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ORIGINAL PAPER

Genetic analysis of invasive populations of *Ventenata dubia* (Poaceae): an assessment of propagule pressure and pattern of range expansion in the Western United States

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Abstract Molecular markers prove to be an invaluable tool in assessing the introduction dynamics, pattern of range expansion, and population genetics of an invasive species. *Ventenata dubia* (Leers) Coss. (Aveneae; ventenata) is a diploid, primarily self-pollinating, annual grass native to Eurasia and Northern Africa. The grass has a detailed herbarium collection history in the western United States since its discovery in eastern Washington in 1952. Genetic analysis of 51 invasive populations (1636 individuals) of *V. dubia,* coupled with historical records, suggests moderate propagule pressure from multiple introductions, followed by local or regional range expansion. Allozyme analysis detected nine multilocus genotypes (MLGs) across eight western US states. A single MLG, referred to as the most common genotype, was detected in 37 of 51 (72.5%) invasive populations across all states. The other eight MLGs were generally found in fewer populations, with limited geographic distributions. Despite multiple introductions, invasive populations exhibit low levels of genetic admixture, low levels of genetic diversity within populations (A = 1.03, %P = 2.94, Hexp = 0.007) and high genetic differentiation among populations (G_{ST}=-0.864). The apparent reduced evolutionary potential of most *V. dubia* populations did not preclude the initial establishment and rapid spread of this species across its new range in the western US.

Keywords Admixture · Allozymes · Genetic diversity and structure · Herbarium specimens · Multilocus genotypes · Multiple introductions

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Introduction

Due to the negative ecological consequences and high economic costs of invasive species (Pimentel et al. 2005; Simberloff et al. 2013; Gaertner et al. 2014; Bellard et al. 2016), the need for predicting which non-native species will become invasive and which native communities will be invaded is of critical importance (Heger and Trepl 2003). As a result, an emerging

concept to best predict establishment success is propagule pressure (introduction effort) (Kolar and Lodge 2001; Lockwood et al. 2005; Simberloff 2009; Ricciardi et al. 2011; Blackburn et al. 2015). Propagule pressure is described as the number of individuals in any specific release event (propagule size), the number of discrete events per unit time (propagule number), as well the overall genetic variability of the founding populations (propagule richness) (Lockwood et al. 2005; Simberloff 2009; Ricciardi et al. 2011). High propagule pressure translates to large founder population sizes, high immigration rate, and/or high genetic diversity which can overcome stochastic processes, resulting in the establishment of non-native species (Simberloff 2009).

Because an invasion can arise from a single, or multiple, introduction(s) and potentially over a long period of time, an interdisciplinary approach can be useful in its reconstruction (Wilson et al. 2009; Estoup and Guillemaud 2010; Estoup et al. 2010; Pawlak et al. 2015). Herbarium (vouchered) specimens provide a chronology of the collection history of a species in a region, and such information has been used to assess the introduction and range expansion of invasive plants (Salo 2005; Chauvel et al. 2006). In addition, the use of molecular markers can provide a detailed picture of the genetic signatures of propagule pressure, the amount and distribution of genetic diversity within and among populations, and the occurrence and consequences of post-introduction events (Kolbe et al. 2004; Novak 2007; 2011; Estoup and Guillemaud 2010). The genetic signatures of high propagule pressure include: (1) the presence of a large number of genotypes/haplotypes among invasive populations, (2) invasive populations that are genetic admixtures (they contain genotypes from different native populations), and (3) similar genetic diversity between native and invasive populations, with little evidence of founder effects (Novak and Mack 2005; Huttanus et al. 2011; Gaskin et al. 2013).

Ventenata dubia (Leers) Coss. (Poaceae, common names ventenata, wiregrass, North Africa grass) is a diploid ($2n = 14$), primarily self-pollinating (hereafter referred to as selfing), winter annual grass in the Aveneae (oat tribe). The species is native to central, southern, and eastern Europe, northern Africa, western Asia, and the Caucasus region (Contu 2013; Prather 2018). There are eight described species in *Ventenata* (Koler) (The Plant List 2013), however *V. dubia* is the only species known to be introduced into the United States (US) (Fryer 2017). The first occurrence record of *V. dubia* was in 1952 in Spokane County, Washington (Flora of North America Editorial Committee 1993), and the grass is now widely distributed across the western US (Wallace et al. 2015). *Ventenata dubia* now occurs throughout much of the Pacific Northwest (Idaho, Oregon, and Washington) as well as California, Utah, Montana, Wyoming and most recently Nevada. In addition, the plant has been reported from Canada, near the Great Lakes, and the northeastern US, however limited records over time suggest that it does not persist in these regions (Fryer 2017).

In its invasive range, *V. dubia* grows in habitats ranging from sea level to mid-elevations (0–1800 m) (Pavek et al. 2011), which receive 350–1120 mm of annual precipitation (Prather 2009). *Ventenata dubia* is most commonly found in dry, open, disturbed areas such as fields, pastures, roadsides and rangelands; however, it can also be found in moist swales, vernal pools and roadside ditches that become dry in the summer (Fryer 2017). In the Pacific Northwest, *V. dubia* replaces native vegetation and endangers native communities such as sagebrush steppe, woodland, and Palouse prairie vegetation (Butler 2011; Wallace et al. 2015; Fryer 2017) because it can form dense stands, and has the potential to increase fuel load, alter fire regimes and promote further invasion, much like *Bromus tectorum* L. (cheatgrass) (D'Antonio and Vitousek 1992). Economic losses associated with *V. dubia* include 20% decrease in crop yields, especially in Kentucky Bluegrass and Timothy hay production. Moreover, because contaminated hay bales are rejected for export, prices of $200–$215/ton are reduced to $70–$100/ton (Fountain 2011).

No previous studies have assessed the genetic diversity, introduction dynamics, and pattern of spread of *V. dubia* in its invasive range, and the species provides an excellent opportunity to obtain insights into the mechanisms of biological invasion and an initial assessment of the population genetic consequences associated with invasion. In this study we will (1) assess the introduction dynamics (single vs multiple introductions) and estimate propagule pressure for the invasion of *V. dubia* in the western US, (2) assess the pattern of range expansion of the species in its new range, and (3) determine the level and structure of genetic diversity within and among invasive

populations of *V. dubia*. Results of this study will allow us to better understand the invasion of *V. dubia* into the western US and will provide the data needed to develop management strategies to control this destructive invasive grass species.

Methods

Herbarium specimens

Despite sampling biases (see Delisle et al. 2003; Daru et al. 2018), herbarium (voucher) specimens that have been properly validated provide a reliable record of the occurrence of a plant species at a certain location and time. *Ventenata dubia* occurrences were compiled through online herbarium databases such as, but not limited to, the Consortium of Pacific Northwest Herbaria (http://www.pnwherbaria.org), Consortium of California Herbaria (http://ucjeps.berkeley.edu/consortium/), Intermountain Regional Herbarium Network (http://intermountainbiota.org), and Global Biodiversity Information Facility (http://www.gbif.org/). Accessions were checked manually to eliminate multiple entries. Because there are native Aveneae species in the study area (e.g., *Deschampsia danthonioides* (Trin.) Munro), verification of specimens was done visually via digitized vouchers, or by species' descriptions available on file. In addition to online databases, state and county records were searched. In many instances, plant surveys at the state and county level were the source of the first county records of the grass, and vouchered specimens were sent to herbaria for verification and processing. Records for which a description or specimen on file were not available, were not considered the first county record because we deemed this information unreliable. Vouchered specimen records from all years were targeted for population sampling, especially the first record of occurrence in each county.

Plant collections and sampling

Across the invasive range of *V. dubia*, mature panicles (or entire plants) were collected from 51 populations spanning eight western states during the months of June–August, prior to seed dispersal. Samples from Oregon were collected in 2014–2016; Idaho, Montana, and Washington during 2015–2016, and California, Nevada, Utah, and Wyoming during 2016. Collection localities were typically located in areas disturbed by human activities, especially roadsides where mowing operations take place. In each population, 27–40 plants were collected haphazardly, based on the size of the population. To lessen the chance of collecting full siblings, individuals were collected 1–3 m apart. For small populations, all individuals were collected. Each panicle (or an entire plant) harboring mature seeds was placed individually in numbered envelopes and stored at room temperature until analysis.

