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RESEARCH ARTICLE

Geographically disjunct populations and widespread genets in an endangered halophilic plant, the Amargosa niterwort (*Nitrophila mohavensis*)

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Abstract *Nitrophila mohavensis*, commonly known as Amargosa niterwort, is a perennial forb classified in the family Chenopodiaceae. The species is restricted to moist alkaline soils of the Ash Meadows National Wildlife Refuge and nearby localities within the Amargosa River basin and is currently listed as endangered. We isolated several polymorphic microsatellite loci and characterized genotypes from individuals spanning the range of the species as a means of inferring reproductive mode and population structure for the species. Out of 178 plants sampled from 35 different sample sites, there were 78 different multilocus genotypes. Most individuals had unique genotypes; however, there were many genotypes that were found in more than one individual and the most common genotype was sampled from 13 individuals. In addition, three genotypes were sampled from individuals across the range of the species, a maximum distance of more than 50 km. The results suggest that *N. mohavensis* reproduces clonally and by outcrossing. The relative frequency of the two reproductive strategies appears to vary among localities. There is not strong genetic differentiation among geographically separated clusters of plants. While the species is locally abundant and does not appear to suffer from inbreeding due to a small number of genets, continued local groundwater extraction coupled with forecasts of increasing regional desiccation due to accelerated climate change may combine to cause future population declines of *N. mohavensis* across its range.

Keywords Amargosa niterwort · Endangered species · Ash Meadows · Reproductive mode · Population genetic structure · Microsatellites

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Introduction

Nitrophila mohavensis, commonly known as Amargosa niterwort, is a perennial forb classified in the family Chenopodiaceae. There are currently two species in the genus *Nitrophila*, although the two species (*N. mohavensis* and *N. occidentalis*) are markedly different and do not cluster together as sister taxa in a molecular phylogeny (Berner et al. 2009). The species is restricted to a relatively small geographic area of the Amargosa River valley from Ash Meadows National Wildlife Refuge (AMNWR) to Tecopa (T), California, a distance of approximately 60 km. Although *N. mohavensis* is recognized as a rare plant owing to its small range, and is listed as an Endangered Species, it is locally common in moist, alkaline soils (BIOWEST 2010). Recent surveys estimated there are ~75,000–100,000 individual plants distributed among several disjunct populations (BIOWEST 2010; BLM 2012).

There are significant gaps in our knowledge of the basic biology of *N. mohavensis*. For instance, it is not known whether reproduction is predominantly asexual or by outcrossing. Although individuals have many small flowers (2.3–3.5 mm) and produce small black seeds (1.2 mm), pollination has not been observed and seed germination rates are low (Pavlik (2010) BMP Ecosciences, Oakland, CA, personal communication). Moreover, it is not known whether seeds are produced by outcrossing, selfing, or by some form of asexual reproduction (i.e. gametophytic apomixis).

Excavation of plants uncovered a network of rhizoids, although the extent of rhizomatous growth has not been measured (BLM 2012) and the size of individual genets is unknown. The existence of rhizoids suggests that ramets of *N. mohavensis* may share resources: a condition termed clonal integration. Clonal integration can increase the success of plants inhabiting environments that impose physiological stress (Evans and Whitney 1992; Pennings and Callaway 2000). *N. mohavensis* lives in an extreme environment characterized by very high heat and high alkalinity and salinity. Temperatures at the surface of the ground in summer can exceed 120 °F and the soils are often salt encrusted (A. Martin, personal observation). Such extreme conditions may explain the low germination rates of *N. mohavensis*.

The success of clonal reproduction in stressful environments suggests that *N. mohavensis* may comprise relatively few genets, and that local patches of *N. mohavensis* may represent single genets that successfully established and proliferated by rhizoid growth. If reproduction is primarily by rhizomatous growth, the species may have had a more continuous distribution in the past and the current patchy distribution reflects mortality of plants. Alternatively, *N. mohavensis* may reproduce by seeds and seedling establishment may occur during periods of exceptional precipitation. In this case, clusters of plants may consist of multiple genets if there is outcrossing or there are clones not connected by rhizomes. In the latter case, the disjunct distribution of plants may reflect seed or fragment dispersal, most likely by water transport (Reveal 1978).

