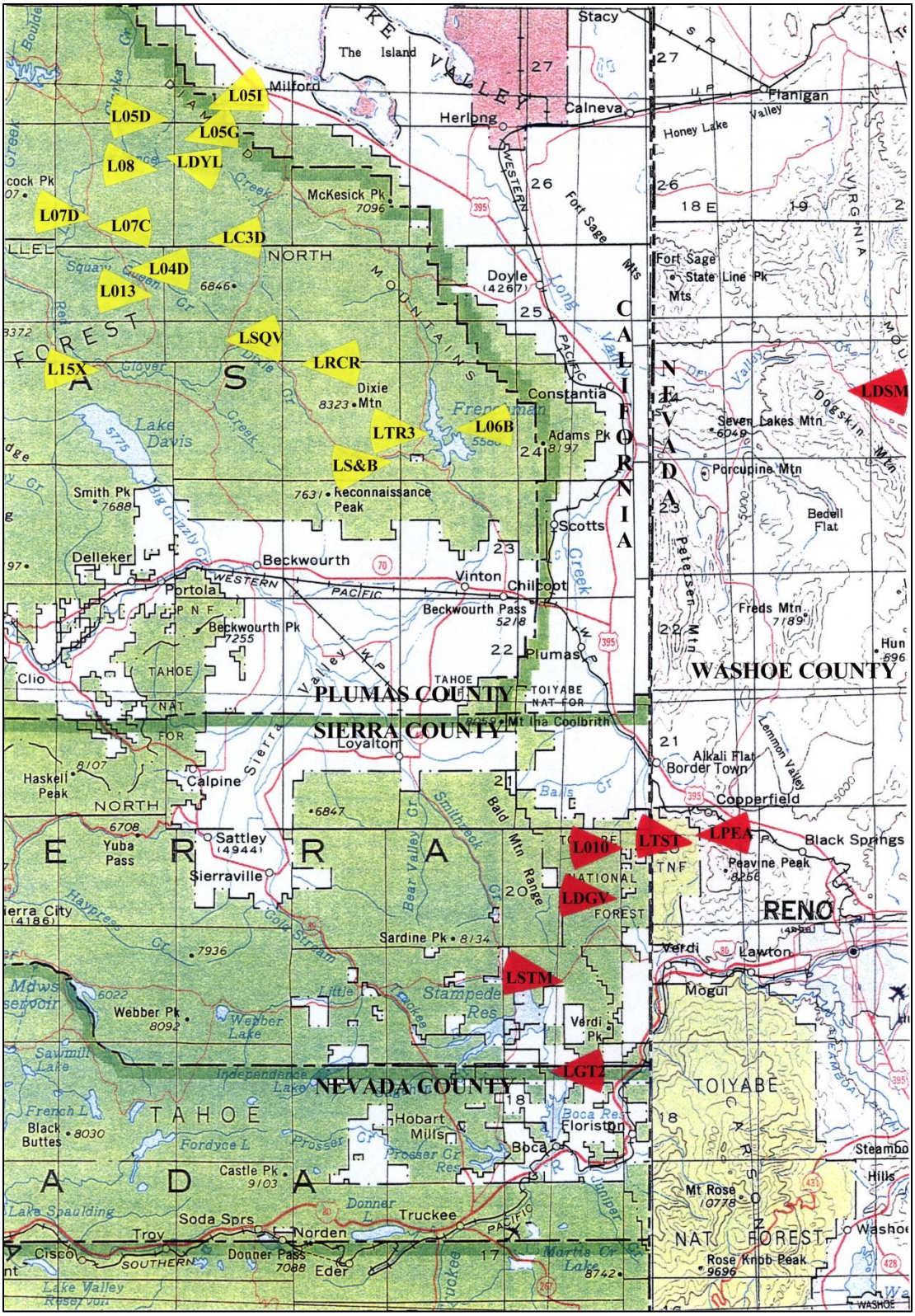

Figure 3. Two clusters of genetic diversity among *Trifolium lemmonii* populations. Eastern populations are designated with red arrows, and western populations are designated with yellow arrows. Map modified from United States Dept. of the Interior Geological Survey, 1981 (California) and 1984 (Nevada).