All populations were collected by the procedure described above, apart from the population from Utah. Due to the time of collection, and the condition of the plants, *V. dubia* litter and debris was collected and placed in individual packets, with each litter sample collected approximately 20 m apart. From the litter and debris, a single *V. dubia* seed was sampled from each packet and germinated for allozyme analysis.

The 51 invasive populations analyzed in this study were chosen based on their historical significance (earliest herbarium specimens, see Fig. 1, Supplemental Information Tables 1 and 2), geographic distribution, as well as having enough viable seeds. These 51 populations were assigned to four sub-regions: (1) Coastal Range: populations generally located west of Cascade/Sierra Nevada Mountains; (2) Columbia Basin: populations from the Columbia Basin in eastern Washington and the Blue Mountains region of eastern Oregon; (3) Great Basin: populations from the Snake River Plains and the Great Basin; and (4) Rocky Mountains: populations from and east of the Northern Rocky Mountains and north-central Wyoming (Supplemental Information Fig. 1). Populations were assigned to these four sub-regions based on geographic features which may prevent seed dispersal (gene flow) among populations from different sub-regions.

Voucher specimens were collected for each population to be digitized and processed at the Snake River Plains Herbarium at Boise State University, Boise, Idaho.

Allozyme analysis

Ventenata dubia caryopses (hereafter referred to as seeds) were stored in the laboratory for at least 3 months to allow for after-ripening. After this time, seeds were extracted from the lemma and three seeds

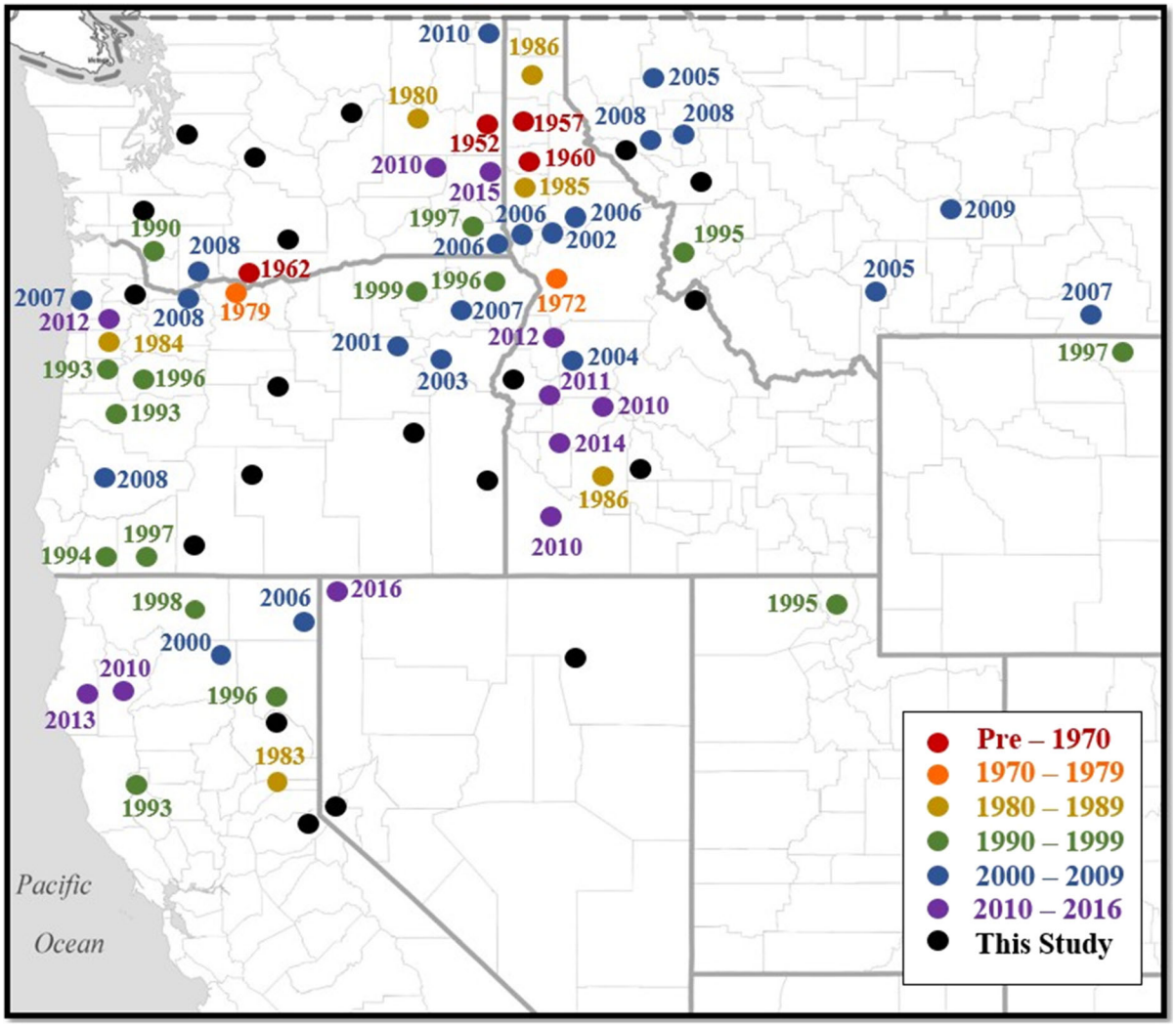


Fig. 1 Date and location of first detection of *Ventenata dubia* in each county in California, Idaho, Montana, Nevada, Oregon, Utah, Washington and Wyoming, based on herbarium specimens. Collection dates are color coded by decade. Black dots with no dates represent new county record specimens acquired over the course of this study (2015–2016)

per individual were germinated in Petri dishes lined with moistened filter paper. Seeds germinated 36–48 h after watering, and seedlings were harvested for analysis after 10–14 days of growth. Due to the small amount of tissue of *V. dubia* seedlings, it was necessary to use two to three seedlings from each maternal plant (family). It was not possible to grow seedlings for longer because of reduced enzyme production and enzyme degradation as they aged. In addition, the highly selfing mating system of *V. dubia* (e.g., low levels of observed heterozygosity, see "Results" section) means that, in almost all cases, all progeny from the same maternal plant are genetically identical.

Allozymes are single-locus, codominant molecular markers, which means that the genotype of individuals (i.e., homozygous versus heterozygous) can be inferred from enzyme banding patterns at each scored locus, thus providing a reliable estimate of allele frequencies within populations. However, allozymes do have several shortcomings, (1) genetic diversity is assessed at only a limited number of enzymes loci (that take part in important metabolic pathways), (2) because synonymous nucleotide sequence substitutions can occur but not alter enzyme banding patterns,

allozymes provide an underestimate of genetic diversity at the DNA sequence level, and (3) because the allozyme technique requires enzymes to be in their native, active state, high-quality tissue samples are required (Rowe et al. 2017). Allozymes were chosen for this study because this technique requires no development time, and it is a relatively inexpensive and rapid method for gathering population genetic data: with each gel run, which requires just a single day, we genotyped approximately 30 individuals at each of 26 allozyme loci (see "Results" section).

Our allozyme analysis was conducted following the procedures of Soltis et al. (1983), with modifications described by Novak et al. (1991). Fresh root and leaf tissues from *V. dubia* seedlings were macerated in Tris–HCl grinding buffer-PVP solution (pH 7.5). Several buffer systems and various enzymes were tested to determine the optimal band visualization conditions for *V. dubia*. After optimization, plants were assessed for their allozyme diversity at 15 enzymes, and these enzymes were visualized using four buffer systems: buffer system 1, isocitrate dehydrogenase (IDH), glyceraldehyde-3-phosphate dehydrogenase (G3PDH) and glucose-6-phosphate dehydrogenase (G6PDH); buffer system 7, alcohol dehydrogenase (ADH), glutamate oxalacetate transaminase (GOT), and phosphoglucoisomerase (PGI); buffer system 8, aldolase (ALD), colorimetric esterase (CE), glutamate dehydrogenase (GDH), leucine aminopeptidase (LAP) and triosephosphate isomerase (TPI); buffer system 9, malate dehydrogenase (MDH), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH, and 6-phosphogluconate dehydrogenase (6PGD).