Additionally, because the species is rare and endangered, it is important to know the ratio of the number of ramets to genets. On average, effective population size is best estimated from the number of genets and is overestimated by the number of ramets. In species that reproduce both asexually and by outcrossing, the reproductive success of a genet is proportional to the number of ramets. If this is true, then effective population size will be maximized when the ratio of ramets to genets is small (Chung et al. 2004). As the ratio of ramets to genets increases, random drift will increase the probability of inbreeding and result in the erosion of genetic variation in populations. Additionally, the endangered status of the species coupled with its disjunct distribution emphasizes the need to know whether the separate occurrences of the species represent genetically different groups of individuals or whether similar genets span multiple localities. If disjunct populations exhibit significant differentiation, then it may be necessary to treat separate occurrences as distinct management units that may require specific conservation actions. By contrast, lack of differentiation would imply that all localities harbor, more or less, equivalent diversity and a broader, range-wide conservation plan is warranted.

Methods

Collection of samples

The species is restricted to a relatively small area of the Amargosa River drainage, with most individuals confined to three localities separated by about 60 km: the AMNWR, an area immediately downstream of the AMNWR but within the Carson Slough (a site called Death Valley junction [DVJ]), and near T, California within the Amargosa River valley (Fig. 1). Within the AMNWR there are many sites where *N. mohavensis* is locally common (BIOWEST 2010). Plants surveys have identified either 13 or 4 separate occurrences of *N. mohavensis* using 160 and 1,000 m mapping distances, respectively (BIOWEST 2010). These are minimum mapping distances for recognition of separate occurrence records as used by the Natural Heritage Program. For this study, the 35 collection sites were grouped into eight geographically separated populations (Fig. 1; Table 1). Six were within the AMNWR: (1) Soda Springs (SS), (2) upstream of Crystal reservoir (AC), (3) immediately downstream of Crystal reservoir (BC), (4) the dunes (D), (5) downstream of Horseshoe reservoir (BH), and (6) downstream from the southern springs complex (Jackrabbit, Point of Rocks, and Big Springs) (SO). The remaining two populations were at DVJ and T. The eight localities were based mainly on geographic separation and their locations relative to drainages. The groupings used for this analysis were similar to the 0.16 km ellipses approach (BIOWEST 2010) with the one exception that the 0.16 km ellipses divided up the occurrences between Crystal dam and the outflow of Horseshoe Reservoir into six different clusters whereas this study treated these occurrences as two separate groups.

The number and distribution of *N. mohavensis* varied across its range. At some sites, only a few plants were found and at others there were thousands of plants distributed over hundreds of meters. For each collection site, we chose to sample plants that were, on average, about 2–4 m apart in part to maximize the probability of sampling different genotypes. For each individual, we removed a small piece of the plant (about 1 cm), put it in an envelope, and stored the sample on ice. Each sample was frozen in liquid nitrogen and pulverized with a mortar and pestle prior to extraction. DNA was extracted using Qiagen and MoBio plant extraction kits.

Ribosomal internal transcribed spacer primers were used to amplify and sequence the gene from representative samples across the range of the species; however, there was no sequence variation among a set of individuals sampled from across the range. Therefore, we pooled the DNA from 12 extractions for construction of genomic libraries enriched for simple sequence repeats (carried out by Genetic