Nei's (1978) unbiased minimum distance measure showed a similar trend, averaging 0.010 for the eastern populations, 0.008 for the western, and 0.051 for between the two geographic regions. When using Nei's (1978) distance measure, however, one of the western populations (L07C) clusters with the eastern populations (Figure 6). This seems to occur largely because of its genotypic ratios for ADH. L07C does not contain alleles novel to the eastern cluster, but has proportionally more of the most common eastern ADH allele (B) than do any of its western neighbors. It is possible that the genotypic ratios for ADH in L07C reflect sampling error, as the sample size of L07C (ten individuals) ended up being about half that of the other populations.

Average genetic identity (Nei 1972) for the eastern populations was 0.986, and for the western populations was 0.988. Average genetic identity between the two geographic regions was 0.940, showing greater genetic identity among the populations within each region rather than between the two regions. Graphed as a dendrogram, genetic identity values separate the populations into eastern and western clusters (Figure 7).

Morphological Results

Principle Coordinate Analysis of the specimens did not delineate any subgroups, indicating that all the populations measured represent one species. No morphological distinction was noted between eastern and western populations (Sommer 1999).

DISCUSSION

Genetic Diversity

Two clusters of *Trifolium lemmonii* populations exist on either side of a broad stretch of largely unsuitable habitat. This geographic division of the species is paralleled by a difference in genotype frequencies, with each of the two clusters of populations (east and west) showing greater similarity within the clusters than between the clusters. It is likely that the geographic distance between the eastern and western populations restricts the gene flow between them. The eastern populations are consistently seen to have less genetic diversity than the western populations. This trend is shown in percentage of polymorphic loci, average number of alleles per locus per population, and presence of uncommon alleles.

The presence of two distinct clusters of *Trifolium lemmonii* populations is supported by F_{ST} values that suggest that much of the genetic partitioning occurs between the eastern and western clusters rather than among all of the populations.

Although there is genetic differentiation between clusters, it is not great and does not warrant separate species status for the two groups, as similarity remains high. The genetic distance between the two clusters is apparent, but not extreme: the most genetically distant populations (L05I and LDSM) retain an 81.6% similarity using Nei's (1972) measure of genetic identity. In addition to the genetic similarity, no obvious morphological distinctions separate the populations.

Similar studies conducted on other clover species allow for comparison with *Trifolium lemmonii*. When compared with rare clovers of fragmented distribution – *T. stoloniferum* Eaton (Hickey et al. 1991) and *T. virginicum* Small (Linscott 1994) – and common, introduced clovers of wide distribution – *T. hybridum* L. and *T. pratense* L. (Hickey et al. 1991) – *T. lemmonii* falls between the rare and common clovers in average number of alleles per polymorphic locus. For measures of average heterozygosity and percentage of polymorphic loci, *T. lemmonii* exhibits greater allelic diversity and heterozygosity than the other clovers. It is possible that *T. lemmonii* would not exceed *T. hybridum* and *T. pratense* in these measures if more populations of the common clovers from a wider range of their distribution had been sampled (Table 6).