Multilocus genotype assignment

Each *V. dubia* individual was assigned a multilocus genotype (MLG) based on the different alleles present at four polymorphic loci. One genotype is referred to as the "most common genotype" (MCG) and occurs most frequently throughout the introduced range of *V. dubia* because it has the most common combination of alleles at all polymorphic loci. Individuals which varied by one allele from the MCG, were considered a different MLG.

Data analysis

Genetic (allozyme) diversity

Allozyme diversity of the 51 invasive populations of *V. dubia* located in the western US was analyzed using the programs POPGENE 1.32 (Yeh and Boyle 1997) and R package "PopGen Report" v 3.0 (Gruber and Adamack 2015). For every individual, allozyme information was entered as their multilocus genotype. Range-wide genetic diversity parameters for *V. dubia* populations in the invasive range include total number of alleles, number of alleles per locus, number of polymorphic loci, percentage of polymorphic loci and percentage of polymorphic populations.

The Index of Association (I_A) was used to test whether loci exhibit linkage disequilibrium (non-random association of alleles between loci) (Brown et al. 1980). The less biased version, rbarD, accounts for the number of loci sampled (Agapow and Burt 2001) where a value of 0.0 indicates no linkage disequilibrium, and a value of 1.0 indicates complete disequilibrium. Both indexes are calculated with the program "poppr" (Kamvar et al. 2015), using 999 permutations. An additional test was run to "clone correct" the data., A pairwise I_A over all loci was performed to ensure that linkage is not the result of a single pair of loci.

Within-population genetic diversity was quantified using the following parameters: the mean number of alleles per locus (A), the number of polymorphic loci within each population (#P), the percent polymorphic loci per population (%P), the expected mean heterozygosity (Hexp) which was calculated using the unbiased estimate method of Nei (1978), the mean observed heterozygosity (Hobs), and the number of homozygous multilocus genotypes (#MLG). Means of these parameters are used to describe the level of genetic diversity (on average) within populations of *V. dubia* in its invasive range in the western US.

To test for deviation from Hardy–Weinberg expectations, Wright's (1965) fixation index ($F = 1 - Hobs/Hexp$) was calculated for each polymorphic locus in a population using POPGENE 1.32 (Yeh and Boyle 1997). The significance of any deviation was determined using a χ^2 test.

Genetic differentiation among populations

The R package "mmod" (Winter 2012) was used to calculate Nei and Chesser (1983) estimators of gene diversity and genetic differentiation. Using mmod, the total gene (allelic) diversity (H_T) was partitioned into the within-population component (H_S) and the among-population component (D_{ST}), with these parameters related by the following equation $H_T = H_S + D_{ST}$. The parameter G_{ST} describes the proportion of the total gene diversity that is partitioned among populations, and was calculated as $G_{ST} = 1 - H_S/H_T$. G_{ST} is a measure of the level of genetic differentiation among populations.

Analysis of molecular variance (AMOVA) was used to estimate the amount of genetic variation partitioned within and among populations. In addition, a hierarchical analysis was performed to determine the amount of genetic variation partitioned within and among populations in the four geographic regions described above. AMOVA was calculated using R package "poppr" (Kamvar et al. 2015).

To graphically show genetic differentiation among populations, an UPGMA phenogram was generated using the program POPGENE 1.32 (Yeh and Boyle 1997) based on Nei's (1978) unbiased genetic distance. This method was used as an alternative to Neighbor joining tree, as the UPGMA procedure assumes the same evolutionary rate for all lineages.

Bayesian assignment analysis

The Bayesian assignment software STRUCTURE (Pritchard et al. 2000) was used to determine the number of genetic clusters (K) for the 51 invasive populations of *V. dubia* using the method of Evanno et al. (2005). In addition, the "hierarchical structure analysis" described by Vähä et al. (2007) and Olafsson et al. (2014) was used to determine the most likely number of genetic clusters. Our initial STRUCTURE analysis included two simulations, one run with K 1-10 with 10,000 iterations and 100,000 Markov Chain Monte Carlo (MCMC) repetitions, and the second set at K 1-8 with 100,000 iterations and 1,000,000 Markov Chain Monte Carlo (MCMC) repetitions. In the second and third round of analysis, populations which were assigned to different subgroups were analyzed separately (Vähä et al. 2007; Olafsson et al. 2014). For invasive populations, individuals with an assignment of 0.8 q or greater were included in the second and third round of analysis, while individuals with < 0.8 q assignment were not used in subsequent runs. Hierarchical substructuring was completed when K was determined to be unequivocal (q assignment was equal among groups).

STRUCTURE results were run with the program "Pophelper 2.0", an R package program specifically designed to analyze and visualize population structure as well as the online web app (http://pophelper.com/) (Francis 2017). Additional graphical representation was obtained using STRUCTURE HARVESTER (Earl 2012) by web service at http://taylor0.biology.ucla.edu/structureHarvester/#.

Genotypic diversity, richness, and evenness

The R package "poppr" (Kamvar et al. 2015) was used to calculate the mean values as measures of genetic diversity, richness, and evenness: Shannon–Wiener Index of MLG diversity (H) (Pielou 1966; Grünwald et al. 2003), Simpson's Index lambda (λ) (Simpson 1949) and E5 (Hill's modified ratio) (Alatalo 1981; Ludwig and Reynolds 1988), where Simpson's Index lambda (λ) is calculated as one minus the sum of squared genotype frequencies and range between 0 (no genotypes are different) to 1 (all genotypes are different). Additionally, the measure of genotypic richness was calculated by direct observation of the number of unique genotypes contained in populations (#MLGs).

Results

Herbarium specimens

In Supplemental Information Table 1 we list the earliest specimens of *V. dubia*, in our dataset, within counties across eight western US states, and this information reveals several patterns about its introduction and range expansion across the region (Fig. 1). The grass was first collected in Spokane County, Washington [No accession] in 1952, with the next specimens collected in Kootenai County, Idaho [WTU273715] in 1957 and in Benewah County, Idaho [WTU273743] in 1960. Another pre-1970 specimen of the grass was from south-central Washington

(Klickitat County [WS247993] in 1962). In the decades of the 1970s and 1980s, several collections were made in areas near these five pre-1970 reports of the grass, as well as a few isolated specimens from California and southern Idaho (Placer County, California [UCD94425] 1983, Elmore County, Idaho [ID037447] 1986). In the 1990s, there was an increase in the number of *V. dubia* herbarium specimens across seven states in the western US: California, Idaho, Montana, Oregon, Utah, Washington, and Wyoming. Four specimens were collected in western Oregon (Willamette Valley) and three specimens were collected in northern California, with additional specimens collected in Washington and northeast Oregon. Simultaneously, several new state records also occurred in Montana, Utah and Wyoming (Ravalli County, Montana [MONT79339] 1995, Cache County, Utah [UTC00216696] 1995, Sheridan County, Wyoming [RM655052] 1997). More recent specimens in most states (2000s, and forward in time) may be the result of expansion from neighboring counties, while most specimens were collected in southern Idaho and northwestern and southern Montana. The most recently collected specimens were from three counties in Nevada in the year 2016 (Washoe County, [V84851], Douglas County, [SRP58611], Elko County, [SRP61395] (Supplemental Information Table 1).

Allozyme diversity patterns

Of the 51 invasive populations (1636 total individuals; 32.1 individuals per population) of *V. dubia* from the western US analyzed at 26 allozyme loci, 15 of 51 populations were polymorphic at one or more loci. Among all 1636 individuals, 30 alleles were identified (1.15 alleles/locus) and four loci were polymorphic (15.4%): *Ce-2, Ce-5, Pgi-2, Tpi-2*. Each polymorphic locus had two alleles. Six of 15 polymorphic populations exhibited diversity at four loci (Supplemental Information Table 3).