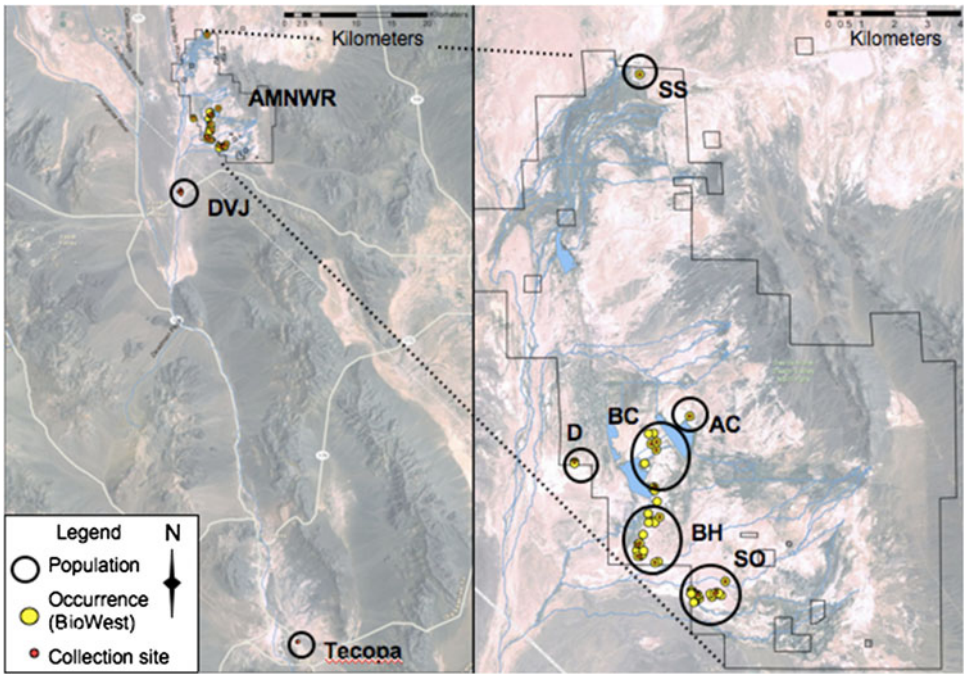

Fig. 1 Map of the distribution of *N. mohavensis* (occurrence points), the location of collection sites, and the delineation of populations. *AMNWR* Ash Meadows National Wildlife Refuge, *DVJ* Death Valley Junction, *SS* Soda Springs, *AC* Above Crystal reservoir, *BC* Below Crystal reservoir, *D* Dunes, *BH* Below Horseshoe reservoir, *SO* Southern springs

Table 1 Population, collection site, latitude and longitude of collection site, and sample size

Locality	Site	Latitude	Longitude	N
Soda Springs (SS)	41	36.48695	−116.33386	3
Above Crystal (AC)	37	36.41247	−116.32020	5
Below Crystal (BC)	17	36.39705	−116.32992	10
	20	36.40687	−116.32919	9
	34	36.40515	−116.32936	5
	35	36.40641	−116.33071	2
Dunes (D)	21	36.40246	−116.35146	8
	36	36.40251	−116.35150	9
Below Horseshoe (BH)	4	36.38470	−116.33382	15
	22	36.38045	−116.32965	5
	24	36.38186	−116.33440	10
	29	36.39050	−116.32846	5
	38	36.38189	−116.33366	5
	81	36.38430	−116.33444	5
Southern springs (SO)	8	36.37646	−116.31053	16
	9	36.37349	−116.31814	12
	31	36.37407	−116.31288	8
	32	36.37370	−116.31352	2
Death Valley (DVJ)	45	36.32813	−116.3696	4
	47	36.32855	−116.36862	2
	49	36.33125	−116.36632	1
	50	36.32829	−116.36651	1
	51	36.3277	−116.36631	2
	52	36.32613	−116.36734	1
	78	36.33018	−116.36725	5
Tecopa (T)	54	35.87198	−116.22020	3
	55	35.87201	−116.22020	1
	56	35.87200	−116.22014	4
	58	35.87181	−116.22018	4
	59	35.87189	−116.22033	2
	60	35.87176	−116.22018	3
	62	35.87195	−116.21991	2
	67	35.87165	−116.22059	2
	74	35.87191	−116.22017	1
	75	35.87195	−116.21991	5

Identification Services, Inc, Topanga, CA). We initially screened 40 primer sets and settled on eight loci that yielded readily and routinely interpretable genotypes. The eight loci were genotyped in two panels using multiplex PCR at the University of Nevada Core Facility. Genotypes were scored using ABI's Genotyper software.

Identification of distinct multilocus genotypes (MLG) and assignment of each individual to a genotype was accomplished using Genodive (Meirmans and Van Tienderen 2004) for the data set that included missing data for some individuals and genotypes and using GenClone (Arnaud-Haond and Belkhir 2007) for a data set without missing data. The data were tested for conformation to Hardy–Weinberg expectations and for linkage

disequilibrium between pairs of loci. We calculated summary diversity statistics, including heterozygosity, and number of alleles for each locus. With GenClone, we calculated the probability of occurrence of each MLG, p_{gen} (F_{IS}), and the probability that an individual ramet of a MLG was derived from an independent sexually recombining reproductive event was estimated using p_{sex} (Arnaud-Haond and Belkhir 2007). Additionally, we used GenClone to determine the number of genotypes that were 1 mutation step from another genotype assuming that some of the one-step away genotypes may reflect scoring error, although most probably resulted from mutation (Chenault et al. 2011). For each locality we calculated genotypic diversity ($1 - \sum p_i^2$), the number of genotypes normalized to a sample size of 17 (for comparative purposes), the ratio of the number of individuals (N) to the number of genotypes (G) sampled (N−1)/(G−1), and the proportion of unique genotypes.

We estimated genetic differentiation among the eight localities using all individuals and for a reduced data set that only included genetically unique individuals from each population. We omitted genetically redundant individuals within a population because the presence of multiple individuals with the same genotype (clones) deflates levels of within population variation and therefore upwardly biases estimates of differentiation. We calculated Θ_{ST} (an analog of F_{ST}) using GenoDive. Significant differentiation was inferred if the calculated *p* value was less than 0.01. We also used the first two axes of principle components analysis to visually assess whether there were clusters of similar genotypes across all individuals (using GenoDive).