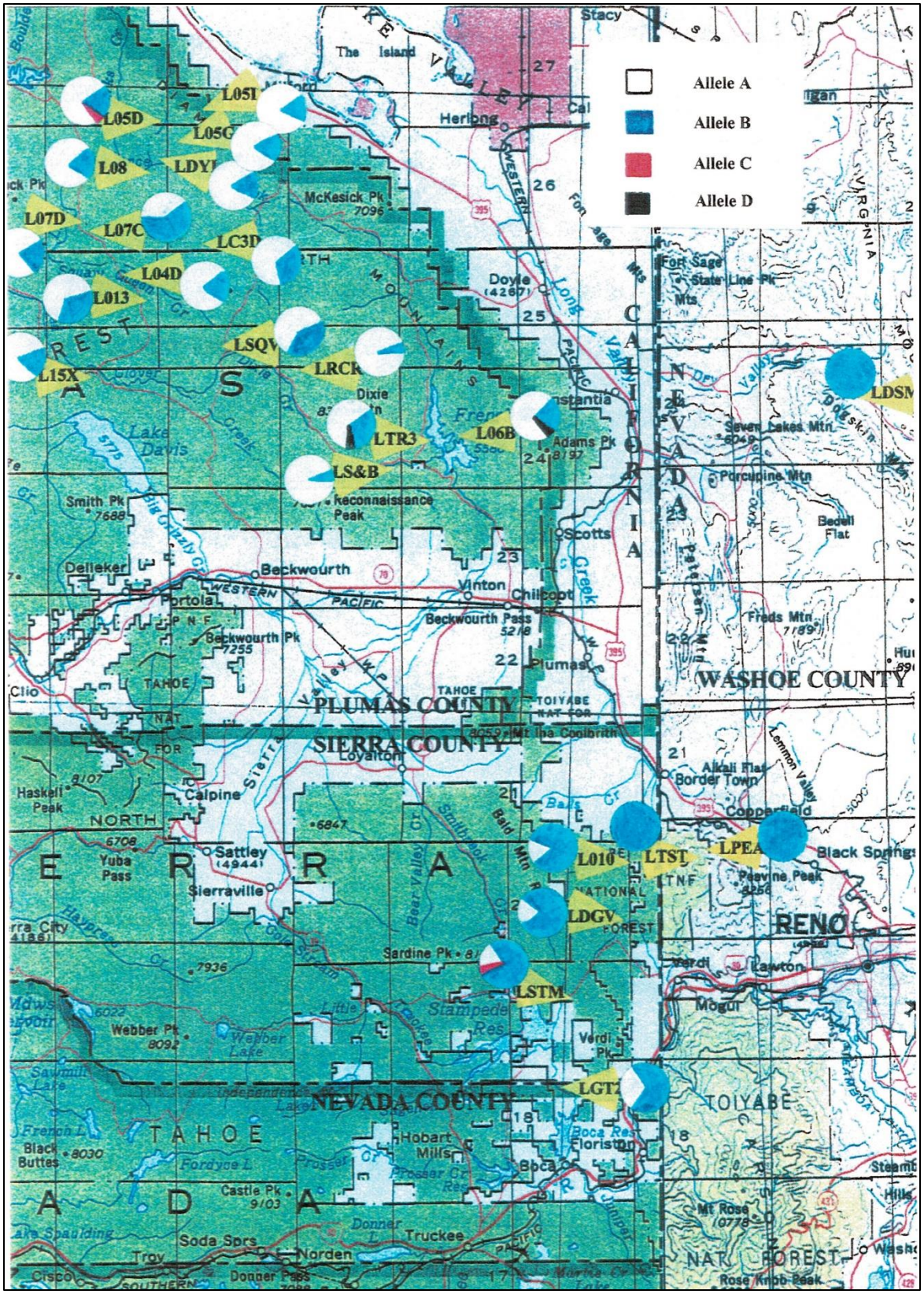

Figure 4. ADH allele frequencies for *Trifolium lemmonii* populations. Map modified from United States Dept. of the Interior Geological Survey, 1981 (California) and 1984 (Nevada).