An analysis of the modified index of association (rbarD) revealed varying degrees of linkage disequilibrium among polymorphic loci, and provide significant support for the hypothesis that overall, alleles across different loci are linked (Supplemental Information Fig. 2a). Clone corrected data confirmed these results (Supplemental Information Fig. 2b), further supporting the evidence of significant linkage disequilibrium among loci. A graphical representation of the degree of linkage among loci reveals that most alleles observed at different loci (four of six loci pairs) are associating somewhat randomly in invasive populations of *V. dubia* (Supplemental Information Table 4). While linkage disequilibrium between loci does occur among invasive populations, we did not detect complete disequilibrium (1.0), thus all loci will be retained in all analyses according to the recommendations of Flint-Garcia et al. (2003).

Multilocus genotypes

Across 51 invasive populations of *V. dubia*, nine MLGs were detected (Fig. 2), with 36 of 51 populations contained a single MLG (Supplemental Information Table 5). Twenty-seven of 51 populations were composed of only the MCG (depicted by dark blue) (Fig. 2), while 36 of 51 populations contained at least one individual with the MCG. Of all 1636 individuals analyzed, 1030 were found to have the MCG. Populations containing the MCG are widespread and found in every state: California (five of eight populations), Idaho (all eight populations), Montana (all four populations), Oregon (eight of 16 populations), Washington (seven of 10 populations), and the only MLG detected in Utah, Nevada and Wyoming (Fig. 2).

The second most frequent MLG (depicted by yellow) was found in 11 of 51 populations, with 10 of these populations located in Washington and Oregon (Fig. 2). The yellow MLG was detected in 231 of 1636 individuals analyzed. The other seven MLGs were more locally distributed and occurred at lower frequencies. These low-frequency genotypes occur primarily in the Coastal Range sub-region in western portions of California, Oregon, and Washington. For instance, two MLGs (teal and green) were each found in only one population, in California and Oregon, respectively (Fig. 2).

Genetic diversity within populations

Among the 51 invasive populations analyzed, the mean number of alleles (A) was 1.03, the number of polymorphic loci (#P) and percent polymorphic loci (%P) are 0.76 and 2.94, respectively, expected mean heterozygosity (Hexp) was 0.0072, mean observed heterozygosity (Hobs) was 0.00009, and the number of

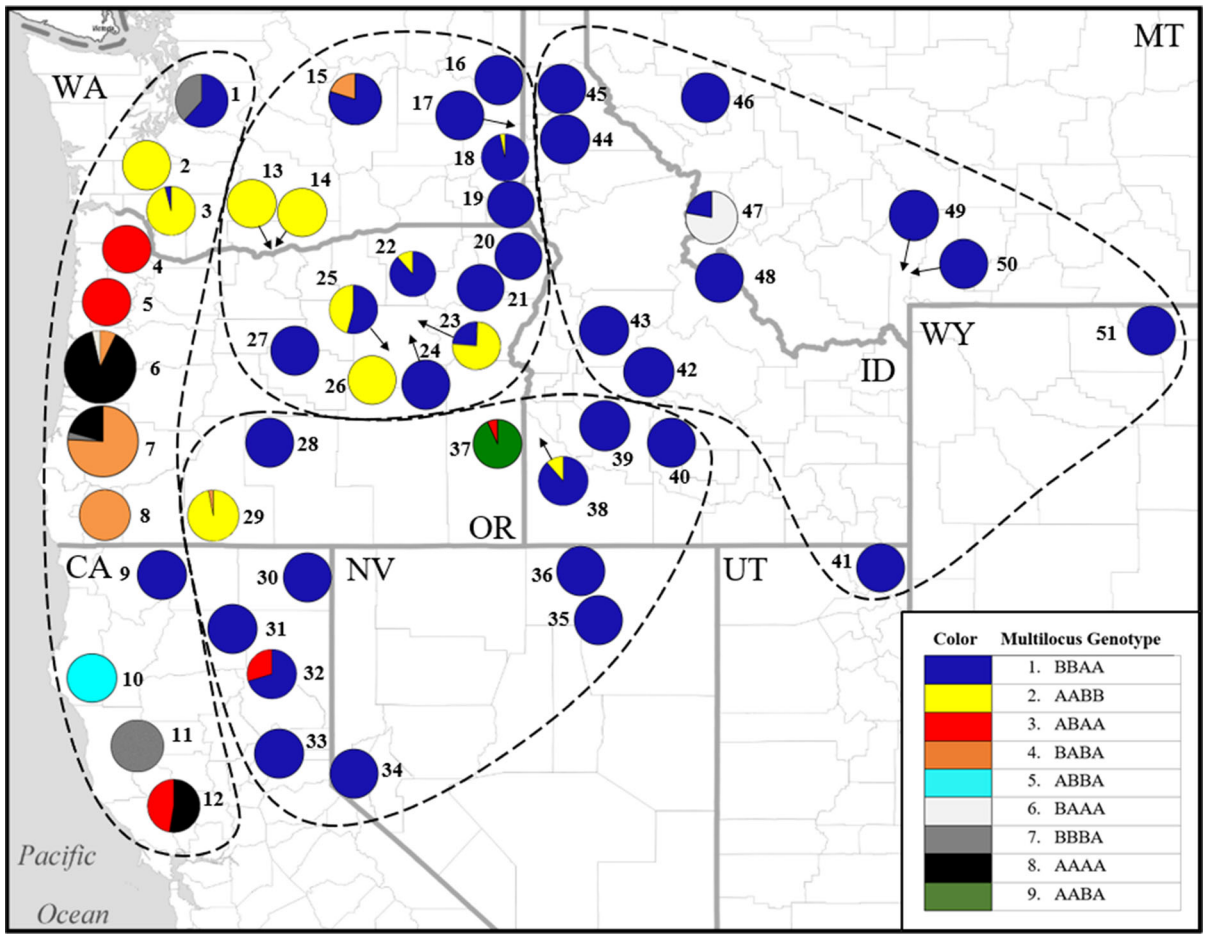


Fig. 2 Map showing the distribution of the nine multilocus genotypes (MLG) detected in 51 invasive populations of *Ventenata dubia*. Color of each multilocus genotype follows the key provided in this figure. Letters in the key represent different alleles at each of four homozygous polymorphic loci: Ce-2, Ce-5, Pgi-2, and Tpi-2. Genotype number are assigned by order of discovery. The most common genotype (MCG) is shown in blue. Sizes of the pie diagrams vary only to enhance legibility. Dashed lines represent regional population groups: Coastal Range, Columbia Basin, Great Basin, and Rocky Mountains

homozygous multilocus genotypes (#MLG) was 1.33 (Table 1). Only two of the 1636 *V. dubia* individuals analyzed in this study were heterozygous, and both individuals were in the population from Mosquito Creek, Oregon (*Hobs* = 0.0044). The populations with the highest amount of genetic diversity were Joe Rausch's Shaketable, Oregon, Mosquito Creek, Oregon, and Starkey, Oregon; all three of these populations contained four polymorphic loci. Other populations with four polymorphic loci included Little Squab Creek, Idaho, Pullman, Washington, and Kalama, Washington. Thirty-eight populations lacked any allozyme diversity (Table 1).

On average, populations from the Columbia Basin sub-region had the highest level of genetic diversity, followed by populations from the Coastal Range sub-region, and the Great Basin sub-region. Populations from the Rocky Mountains sub-region had the lowest genetic diversity (Table 1).