We calculated the geographic range of each genotype that was sampled from more than one population based on the straight-line distances between individuals. Additionally, we assessed whether there was a signal of isolation-by-distance by comparing the genetic differentiation between populations to the log of the geographic distance between localities using a Mantel test (implemented in R). Geographic distance was estimated from the center of the collection sites that comprised a population.

Results

Characteristics of the loci

Although we developed primers for characterizing 40 different loci, only eight sets of primers yielded readily interpretable and repeatable polymorphism. All of the loci contained dinucleotide repeats: five loci were simple repeats, two were interrupted simple repeats, and one had three different motifs. The number of alleles per locus ranged from two to eleven, with 45 total alleles summed across all eight loci. Expected heterozygosity ranged from 0.15 to 0.81 across loci (Table 2).

Hardy–Weinberg expectations

For collection sites with sample sizes of 10 or more individuals, we tested whether genotypes conformed to Hardy–Weinberg expectations (HWE). The results varied across sites and loci. At one site (4), two of the eight loci departed

Table 2 Description of the microsatellite loci used to character the genotypes of individuals

Name	Primers	Motif	Alleles	H_E
A1	5′-AACTGCCAATCAAGCTGA-3′ 5′-AGGGAAGAAAGAGCAGAGAG-3′	$(CA)_{20}$	5	0.707
A103	5′-ATGGGTGACCACGGTTTACA-3′ 5′-GCAACCCAAATTAGGTCCAA-3′	$(AC)_8AT(AC)_{15}AT(AC)_8AT(AC)_9$	9	0.754
A111	5′-AAATTTGTTTGCATGGATATTGG-3′ 5′-ATGGCTAACAAAATTCCTTACTGC-3′	$(TG)_{18}(TC)_{27}(TA)_5$	2	0.147
A123	5′-TGCAATGTAACTGATCGTTCG-3′ 5′-GTGGCTGACCTGGAGAATGT-3′	$(AC)_{24}$	4	0.658
B1	5′-GCCTAAGGCAGTATGGTTCTG-3′ 5′-TCCGTCAGAGCCTATTTCAAC-3′	$(AG)_{11}$	3	0.382
B2	5′-CCACAACATCATCAACTTCC-3′ 5′-ACACAAGAACAGCACATAACAG-3′	$(TC)_{22}$	11	0.811
B5	5′-CACCTTACCCAACTCCTCCA-3′ 5′-CCTCTGACTCCCACCTCTCTT-3′	$(GA)_{17}N_{14}(GA)_7$	3	0.510
B11	5′-GCGACCTTTACCACTGGACT-3′ 5′-AGTTCCGTTTCTTGGCTGAC-3′	$(AG)_{22}$	8	0.714
Total			45	0.585

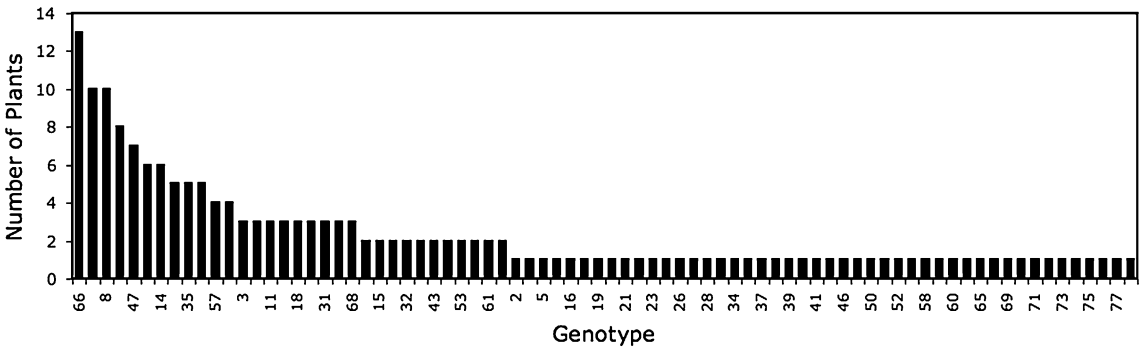

Fig. 2 Histogram showing the number of individuals sampled for each of the 78 genotypes

Table 3 Summary statistics for multiple locus genotypes (MLGs) encountered 2 or more times

