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Genetic diversity and structure of *Crupina vulgaris* (common crupina): a noxious rangeland weed of the western United States

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Abstract

Common crupina (*Crupina vulgaris*) is a federal noxious weed in the western USA that is currently the target of a classical biological control programme using the fungus *Ramularia crupinae*. We first identified and determined the location of populations of the two varieties of common crupina in the western United States and assessed the pattern of genetic diversity and structure of these populations. We found seven AFLP (Amplified Fragment Length Polymorphism) genotypes for 326 plants in 17 populations. AFLP genotypes correlated with two taxa, either *C. vulgaris* var. *vulgaris* or *C. vulgaris* var. *brachypappa*. This annual species is outcrossing, but relies on selfing when pollination does not occur, which may explain why less than 1% of the genetic variation is within populations. We found strong population genetic structuring and can typically predict genotype or variety for a given location. Researchers and managers will be able to predict and survey for differential efficacy of *R. crupinae* on the different genotypes and varieties during initial biological control field releases, thereby increasing the likelihood of successful biocontrol establishment and impact.

Keywords

AFLP, common crupina, *Crupina vulgaris*, biological control

Introduction

A plant invasion can be a very diverse collection of genotypes ranging across a large landmass and be present in multiple, diverse ecological situations. Different lineages of an invasive species, reflecting different evolutionary origins and phenotypes, can be present (Ward et al. 2008) and the resulting complex variation in plant traits can exist at large and small spatial scales. The identification of traits that facilitate the spread of invasions and the interactions amongst these traits, are fundamental challenges in invasion ecology (Pyšek et al. 2015) and important in the development of effective management strategies. Knowing the distribution of varying genotypes across an invaded range can be critical for managing the invasion, especially if phenotypes vary in how they invade or react to weed control methods (Ward et al. 2008; Williams et al. 2020). A classical biological control agent can have different rates of efficacy on different host plant genotypes. This has occurred with fungal agents used in the control of rush skeletonweed (*Chondrilla juncea*; Burdon et al. (1981)), a mite agent of old world climbing fern (*Lygodium microphyllum*; Goolsby et al. (2006)) and insect agents of Brazilian peppertree (*Schinus terebinthifolius*; Manrique et al. (2008)). Similarly, agents may have cryptic subspecies or genotypes that behave differently on the same plant genotype (see examples in Smith et al. (2018)). Mismatching agent and plant genotype can reduce biological control efficacy or lead to a failed release. If the invasive plant species has strong population structuring, i.e. the genotype can be predicted by location, releases can be planned to place the most efficacious agent on the appropriate plant genotype.

Common crupina (*Crupina vulgaris* Pers. ex Cass., Asteraceae) is a federal-listed noxious weed in the USA (USDA APHIS 2022). It is an overwintering annual plant with origins in the Mediterranean Region (CABI 2022). Common crupina is a close relative of the knapweeds (*Centaurea* spp.) and competes with grasses and forbs in grazing and natural areas (Miller and Thill 1983). There are two varieties of common crupina established in the USA: *C. v.* var. *vulgaris* (often incorrectly named *C. v.* var. *typica*) and *C. v.* var. *brachypappa* P. Beauv. (Latin for short pappus; the feather-like hairs on the seed), that can be reliably separated by rosette form and seed morphology (Couderc-LeVaillant 1993; Roché et al. 1997). Common crupina was first reported in the USA near Grangeville, Idaho (1969), with subsequent reports from Sonoma County, California (1975), Chelan County, Washington (1984), Umatilla County, Oregon (1987) and Modoc County, California in 1991 (Garnatje et al. 2002). It increased its range more than 1000-fold in 30 years and now occupies > 25,000 ha (Garnatje et al. 2002) and is established in multiple counties in California, Washington, Oregon and especially Idaho (EDDMaps 2022; SDA NRCS 2022).