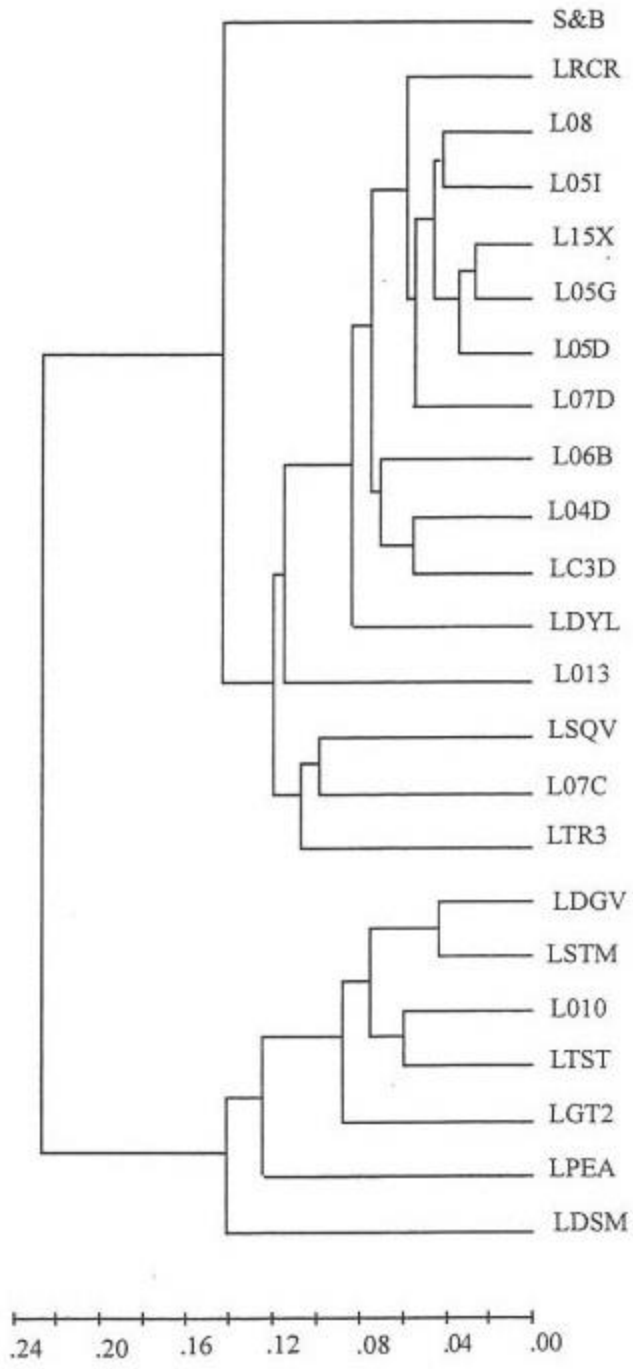

Figure 5. Dendrogram showing Modified Rogers Distance (Wright 1978) constructed using the unweighted pair group method of cluster analysis. Image is modified from BIOSYS-1 output (Swofford and Selander 1981).