MLG	One step MLG (number of individuals)	$P_{gen(FIS)}$	Nramets	P_{sex}	Populations
7	6 (1)	7.9×10^{-8}	13	3.2×10^{-6}	DVJ
26		3.1×10^{-5}	10	5.2×10^{-3}	BC, BH, D
70		2.0×10^{-6}	10	3.4×10^{-4}	BC, BH, D, T
19		5.4×10^{-6}	7	9.1×10^{-4}	BH, T
38		2.3×10^{-6}	7	4.0×10^{-4}	BC
57	50 (1)	2.9×10^{-5}	7	5.0×10^{-3}	BH, DVJ, T
3	4 (1)	9.8×10^{-7}	5	8.0×10^{-4}	BH, D, SO
28		8.5×10^{-5}	5	1.4×10^{-2}	BC, DVJ, T
44		2.6×10^{-6}	5	4.3×10^{-4}	BH
16		7.6×10^{-7}	4	1.3×10^{-4}	SO
11	9 (1)	4.8×10^{-9}	3	8.1×10^{-7}	BH
22	24 (2)	5.9×10^{-6}	3	8.4×10^{-4}	SO
36	40 (2)	4.9×10^{-6}	3	8.2×10^{-4}	BC, D
37		6.5×10^{-6}	3	1.1×10^{-3}	BC, BH
49		1.2×10^{-5}	3	2.1×10^{-3}	DVJ
56		1.6×10^{-6}	3	2.8×10^{-4}	BC, SO
60		1.7×10^{-5}	3	2.9×10^{-3}	T
61		1.2×10^{-6}	3	2.1×10^{-4}	BH
62	53 (1)	8.1×10^{-6}	3	1.4×10^{-3}	BH, SO
1		1.9×10^{-7}	2	3.3×10^{-5}	BH
12		9.3×10^{-8}	2	1.6×10^{-5}	SO
20	21 (1)	2.1×10^{-5}	2	3.5×10^{-3}	SO
24	22 (3), 23 (1)	9.4×10^{-6}	2	1.5×10^{-3}	BH, SO
32		1.3×10^{-5}	2	2.0×10^{-3}	SO
33	34 (1)	2.7×10^{-5}	2	4.5×10^{-3}	DVJ
40	36 (3)	2.1×10^{-6}	2	3.6×10^{-4}	BC, SO
47		7.5×10^{-7}	2	1.3×10^{-4}	SO

from expectations with significantly positive F_{IS} values. At another site (T), all loci conformed to HWE for the 27 individuals sampled. By contrast, three of the loci deviated from HWE for samples collected from a relatively small area at DVJ, and for each locus, F_{IS} was negative. When we performed the analysis after pooling collection sites within the eight geographically separated localities, the results were similar: 22 of the 58 locus × 8 locality values differed significantly from HW expectations with some loci exhibiting positive F_{IS} and others negative F_{IS} values. Furthermore, there was significant disequilibrium between all pairs of loci each collection locality and for the eight geographically separated localities. Departure from HWE and linkage equilibrium is expected when there is asexual reproduction.

Table 4 The number of individuals (N), the number of multi-locus genotypes (G), the number of genotypes (±95 % confidence interval) normalized to a sample size of 17 (G_{NORM}), genotype diversity (D), the ratio of the number of individuals (ramets) to the number of genotypes (genets)(N−1)/(G−1), and the proportion of unique genotypes (PUG)

Locality	N	G	G_{NORM}	D	(N−1)/(G−1)	PUG
SS	3	3	–	1.0	1.0	1
AC	5	3	–	0.64	2.0	1
BC	27	15	9.5 ± 3.9	0.91	1.9	0.53
BH	45	23	8.7 ± 4.2	0.93	2.0	0.78
D	17	8	8.0 ± 4.0	0.80	2.3	0.25
SO	37	23	10.5 ± 4.0	0.94	1.6	0.79
DVJ	17	6	6.0 ± 3.8	0.66	3.2	0.33
Tecopa	27	17	10.1 ± 4.0	0.91	1.6	0.63
Total	178	78	7.3 ± 4.1	0.97	2.28	–

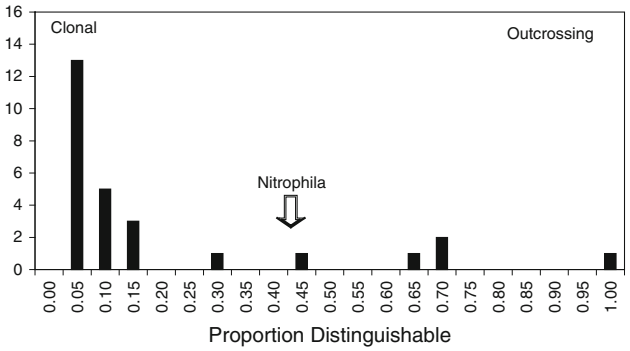

Fig. 3 Summary of the proportion of plants distinguishable (G/N) scores for various species of plants that range from clonal reproducers to obligate outcrossers (Ellstrand and Roose 1987; Jacquemyn et al. 2006). The value for all *N. mohavensis* individuals is indicated