All 15 populations which contained at least one polymorphic locus showed a significant deviation from Hardy–Weinberg equilibrium. Significant deviations from Hardy–Weinberg equilibrium were observed at all 39 polymorphic loci in these populations. Wright's fixation index (F) values for *Pgi*-2 and *Tpi*-2 in the population from Mosquito Creek, Oregon,

Table 1 Within-population genetic diversity parameters for 51 invasive populations of *Ventenata dubia* from the western US analyzed in this study

Region	Population	N	A	#P	%P	Hobs	Hexp	#MLG
Coastal range	1. Wilderness Village, WA	34	1.04	1	3.85	0.0000	0.0182	2
	2. Toledo, WA	28	1.00	0	0.00	0.0000	0.0000	1
	3. Kalama, WA	25	1.15	4	15.38	0.0000	0.0118	2
	4. Sherwood, OR	30	1.00	0	0.00	0.0000	0.0000	1
	5. Monmouth, OR	33	1.00	0	0.00	0.0000	0.0000	1
	6. Eugene, OR	42	1.08	2	7.69	0.0000	0.0117	3
	7. Roseburg, OR	39	1.12	3	11.54	0.0000	0.0292	3
	8. Bigelow, OR	32	1.00	0	0.00	0.0000	0.0000	1
	9. Tenant Rd, CA	30	1.00	0	0.00	0.0000	0.0000	1
	10. Hidden Valley Rd, CA	30	1.00	0	0.00	0.0000	0.0000	1
	11. Lake Pillsbury, CA	32	1.00	0	0.00	0.0000	0.0000	1
	12. Lower Lake, CA	38	1.04	1	3.85	0.0000	0.0192	2
Coastal range mean		33	1.04	0.9	3.53	0.0000	0.0075	1.6
Columbia Basin	13. Husum, WA	30	1.00	0	0.00	0.0000	0.0000	1
	14. Lyle, WA	35	1.00	0	0.00	0.0000	0.0000	1
	15. Sims Corner, WA	35	1.08	2	7.69	0.0000	0.0246	2
	16. Spangle, WA	35	1.00	0	0.00	0.0000	0.0000	1
	17. Rosalia, WA	30	1.00	0	0.00	0.0000	0.0000	1
	18. Pullman, WA	35	1.15	4	15.38	0.0000	0.0166	2
	19. Anatone, WA	30	1.00	0	0.00	0.0000	0.0000	1
	20. Flora, OR	30	1.00	0	0.00	0.0000	0.0000	1
	21. Mill Creek, OR	33	1.00	0	0.00	0.0000	0.0000	1
	22. Starkey, OR	35	1.15	4	15.38	0.0000	0.0311	2
	23. Mosquito Creek, OR	35	1.15	4	15.38	0.0044	0.0608	2
	24. Keeny Meadows, OR	19	1.00	0	0.00	0.0000	0.0000	1
	25. J. Rausch's Shaketable, OR	35	1.15	4	15.38	0.0000	0.0764	2
	26. North Finger Rd, OR	35	1.00	0	0.00	0.0000	0.0000	1
	27. Ochoco Mountains, OR	30	1.00	0	0.00	0.0000	0.0000	1
Columbia Basin mean		32	1.05	1.2	4.61	0.0003	0.0140	1.3
Great Basin	28. Silver Lake, OR	34	1.00	0	0.00	0.0000	0.0000	1
	29. Klamath Lake, OR	31	1.08	2	7.69	0.0000	0.0048	2
	30. Pine Creek, CA	30	1.00	0	0.00	0.0000	0.0000	1
	31. Ahjumawi Lava SP, CA	30	1.00	0	0.00	0.0000	0.0000	1
	32. Susanville, CA	27	1.04	1	3.85	0.0000	0.0160	2
	33. Emigrant Gap, CA	30	1.00	0	0.00	0.0000	0.0000	1
	34. Stateline, NA	31	1.00	0	0.00	0.0000	0.0000	1
	35. Elko, NV	36	1.00	0	0.00	0.0000	0.0000	1
	36. Mountain City, NV	30	1.00	0	0.00	0.0000	0.0000	1
	37. JB Charbonneau GS, OR	39	1.08	2	7.69	0.0000	0.0109	2
	38. Little Squab Creek, ID	35	1.15	4	15.38	0.0000	0.0241	2
	39. Lucky Peak, ID	36	1.00	0	0.00	0.0000	0.0000	1
	40. Hill City, ID	30	1.00	0	0.00	0.0000	0.0000	1
Great Basin mean		32	1.02	0.7	2.66	0.0000	0.0043	1.3
Rocky mountains	41. Old Sardine Canyon Rd, UT	30	1.00	0	0.00	0.0000	0.0000	1

Table 1 continued

Region	Population	N	A	#P	%P	Hobs	Hexp	#MLG
	42. Kirkham Campground, ID	30	1.00	0	0.00	0.0000	0.0000	1
	43. Sugarloaf Peninsula, ID	31	1.00	0	0.00	0.0000	0.0000	1
	44. Tensed, ID	35	1.00	0	0.00	0.0000	0.0000	1
	45. Beauty Bay, ID	30	1.00	0	0.00	0.0000	0.0000	1
	46. Bison Range, MT	33	1.00	0	0.00	0.0000	0.0000	1
	47. Hamilton, MT	36	1.04	1	3.85	0.0000	0.0133	2
	48. Gibbonsville, ID	30	1.00	0	0.00	0.0000	0.0000	1
	49. Bozeman, MT	30	1.00	0	0.00	0.0000	0.0000	1
	50. Triple Tree, MT	30	1.00	0	0.00	0.0000	0.0000	1
	51. Soldier Creek, WY	27	1.00	0	0.00	0.0000	0.0000	1
Rocky Mountains mean		31	1.00	0.1	0.35	0.0000	0.0012	1.1
Overall mean		32	1.03	0.76	2.94	0.00009	0.00723	1.33

Parameters are the sample size of each population (N), mean number of alleles per locus (A), number of polymorphic loci per population (#P), percent polymorphic loci per population (%P), mean observed heterozygosity (Hobs), expected mean heterozygosity (Hexp) (unbiased estimate method of Nei (1978), and the number of homozygous multilocus genotypes (#MLG). Populations are organized based on the four geographic regions, and the regional mean values and total mean of all parameters are provided

are due to the presence of two heterozygous individuals at both loci (Table 2).

Genetic differentiation among populations

Averaged across the four polymorphic loci, Nei's (1987) total gene (allelic) diversity (H_T) is 0.349, the within-population component of gene diversity (H_S) is 0.048, and the among–population component of gene diversity (D_{ST}) is 0.301. The proportion of total gene diversity partitioned among populations (G_{ST} = 0.864) indicate that 86.4% of the total allelic diversity is partitioned among populations (Table 3). All four polymorphic loci had relatively high values for total gene diversity (H_T), and values for the proportion of total gene diversity partitioned among populations (G_{ST}) indicate high genetic structure at all four loci (Table 3).

Results of Analysis of molecular variance (AMOVA) reveal that 14.59% of the genetic diversity was partitioned within populations, while 85.23% of the genetic diversity was partitioned among populations Table 4a). A second AMOVA analysis showed that 14.14% of the genetic diversity was partitioned within populations, 72.66% of the diversity was partitioned among populations within regions, and 13.03% of the diversity was partitioned among regions (Table 4b).

The UPGMA dendrogram, based on Nei's (1978) genetic distance values, showed that most populations of *V. dubia* analyzed in this study occurred in four distinct clusters (Fig. 3). The largest cluster contains populations representing predominantly the Columbia Basin, Great Basin, and the Rocky Mountains sub-regions. The second and third cluster contains populations which are distributed in the Coastal Range sub-region exclusively. Cluster 4 contains populations predominantly assigned to the Columbia Basin and Coastal Range sub-regions. Populations from Hamilton, Montana, Joe Rausch's Shaketable, Oregon, Lake Pillsbury, California, and JB Charbonneau GS, Oregon, were excluded from clustering assignment as the populations were the only populations on their respective branches.