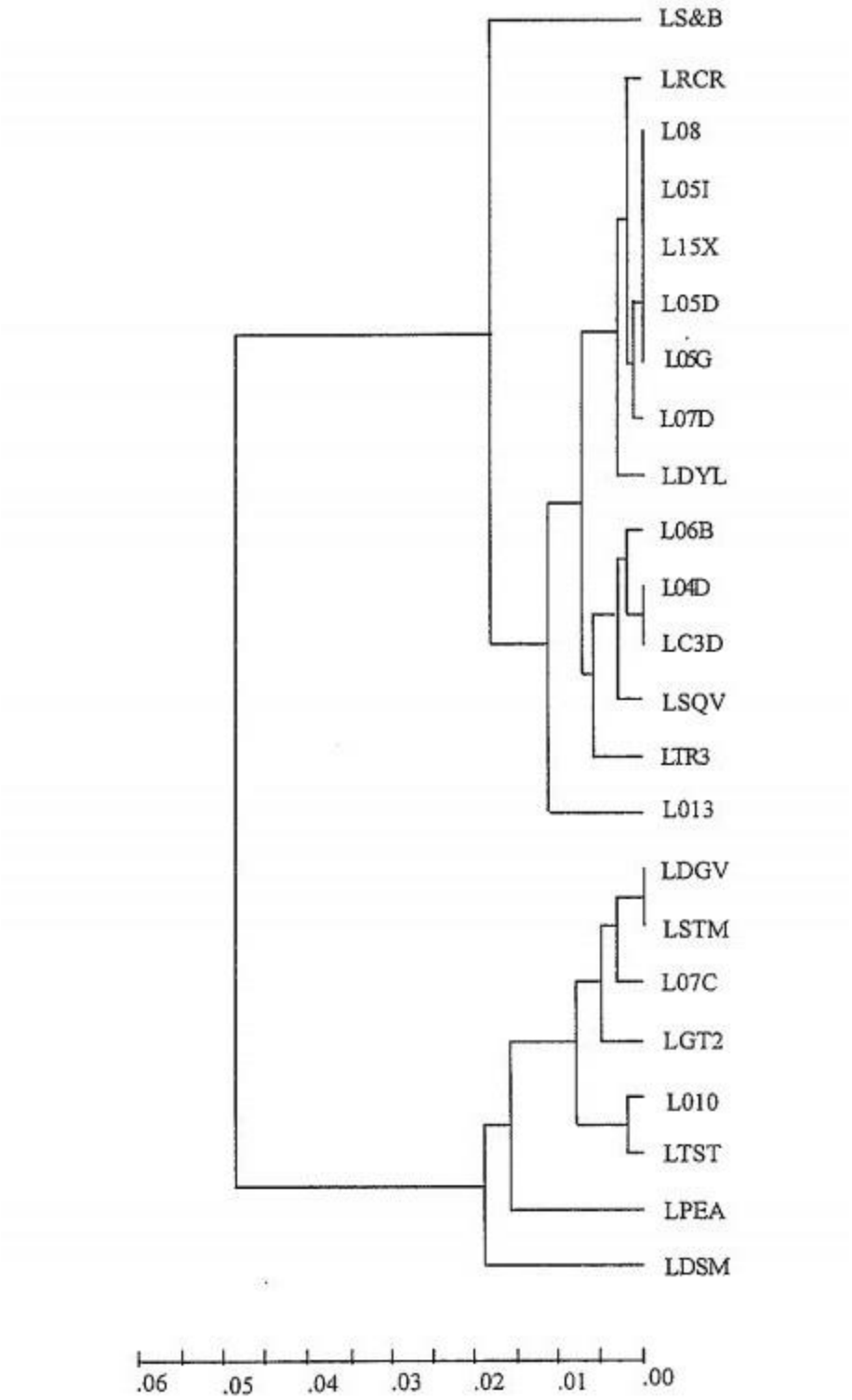

Figure 6. Dendrogram showing Nei's (1978) genetic distance constructed using the unweighted pair group method of cluster analysis. The lower branch represents the eastern populations, with one western population (L07C) nested within. The upper branch represents the remainder of the western populations. The image is modified from BIOSYS-1 output (Swofford and Selander 1981).