Genotypic diversity

There were a total of 78 different MLGs identified in the 178 individuals surveyed. Of the 78 MLGs, 32 were sampled from more than 1 individual and the maximum number of individuals sampled for one MLG was 13 (Fig. 2). The numbers were similar after omitting one locus and several individuals for which there were missing data. For each MLG that was sampled from more than one individual plant, the probability of occurrence ranged from 4.8×10^{-9} to 8.5×10^{-9} and for each MLG, the probability that putative ramets were derived form an independent reproductive event ranged from 0.014 to 3.2×10^{-6} (Table 3). The ratio of the number of individuals to the number of MLGs ranged from 1 to 3.2 across the eight populations surveyed (Table 4). The reciprocal of the ratio of the number of ramets to genets, namely the proportion of plants distinguishable, was 0.43, a value in the middle of the distribution of values reported for other putatively

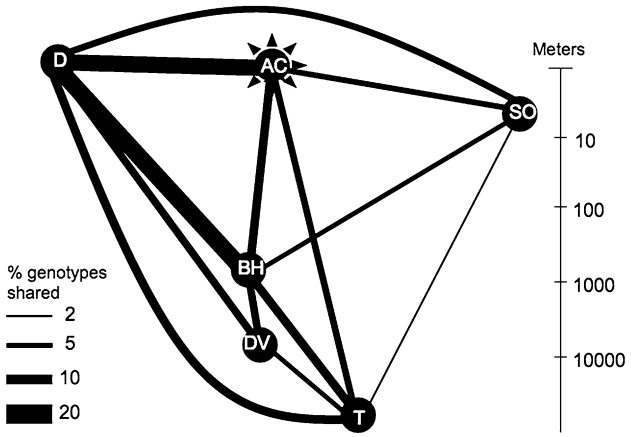

Fig. 4 Network summarizes the percentage of genotypes shared among populations. The populations are drawn relative to their distance (in meters) from the AC populations (indicated by the black sun). The thickness of the line is proportion to the percentage of shared genotypes

Table 5 Number of shared multi-locus genotypes (MLGs) between pairs of populations (above diagonal), total number of MLGs (along diagonal), and proportion of MLGs shared (below diagonal)

	BC	D	BH	SO	AC	SS	DVJ	T
BC	**15**	3	3	2	0	0	0	2
D	0.176	**8**	4	0	0	0	1	1
BH	0.094	0.174	**23**	2	0	0	2	3
SO	0.059	0.074	0.048	**23**	0	0	0	1
AC	0	0	0	0	**3**	0	0	0
SS	0	0	0	0	0	**3**	0	0
DVJ	0	0.083	0.080	0	0	0	**6**	1
T	0.071	0.095	0.088	0.026	0	0	0.048	**17**

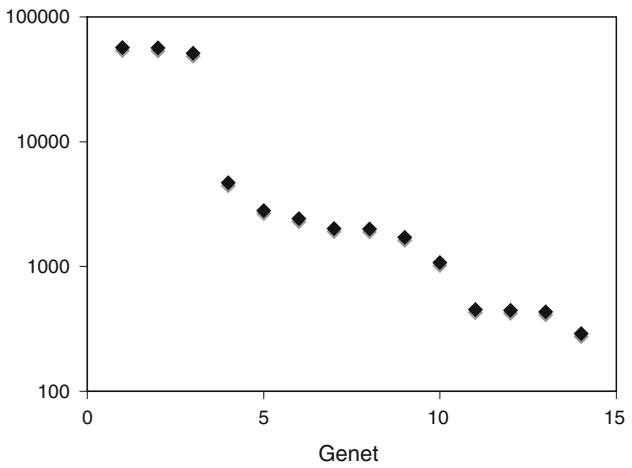

Fig. 5 The range (in meters) measured as the greatest straight-line distance between individuals with identical genotypes for the genotypes sampled from more than one population

Table 6 Estimates of pairwise differentiation (Θ_{ST}) between the eight populations

	BC	D	BH	SO	AC	SS	DVJ	T
BC		−0.022	0.009	0.058	0.160	0.051	0.063	0.045
D	0.060		−0.007	0.089	0.120	0.030	0.010	0.025
BH	0.038**	0.033		0.052*	0.097	0.080	0.023	0.017
SO	0.095***	0.105**	0.066***		0.223**	0.149	0.155**	0.118***
AC	0.232***	0.169	0.126**	0.246***		0.246	0.062	0.104
SS	0.066	0.104	0.056	0.153***	0.288		0.060	0.076
DVJ	0.175***	0.141***	0.092**	0.210***	0.194***	0.162		0.020
T	0.096***	0.116***	0.032***	0.131***	0.165**	0.079	0.124***	