Bayesian assignment analysis

Two initial STRUCTURE analyses were run for invasive populations of *V. dubia* using the method of Evanno et al. (2005) to determine the number of genetic clusters (K) (Supplemental Information: detailed description of the Bayesian assignment analysis). Both analyses resulted in support for K = 2 (Supplemental Information Fig. 3a, b), with the second simulation resulting in two clusters, shown in red and green in Fig. 4a. The hierarchical structure

Table 2 Fixation indices (F) for 39 polymorphic loci in 51 invasive populations of *Ventenata dubia* from the western US

Population	Locus	F*
Wilderness Village, WA	*Pgi-2*	1.0
Kalama, WA	*Ce-2*	1.0
	Ce-5	1.0
	Pgi-2	1.0
	Tpi-2	1.0
Eugene, OR	*Ce-2*	1.0
	Pgi-2	1.0
Roseburg, OR	*Ce-2*	1.0
	Ce-5	1.0
	Pgi-2	1.0
Lower Lake, CA	*Ce-5*	1.0
Sims Corner, WA	*Ce-5*	1.0
	Pgi-2	1.0
Pullman, WA	*Ce-2*	1.0
	Ce-5	1.0
	Pgi-2	1.0
	Tpi-2	1.0
Starkey, OR	*Ce-2*	1.0
	Ce-5	1.0
	Pgi-2	1.0
	Tpi-2	1.0
Mosquito Creek, OR	*Ce-2*	1.0
	Ce-5	1.0
	Pgi-2	0.8504
	Tpi-2	0.8504
Joe Rausch's Shaketable, OR	*Ce-2*	1.0
	Ce-5	1.0
	Pgi-2	1.0
	Tpi-2	1.0
Klamath Lake, OR	*Ce-2*	1.0
	Tpi-2	1.0
Susanville, CA	*Ce-2*	1.0
JB Charbonneau, OR	*Ce-5*	1.0
	Pgi-2	1.0
Little Squab, OR	*Ce-2*	1.0
	Ce-5	1.0
	Pgi-2	1.0
	Tpi-2	1.0
Hamilton, MT	*Ce-5*	1.0

Values of 1.00 indicate complete deviation from Hardy–Weinberg equilibrium through non-random mating. Positive values of F indicate a deficit of heterozygous individuals compared to Hardy–Weinberg expectations, due to high levels of selfing. All values are significant at $P < 0.001$

Table 3 Nei's (1987) gene diversity statistics of 51 invasive populations of *Ventenata dubia* from the western US

Locus	H_T	H_S	D_{ST}	G_{ST}
Ce-2	0.395	0.049	0.346	0.876
Ce-5	0.376	0.056	0.320	0.851
Pgi-2	0.375	0.057	0.318	0.849
Tpi-2	0.248	0.030	0.218	0.881
Mean	0.349	0.048	0.301	0.864

See text for a description of the Nei's gene diversity statistics parameters

analysis (Vähä et al. 2007; Olafsson et al. 2014) of the green cluster resulted in a SubK = 2, (Supplemental Information Fig. 1c). Approximately 62% of these individuals were assigned to the light blue cluster, and approximately 38% were assigned to the orange cluster (Fig. 4b). Further analysis of the light blue cluster showed a SubK = 2 (Supplemental Information Fig. 1d), which assigned 43% of individuals to the dark blue cluster and 39% of individuals to the lilac cluster, with 18% of individuals discarded from the analysis (Fig. 4c). Thus, four genetic clusters were identified in this hierarchical structure analysis of 51 invasive populations of *V. dubia* (indicated in red, orange, dark blue, and lilac in Fig. 4). These four genetic clusters do not correspond to the four population sub-regions; rather membership in these clusters appears to be based on MLGs and pattern of admixture in invasive populations of *V. dubia*. (Supplemental Information: detailed description of the Bayesian assignment analysis).

Genotypic richness, diversity, and evenness

Shannon–Wiener index of MLG diversity ranged from 0.14 to 0.74 in polymorphic populations, and was highest in Mosquito Creek, Oregon, followed by Joe Rausch's Shaketable, Oregon, and Lower Lake, California. Simpson's Index (λ) ranged from 0.06 to 0.50 in genetically variable populations and was highest in Joe Rausch's Shaketable, Oregon and Lower Lake, California, followed by Wilderness Village, Washington. Hill's modified ratio for evenness (E5) ranged from 0.44 to 1.0, among polymorphic populations, with an overall mean value of 0.69 (Supplemental Information Table 6). The 36 populations which had

no allozyme variability had a value of zero for both indices, while evenness cannot be computed for populations lacking genetic diversity. Populations which had multilocus genotypes in near equal abundance were detected at Lake Pillsbury, California, followed by Joe Rausch's Shaketable, Oregon, and Wilderness Village, Washington.

Populations from the Coastal Range had the highest diversity values, followed by populations from the Columbia Basin, while populations from the Rocky Mountains had the lowest diversity. Populations from the Rocky Mountains showed the highest values for evenness, followed by populations from the Columbia Basin; populations from the Great Basin had the lowest evenness value (Supplemental Information Table 6).

Discussion

Our genetic analysis of 51 invasive populations (1636 individuals) of *V. dubia,* coupled with historical records, suggests a moderate propagule pressure from multiple introductions, followed by local or regional range expansion. In addition, our allozyme analysis detected nine multilocus genotypes (MLGs) across eight western US states. A single MLG, referred to as the most common genotype (MCG), was detected in 37 of 51 invasive populations across all US states. The other eight MLGs were generally found in fewer

Fig. 3 Unweighted pair-group method with arithmetic averaging (UPGMA) phenogram for the 51 invasive populations of *Ventenata dubia* analyzed in this study. Populations indicated by (*) are the only populations on their respective branches and are therefore not assigned to a cluster

populations, with limited geographic distributions. Despite multiple introductions, invasive populations exhibit low levels of genetic admixture, low levels of genetic diversity within populations, and high genetic differentiation among populations.

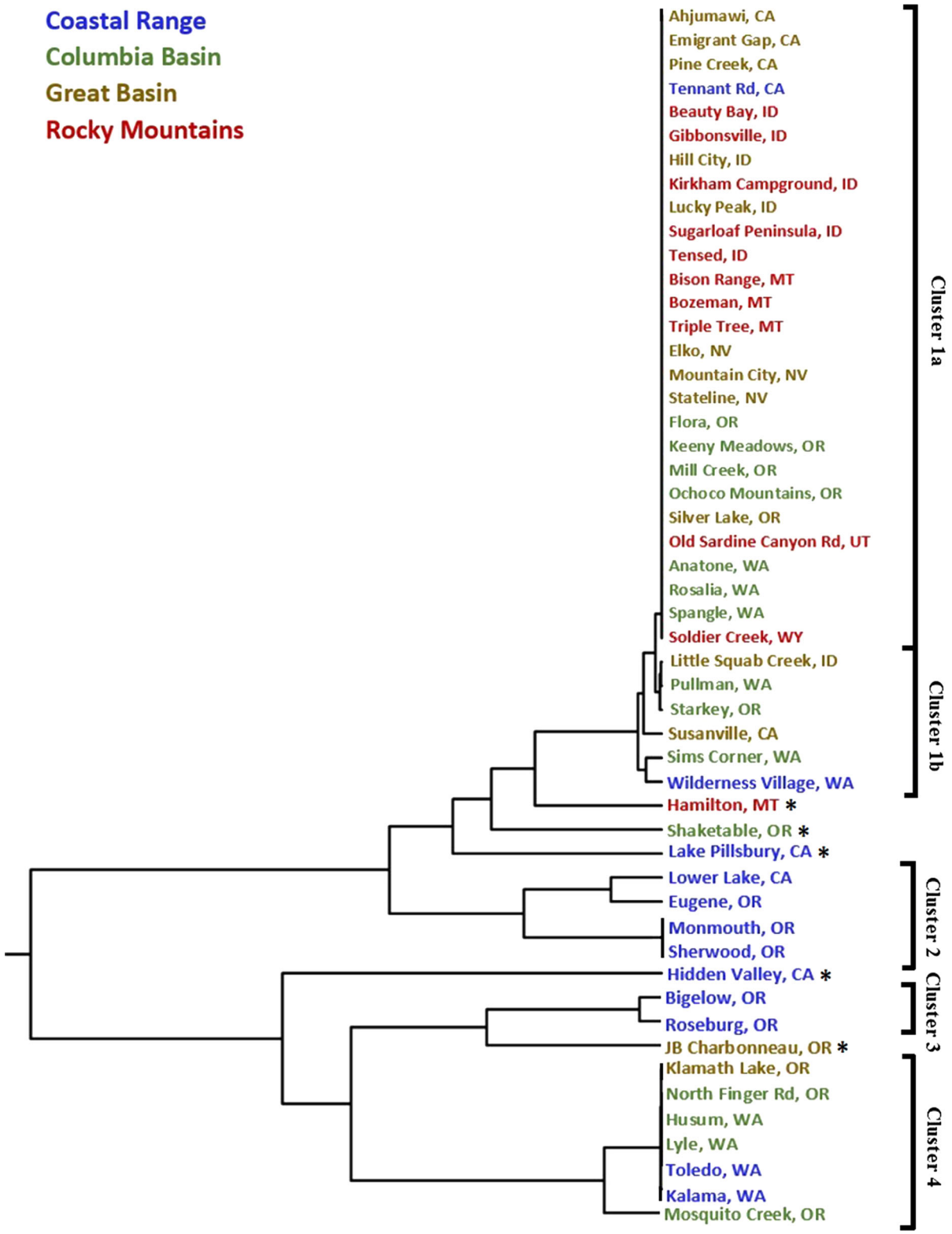
As outlined above, validated herbarium specimens provide reliable information concerning the occurrence of a plant at a certain place and time. *Ventenata dubia* has a detailed collection history in the western US, and this history is consistent with the pattern often associated with multiple introductions and local or regional range expansion (Salo 2005; Chauvel et al. 2006). Based on herbarium records and population collections made during this study, we show that *V. dubia* now occurs in at least 22 Oregon, 18 Idaho, 15 Washington, 10 California, nine Montana, three Nevada, one Wyoming, and one Utah counties.