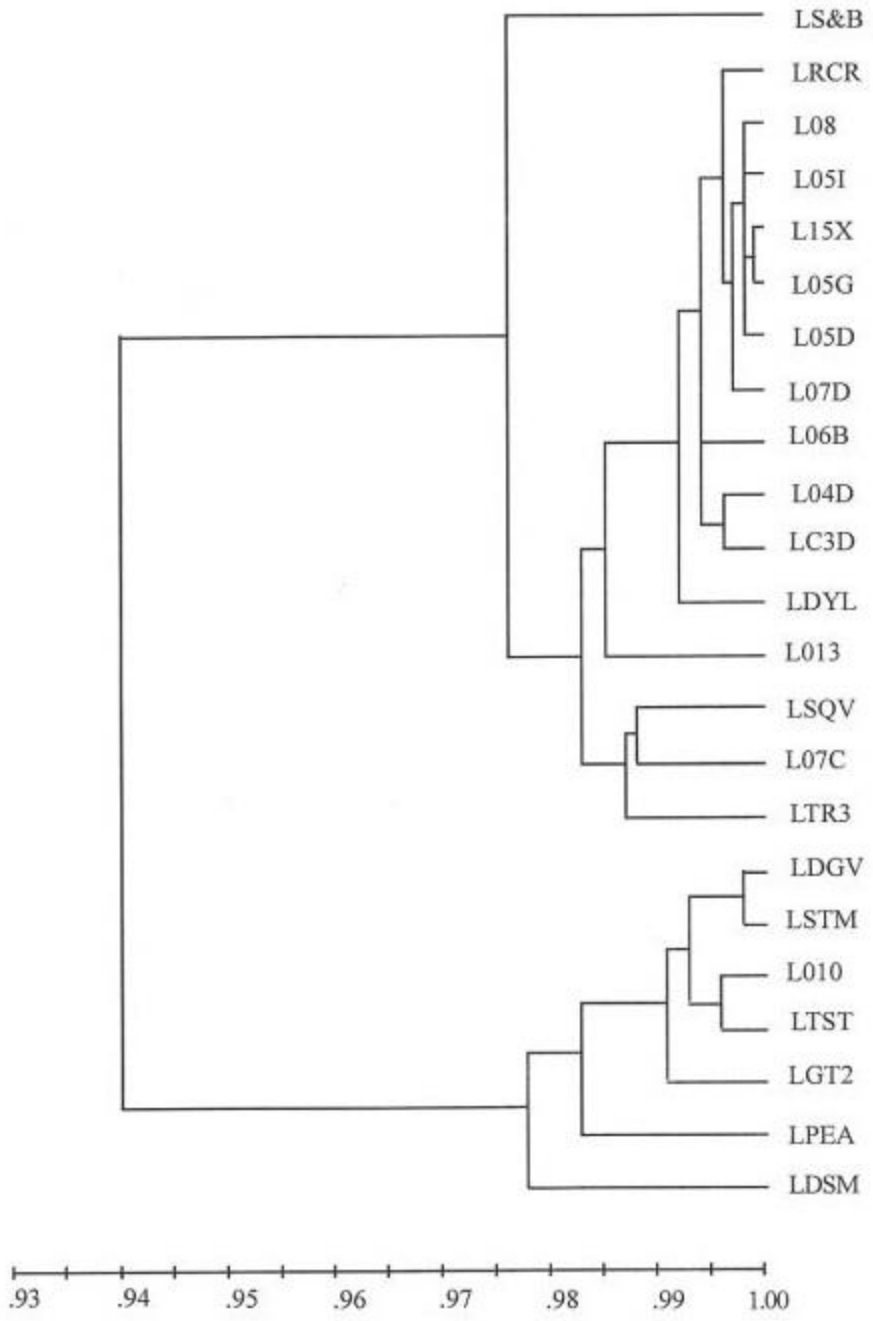

Figure 7. Dendrogram showing Nei's (1972) genetic identity constructed using the unweighted pair group method of cluster analysis. The lower branch represents the eastern populations, and the upper branch represents the western populations. The image is modified from BIOSYS-1 output (Swofford and Selander 1981).

Overall F_{ST} values of *Trifolium lemmonii* (F_{ST} = 0.213) are similar to those of *T. stoloniferum* (F_{ST}=0.206) (Hickey et al. 1991) and *T. virginicum* (F_{ST}=0.292) (Linscott 1994), indicating high levels of partitioning of the genetic diversity. When *T. lemmonii* is split into eastern (F_{ST}= 0.096) and western clusters (F_{ST}=0.087), F_{ST} values for each regional cluster fall between those of the rare and common clovers (Table 6).

Habitat observations

Based on population size and number, *Trifolium lemmonii* seems to be thriving within its limited range. Populations are numerous and often large, and contain both flowering and non-flowering individuals. In the larger populations, *T. lemmonii* is significant in its communities and seems to thrive in somewhat disturbed habitats.

Table 6. F-statistics and measures of genetic diversity for five clover species. Data for *Trifolium virginicum* (Linscott 1994). Data for *T. stoloniferum, T hybridum, T. pratense* (Hickey et al. 1991).

Species	Mean number of alleles per locus	Mean direct count of heterozygosity	Percent of loci with allelic variation in at least one population	F_{IS}	F_{IT}	F_{ST}
T. stoloniferum	1.10	0.047	15.0	0.222	0.030	0.206
T. virginicum	1.1	0.022	69.2	0.182	0.421	0.292
T. hybridum	1.73	0.066	53.3	0.178	0.208	0.036
T. pratense	1.93	0.079	53.3	0.041	0.061	0.021
T. lemmonii	1.61	0.099	81.8	0.069	0.267	0.213

Plumas National Forest grazing records for the LSQV population show continuous grazing for the 50 years prior to the study, yet the *Trifolium lemmonii* population is very large and has both larger flowering individuals and smaller non-flowering individuals, suggesting multiple age classes. An historically documented grazing route in the Toiyabe National Forest shows sheep movement through three of the seven eastern populations sampled for allozyme analysis (USFS 1994). The grazing route is within a few miles of three other eastern populations sampled in the study.

Trifolium lemmonii has successfully colonized road sides and dirt tire tracks near or in established populations. On several sites, individuals grow well away from the main colony along compacted tire tracks.

Heavy disturbance can be a threat to the populations, however, as was observed with a Plumas County occurrence (Forest Service record TRLE 11-19), that was located during the study. The population grew on a little-used road bed. A few years after this study, the author visited this site and could not find any *Trifolium lemmonii* there. The road bed had seen extensive use in the intervening time, and no clover remained on it.

Conservation Status

Trifolium lemmonii is surprisingly robust given its highly restricted distribution. It has moderate genetic variation and allelic variation, tolerates some disturbance, is abundant in eastern Plumas County, and occurs in some large populations. Overall, *T. lemmonii* seems to be prospering within its limited range. From a conservation perspective, *T. lemmonii* does not appear to merit special status or listing beyond a watch list status. The main concern for the species lies in its restricted range, with habitat loss and heavy disturbance being the only obvious potential threats.

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LITERATURE CITED

California Native Plant Society (CNPS), Rare Plant Program. 2021. Inventory of Rare and Endangered Plants of California (online edition, v9-01 1.0). <https://www.rareplants.cnps.org>

Ellison, N.W., A. Liston, J.J. Steiner, W.M. Williams, and N.L. Taylor. 2006. Molecular phylogenetics of the clover genus (*Trifolium*-Leguminosae). Molec. Phylog. Evol. 39: 688–705.