Values above diagonal are based on a reduced data set including only unique genotypes at each locality; values below diagonal are for all sampled individuals. Estimated p values: * 0.01; ** 0.005; *** 0.001

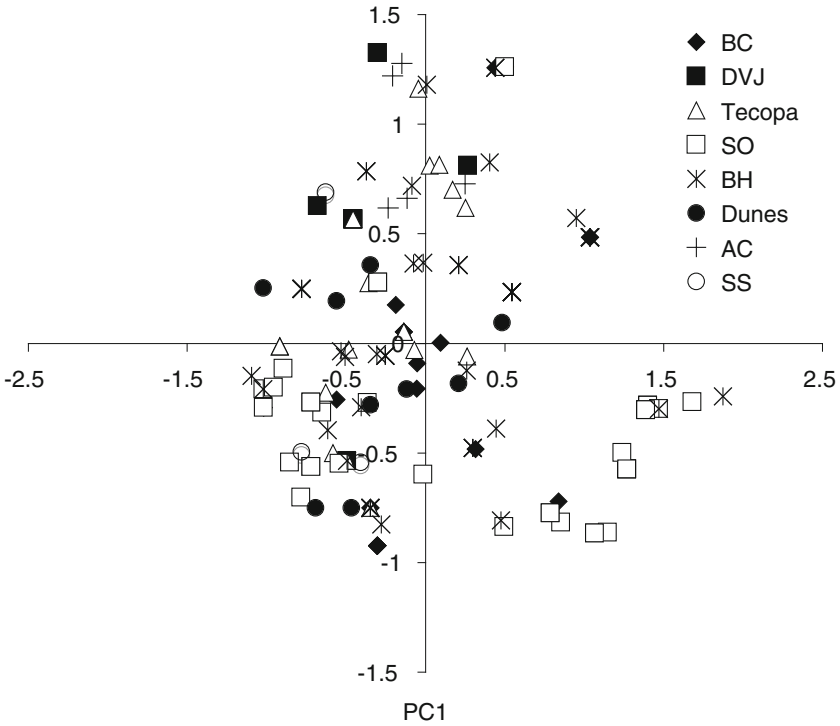

Fig. 6 Plot of principle components scores for all individuals based on multi-locus genotypes. Population codes are provided in the legend of Fig. 1 and in Table 2

clonal plant species (Fig. 3). The number of MLGs in each population ranged from 3 to 23, although all populations harbored similar numbers of genotypes when the estimates were normalized to a sample size of 17; the one exception was DVJ that had fewer genotypes than the other populations (Table 4). The proportion of genotypes that were unique to a particular population ranged from 0.25 to 1, although the two localities in which none of the genotypes were found elsewhere in the range had small sample sizes (Table 4).

Geographic distribution of genotypes

All of the populations for which we sampled a reasonably large number of individuals shared genotypes (Fig. 4; Table 5). Depending on the pair-wise comparison, the proportion of shared genotypes ranged from 0 to 0.18. More interestingly, three of the genotypes were shared between localities separated by more than 50 km (Figs. 4, 5). Tests of differentiation between all pairs of localities based on unique genotypes only (multiple clones from each site were removed) revealed one site that was consistently different from all other sites: the southern springs outflow (SO). All other pair-wise comparisons were not significant (Table 6). However, when we included all individuals in the analysis, nearly all of the pair-wise comparisons between localities were statistically significant (Table 6); the only exception was the Soda Springs population and this population was most likely not significantly different from other populations because we only successfully genotyped three individuals from a single collection site. A plot of the first two principle components scores based on

the genotypes of all individuals revealed little evidence of geographic structure; for instance, samples from the most geographically remote locality (T) did not cluster together to the exclusion of individuals from other localities (Fig. 6). There was no evidence of isolation by distance ($p > 0.5$).

Discussion

Inferences about modes of reproduction

It is clear from excavations that separate plants may be connected below ground by rhizomes. The existence of separate clusters of plants suggested that most plants comprising a cluster may be ramets of a single genet. However, at most collection sites where there were patches of *N. amargosa*, we discovered multiple genotypes, suggesting that clusters of plants consist of more than one genet. Given the high mutation rate for microsatellite loci, it is possible that the existence of multiple genotypes within a single large cluster of plants reflects somatic mutation; however, in many cases, this explanation seems unlikely because co-occurring genotypes often differed at two or more loci. Only 12 of the 78 genotypes were 1 mutation away from another genotype.