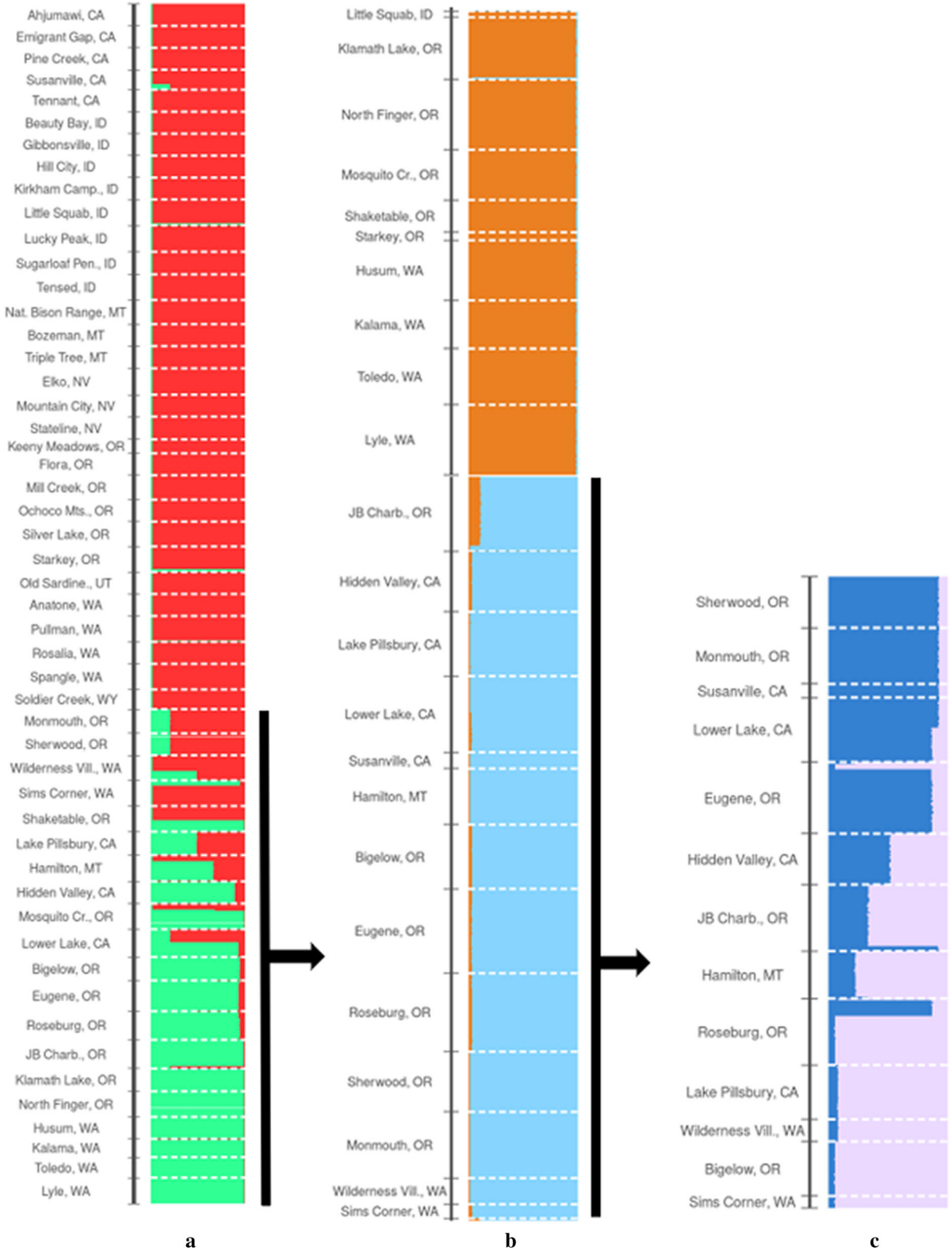
Any inferences drawn from the collection history of an invasive species can be further assessed using molecular data. The detection of nine homozygous MLGs among the 51 invasive populations of *V. dubia* analyzed in this study suggests that multiple and separate introduction events into the western US have occurred. The MCG (blue color) was detected in

Table 4 Analysis of molecular variance (AMOVA) using 'poppr' (Kamvar et al. 2015) of 51 invasive populations of *Ventenata dubia* from the western US

	df	Sum of squares	Variation component	Percentage variation
(a)				
Among populations	50	1980.995	0.61442	85.23
Within populations	1586	335.415	0.10519	14.59
Within individuals	1636	2.000	0.00122	00.16
Total	3271	2318.411	0.72084	–
(b)				
Among regions	3	342.7165	0.09697	13.03
Among populations within regions	47	1638.2789	0.54059	72.66
Among samples within populations	1585	335.4153	0.10520	14.14
Within samples	1636	2.0000	0.00122	00.16
Total	3271	2318.4108	0.74399	–

Part (a) shows the amount of genetic variation partitioned within and among populations; and (b) is a hierarchical analysis for the amount of genetic variation partitioned within populations, among populations within regions, and the four geographic regions


Coastal Range
Columbia Basin
Great Basin
Rocky Mountains
Ahjumawi, CA
Emigrant Gap, CA
Pine Creek, CA
Tennant Rd, CA
Beauty Bay, ID
Gibbonsville, ID
Hill City, ID
Kirkham Campground, ID
Lucky Peak, ID
Sugarloaf Peninsula, ID
Tensed, ID
Bison Range, MT
Bozeman, MT
Triple Tree, MT
Elko, NV
Mountain City, NV
Stateline, NV
Flora, OR
Keeny Meadows, OR
Mill Creek, OR
Ochoco Mountains, OR
Silver Lake, OR
Old Sardine Canyon Rd, UT
Anatone, WA
Rosalia, WA
Spangle, WA
Soldier Creek, WY
Little Squab Creek, ID
Pullman, WA
Starkey, OR
Susanville, CA
Sims Corner, WA
Wilderness Village, WA
Hamilton, MT *
Shaketable, OR *
Lake Pillsbury, CA *
Lower Lake, CA
Eugene, OR
Monmouth, OR
Sherwood, OR
Hidden Valley, CA *
Bigelow, OR
Roseburg, OR
JB Charbonneau, OR *
Klamath Lake, OR
North Finger Rd, OR
Husum, WA
Lyle, WA
Toledo, WA
Kalama, WA
Mosquito Creek, OR
Cluster 1a
Cluster 1b
Cluster 2
Cluster 3
Cluster 4