Gillett, J.M. 1972. Taxonomy of Trifolium (Leguminosae). IV. The American species of section *Lupinaster* (Adanson) Seringe. Canad. J. Bot. 50: 1975–2007.

Hickey, R.J., M.A. Vincent, and S. I. Guttman. 1991. Genetic variation in running buffalo clover (*Trifolium stoloniferum*, Fabaceae). Conserv. Biol. 5: 309–316.

Latschaschvili, I. 1976. Zametki po sistematike i geografii rastenii. Not. Sist. Geogr. Inst. Bot. Thbilissiensis 32: 14–35.

Lessios, H.A. 1992. Testing electrophoretic data for agreement with Hardy-Weinberg expectations. Marine Biol. 112: 517–523.

Linscott, T.M. 1994. Morphological and genetic diversity of *Trifolium virginicum* populations using quantitative and allozyme studies. M.S. Thesis. Miami University, Oxford, Ohio.

McDermott, L.F. 1910. An Illustrated Key to the North American Species of *Trifolium*. Cunningham, Curtiss & Welch, San Francisco.

Martin, J.S. 1943. A revision of the native clovers of the United States. Ph.D. dissertation. Univ. of Washington, Seattle.

Mozingo, N.H. and M. Williams. 1980. Threatened and Endangered Plants of Nevada: An Illustrated Manual. U.S. Fish and Wildlife Service and Bureau of Land Management.

Nei, M. 1972. Genetic distance between populations. Amer. Natur. 106: 283–292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Nevada Natural Heritage Program. 2021. Plant and Animal Watch List July 2021. Nevada Natural Heritage Program, Carson City.

Rohlf, F.J. 1997. NTSYSpc Numerical Taxonomy and Multivariate Analysis System Version 2.00 User Guide. Exeter Software, Setauket, New York.

Skinner, M.W. and B.M. Pavlik. 1994. California Native Plant Society's Inventory of Rare and Endangered Vascular Plants of California. 5th edition. California Native Plant Society. Sacramento.

Soltis, D.E., C.H. Haufler, D.C. Darrow, and G.J. Gastony. 1983. Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. 73: 9–27.

Sommer, S.J. 1999. Genetic diversity within and among populations of *Trifolium lemmonii* S.Wats. M.S. Thesis, Dept. of Botany, Miami University, Oxford, Ohio.

Swofford, D.L. and R.B. Selander. 1981. BIOSYS-1: A Fortran program for the comprehensive analysis of electrophoretic data in populations genetics systematics. J. Hered. 72: 281–283.

Taylor, M.S. 1981. Lemmon's Clover *Trifolium lemmonii* Watson. Status Report. U.S. Forest Service, Plumas National Forest. Quincy, California.

Thiers, B. 2021 [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/science/ih/>

True, G.H. 1974. Lemmon's clover rediscovered. Four Seasons 4(4): 22–23.

United States Department of the Interior Geological Survey. 1981. State of California, North Half. Scale 1:500,000. Denver, Colorado.

United States Department of the Interior Geological Survey. 1984. State of Nevada. Scale 1:500,000. Denver, Colorado.

United States Forest Service (USFS). 1994. Toiyabe National Forest: Carson Ranger District. Scale 1:126,720. Washington, DC.

Vincent, M.A. and D. Isley. 2012. *Trifolium*. Pp. 789–798, in B.G. Baldwin, D.H. Goldman, D.J. Keil, R. Patterson, and T.J. Rosatti (eds.). 2012. The Jepson Manual: Vascular Plants of California (ed. 2). Univ. of California Press, Berkeley.

Watson, S. 1876. Description of new species of plants, chiefly Californian, with revisions of certain genera. Proc. Amer. Acad. Arts 11: 127–130.

Watson, S. 1880. California Geologic Survey Vol. 2. Bot. Calif. 2: 440.

Wright, S. 1978. Evolution and the genetics of populations, a treatise in four volumes: Volume 4, variability within and among natural populations. Univ. of Chicago Press, Chicago.

Zohary, M. and D. Heller. 1984. The Genus *Trifolium*. Israel Academy of Sciences and Humanities, Jerusalem.