The existence of multiple genets within a single cluster of plants suggests that *N. amargosa* may reproduce asexually and sexually. The estimated proportion of individuals distinguishable (PD) for *N. amargosa* is similar to other species that reproduce both clonally and by outcrossing (Ellstrand and Roose 1987). Recombination during sexual reproduction probably explains most genotype diversity. Direct evidence of sexual reproduction is lacking, however. Additionally, germination rates are low suggesting sexual reproduction may by limited by low seedling establishment (Reveal 1978). Nonetheless, the existence of high genotypic diversity within populations and a relatively low ratio of ramets to genets (2.3) clearly suggest outcrossing occurs and that germination and seedling establishment happens.

It is unknown whether seeds are produced via germination, either by outcrossing or selfing, or whether seeds are produced asexually. A variety of different reproductive strategies are known to exist within the Chenopodiaceae. Both diplosporic and aposporic gametophytic apomixis (asexual reproduction) have been described for species within the family (Szkutnik 2010). The difference between the two forms is that the non-reduced 2N cells that become the seed can be derived either from megaspore mother cell after a failure of meiosis (diplospory) or from a nuclear cell (apospory). Gametophytic apomixis is typically restricted to polyploids, although it does occur in some diploids. There is no evidence that *N. amargosa* is a polyploid based on the microsatellite genotypes. DNA content flow cytometry is necessary for establishing whether plants are polyploid or diploid, however. If *N. amargosa* produces seed apomictically, then the widespread distribution of identical genets may be the result of asexually produced seed and dispersal by either water or wind.

If seed production occurs apomictically, it is unlikely that sexual reproduction by outcrossing created the different genotypes we discovered. It is conceivable, however, that *N. amargosa* is facultatively apomictic. Facultative apomixis has been described for a number of different plants (Barcaccia et al. 2006; Bicknell et al. 2003; Durand et al. 2000; Overath and Asmussen 2000; Szkutnik 2010), including members of the Chenopodiaceae (Szkutnik 2010), and some species are know to produce seed asexually and sexually in the same ovule (Harlan et al. 1964). In the genus *Paspalum* (a grass), plants produce seed apomictically in the absence of pollen, but produce seeds sexually when pollen is present (Siena et al. 2008). Facultative apomictic production of seed can explain the occurrence of widespread clones and abundant genotypic diversity within populations of *N. amargosa*, similar to explanations for other plants (Cosendai and Horandl 2011).

Another possibility is that seed production in *N. amargosa* is obligately sexual but that reproduction may involve selfing, outcrossing, or both. If reproduction occurs sexually by selfing, it is difficult to explain the presence of identical genotypes across multiple populations and more than 50 km apart, however. Multi-locus genotype 66 (based on all the data) provides an instructive case. The genotype is heterozygous at all eight loci surveyed. The probability of sampling an identical genotype by selfing that is heterozygous for all eight loci in two localities is $0.5^8 = 0.0039$. Thus, while it is possible that clones sampled from DVJ and T were identical because of selfing, it seems highly unlikely.

Finally, it is possible that the existence of identical genotypes in different populations may be the consequence of fragmentation (Reveal 1978). There is no evidence of fragmentation, however, even within localities. Overall, the genetic data suggest the possibility that *N. amargosa* may be facultatively apomictic but convincing data are lacking.

Conservation implications

Relatively high genetic diversity within all sampled populations suggest that inbreeding depression caused by the loss of variation from heightened rates of genetic drift expected in small populations (Bustamante et al. 2002; Hammerli and Reusch 2003) is probably not a problem in this species. Furthermore, the relatively low ratio of ramets (genetically identical plants) to genets (different genotypes) suggest that effective populations sizes are not small

and that census counts of *N. amargosa* abundance overestimate the number of reproductive plants by only twofold or threefold.

Additionally, lack of differentiation among populations implies that there is little incentive to recognize the geographically separated populations as demographically or evolutionary isolated. Nonetheless, while the populations within the AMNWR appear robust, the population surviving at the T site is not large and the plants are much less densely distribution across the landscape than in other localities (A. Martin, pers. obs.). The nearby spring has been modified into a hot tub of sorts and it is unclear whether this modification has changed soil moisture in a way that challenges *N. amargosa* growth and reproduction and that may ultimately cause the extirpation of this population.

Although the populations of *N. amargosa* across its range appear robust (large numbers of individuals and abundant genetic variability), the fact that the species depends on a high water table (Hasselquist and Allen 2009) suggests the species may suffer declines in number and distribution if local groundwater mining continues to increase (Deacon et al. 2007; Fuller and Harhay 2012) and the predictions for increased desiccation of the southwestern United States happen as a consequence of global warming (Cayan et al. 2011; Seager et al. 2007). All of these conservation issues suggest that the species should be consistently monitored to assess whether there are trends in abundance and shifts in the ratio of ramets to genets. With the existence of a set of readily characterized genotypes, detailed monitoring should be routine and informative.

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