Ahjumawi, CA
Emigrant Gap, CA
Pine Creek, CA
Susanville, CA
Tennant, CA
Beauty Bay, ID
Gibbonsville, ID
Hill City, ID
Kirkham Camp., ID
Little Squab, ID
Lucky Peak, ID
Sugarloaf Pen., ID
Tensed, ID
Nat. Bison Range, MT
Bozeman, MT
Triple Tree, MT
Elko, NV
Mountain City, NV
Stateline, NV
Keeny Meadows, OR
Flora, OR
Mill Creek, OR
Ochoco Mts., OR
Silver Lake, OR
Starkey, OR
Old Sardine., UT
Anatone, WA
Pullman, WA
Rosalia, WA
Spangle, WA
Soldier Creek, WY
Monmouth, OR
Sherwood, OR
Wilderness Vill., WA
Sims Corner, WA
Shaketable, OR
Lake Pillsbury, CA
Hamilton, MT
Hidden Valley, CA
Mosquito Cr., OR
Lower Lake, CA
Bigelow, OR
Eugene, OR
Roseburg, OR
JB Charb., OR
Klamath Lake, OR
North Finger, OR
Husum, WA
Kalama, WA
Toledo, WA
Lyle, WA
Little Squab, ID
Klamath Lake, OR
North Finger, OR
Mosquito Cr., OR
Shaketable, OR
Starkey, OR
Husum, WA
Kalama, WA
Toledo, WA
Lyle, WA
JB Charb., OR
Hidden Valley, CA
Lake Pillsbury, CA
Lower Lake, CA
Susanville, CA
Hamilton, MT
Bigelow, OR
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Wilderness Vill., WA
Sims Corner, WA
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Lower Lake, CA
Eugene, OR
Hidden Valley, CA
JB Charb., OR
Hamilton, MT
Roseburg, OR
Lake Pillsbury, CA
Wilderness Vill., WA
Bigelow, OR
Sims Corner, WA
a
b
c

◀**Fig. 4** STRUCTURE (Pritchard et al. 2000) bar plots of the genetic clusters identified for invasive populations of *Ventenata dubia*. **a** the initial partitioning analysis of 51 invasive populations (K = 2), **b** results for invasive populations based on the hierarchical structuring analysis of the green cluster (subK = 2), and **c** results for invasive populations based on the hierarchical structuring analysis of light blue cluster (subK = 2). The dark lines and arrows indicate which genetic clusters were included in the next round of analysis

V. dubia populations in every western US state. Moreover, this genotype occurs in localities where the grass was first collected (Spokane County, Washington and Kootenai County, Idaho) and now predominates in populations in the Rocky Mountains, Great Basin and Columbia Basin sub-regions. The occurrence of the MCG across this large area may reflect the following sequence of events: the introduction of this MLG into Spokane County, and its subsequent spread as range expansion of *V. dubia* proceeded eastward and southward through several major highways. An alternative scenario for the widespread distribution of the MCG in the eastern portion of the study area involves independent (multiple) introductions of this MLG into various locations in the region. Such a hypothesis may explain the occurrence of the MCG in the isolated and localized populations of the grass in Wyoming and Utah. Although our allozyme data do not allow us to differentiate between these two scenarios, this latter scenario appears less parsimonious.

Multiple introductions likely explain the number and distribution of MLGs within and among the 12 populations in the Coastal Range sub-region: eight of the nine MLGs detected among all 51 invasive populations from the western US were detected among the populations of this sub-region. Three of these eight genotypes (dark grey, black, and teal) were only detected within populations from this sub-region. In general, the genotypes detected among Coastal Range sub-region populations have limited geographical distributions. For instance, in Oregon, the red genotype was detected in Monmouth (where the grass was first collected in 1984) and Sherwood, and the black genotype was detected in Eugene and Roseburg. The red and black genotypes were also detected in the population sampled near Lower Lake, California. These results suggest that the red and black genotypes may have been introduced independently into Oregon and California.

The number of multilocus genotypes or haplotypes found among invasive populations can provide an estimate of propagule pressure (Novak and Mack 2005; Huttanus et al. 2011). The detection of nine MLGs among these 51 invasive populations of *V. dubia* suggests moderate propagule pressure for the introduction of this species into the western US. And if each MLG is the product of an independent introduction event, the detection of nine genotypes translates into a minimum of nine separate introduction events. Our estimate of propagule pressure is bolstered by the detection of different genotypes in localities associated with the earliest collection records of the grass in the western US.

Similar with collection history data, the distribution of these MLGs among invasive populations suggests that range expansion of *V. dubia* in its invasive range has occurred at a regional or local geographical scale. Evidence for this range expansion is provided by the low incidence of genetic admixture among invasive populations: only 15 of 51 (29.4%) invasive populations have two or more MLGs; and of these 15 populations, only the populations from Eugene and Roseburg, Oregon, have three MLGs.

The genetic diversity and structure of invasive populations is influenced by multiple factors: the level and structure of genetic diversity within and among native populations, propagule pressure, and the pattern of range expansion of a species in its new range (Taylor and Keller 2007; Keller and Taylor 2008; Novak and Mack 2016). With low propagule pressure (i.e., small founder population size) and local range expansion, invasive populations often exhibit reduced genetic diversity and increased genetic differentiation, in comparison to native populations (Brown and Marshall 1981; Novak and Mack 2005; Wares et al. 2005; Barrett 2015).

The allozyme data reported here provide an initial assessment of the genetic consequences of the introduction and range expansion of *V. dubia* in the western US. Despite evidence of moderate propagule pressure, the level of genetic diversity, on average, within the 51 invasive populations of *V.* dubia reported here is low in comparison with the level of diversity reported for other plant species (Hamrick and Godt 1996). The low level of genetic diversity detected within these invasive populations likely stems from local and/or regional pattern of range expansion. With local and/or regional range expansion, the multilocus

genotype(s) introduced into a geographic area would not intermix with the genotype(s) introduced into another area, and within-population genetic diversity would be low. In addition, all the factors mentioned here have, in combination, resulted in the high level of genetic differentiation among the 51 invasive populations reported in this study.

The level and structure of invasive (and native) populations is strongly influenced by the reproductive mode and mating system of a species (Barrett et al. 2008; Pannell 2015). For instance, plant species with higher rates of selfing have lower levels of genetic diversity within populations and higher genetic differentiation among populations, compared to predominantly outcrossing species (Brown and Burdon 1987; Hamrick and Godt 1996). Thus, the low level of genetic diversity and high genetic structure detected within these invasive populations is likely also influenced by the highly selfing mating system of *V. dubia*. Evidence for the selfing mating system of this species is provided by the detection of only two heterozygous individuals among all 1636 individuals from the western US we analyzed, in a single population, Mosquito Creek, Oregon. Additionally, the mean value of Hobs for all 51 invasive populations is very low (0.00009).

Conclusions

The genetic diversity and structure parameters reported here for *V. dubia* are similar to the values reported for invasive populations of *B. tectorum* (Novak and Mack 2016) and *Taeniatherum caput-medusae* (medusahead) (S.J. Novak, unpublished data), two highly selfing, annual grasses invasive in the western US, also analyzed using allozyme markers. Much like these two invasive annual grasses, results of the current study indicate that *V. dubia* was introduced multiple times into the western US. Despite multiple introductions, invasive populations of *V. dubia* exhibit low levels of genetic admixture, low levels of genetic diversity within populations, and high genetic differentiation among populations. This putatively low evolutionary potential however did not preclude the initial establishment of *V. dubia* and has not limited its rapid spread across its new range.

Gaining insights into other aspects of this invasion will require the genetic analysis of native populations, using allozymes (Bossdorf et al. 2005; Novak and Mack 2001, 2005). Identifying the same multilocus genotypes within and among native populations will provide evidence in support of the multiple introduction hypothesis and will aid in identifying the geographic origins (source populations) of this invasion (Novak 2011). Population genetic data from native populations will also allow a more precise estimate of the degree to which founder effects have influence the genetic diversity and structure of invasive populations (Novak and Mack 2005; Dlugosch and Parker 2008), and will allow an assessment of the role of post-introduction evolution versus prior adaptation in this invasion (Hufbauer et al. 2011; Rey et al. 2012). Finally, the genetic analysis of native and invasive populations within the same experimental framework can inform programs aimed at managing the invasion of *V. dubia* in the western US, especially efforts to search for effective and specific biological control agents (Gaskin et al. 2011).

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