Common crupina is an outcrossing species that attracts generalist insect pollinators with pollen and nectar, but when conditions are not favourable for cross pollination (e.g. cooler weather, low common crupina density, pollinators attracted to other plant species etc.), common crupina relies on selfing (i.e. self-pollination) to produce seeds (Couderc-LeVaillant 1984) without notable loss in fecundity (Roché 1996). It is unknown which mating system dominates in the invasion. The mating system has a bearing on invasion

success; sexual reproduction via outcrossing provides new genetic combinations, which may be selected for when environments are variable or when expanding range into different environments. Plants that rely mainly on selfing have reduced genetic variation, but may have an advantage for persisting in environments similar to their parental origins and a single or few individuals can reproduce and start a population without relying on pollen from another individual (Barrett et al. 2008; Razanajatovo et al. 2016). Knowledge of the mating system in an invasion allows better niche targeting (what part of the plant to attack) when selecting biological control agents (Gaskin et al. 2011).

This weed species is currently a target of classical biological control using the federally approved leaf- and stem-spotting fungus, *Ramularia crupinae* Dianese, Hasan & Sobhian (Deuteromycotina) (Bruckart et al. 2014). Previous genetic studies investigating the origins and invasion of common crupina were performed on five populations (five plants per population; Roché et al. (2003) and Garnatje et al. (2002)) and showed that the two varieties are genetically distinct. Moreover, accessions of *C. v.* var. *brachypappa* showed significant differences in susceptibility to a previously proposed, non-approved biological control agent, *Puccinia crupinae* Ranoj. (Bruckart et al. 2006). Due to the differential susceptibility of varieties and genotypes to fungal attack, the goals of this research are to support the newly-approved biological agent, *R. crupinae*, with a more extensive molecular analysis of the genetic diversity and population structure of the common crupina invasion. Our specific objectives were to: 1) determine distribution of taxonomic varieties using morphological characteristics and genetic data; and 2) describe the amount and structure of genetic diversity within and amongst the two varieties and invasive populations of common crupina in the western USA invasion.

Methods

Leaf material was collected from 326 plants (17 locations, mean = 19.2 plants per location) (Fig. 1, Table 1, Suppl. material 1; Population Data tab). Our survey of populations was relatively complete, with no other known invasions in California, Washington or Oregon. There may be other populated counties in Idaho (EDDMaps 2022), but we were unable to find or obtain specimens from Adams, Bingham, Fremont, Gem or Washington Counties. Plants were sampled haphazardly across an invasion patch and at least 1 m apart, except for population 17, which was received from the USDA ARS laboratory in Ft. Detrick, Maryland and was sourced from Chelan County, WA in 2001. We extracted genomic DNA from approximately 20 mg of silica-dried leaf material using a modified CTAB method (Hillis et al. 1996). Our amplified fragment length polymorphism (AFLP) method followed Vos et al. (1995) with modifications as in Gaskin and Kazmer (2009). All 15 selective primer combinations of MseI + CAA, CAC, CAT, CTA or CTA and EcoRI + AAG, ACC or ACT were pre-screened for PCR product quality and number of variable loci using eight plant samples and the two primer pairs with the most polymorphic loci were chosen (MseI + CAT/ EcoRI + ACT and MseI + CAC/ EcoRI + AGG). AFLP data were generated on an Applied Biosystems (ABI, Foster City,

Table 1. Location and plant information for *Crupina vulgaris* collections.

Population	State	County	Location	N[1]	Genotypes present	G[2]	G/N	PLP[3]	L[4]
1[v]	CA	Sonoma	Santa Rosa	20	G1	1	0.05	0.00	
2[b]	CA	Modoc	Kelly Springs	20	G3	1	0.05	0.00	5.9
3[v]	ID	Idaho	Slate Creek	20	G2	1	0.05	0.00	7.2
4[v]	ID	Idaho	Harpster	20	G1, G2	2	0.10	0.02	7.9
5[v]	ID	Clearwater	Orofino	19	G1	1	0.05	0.00	7.4
6[v]	ID	Nez Perce	Waha	20	G1	1	0.05	0.00	8.1
7[v]	ID	Idaho	Gil Gulch	20	G1, G6, G7	3	0.15	0.04	7.8
8[v]	OR	Wallowa	Joseph Creek	20	G2	1	0.05	0.00	7.7
9[v]	OR	Wallowa	Grouse Creek	20	G1	1	0.05	0.00	
10[v]	OR	Baker	Halfway	20	G1	1	0.05	0.00	8.1
11[v]	OR	Baker	Pine Creek	20	G1	1	0.05	0.00	
12[v]	OR	Umatilla	Tollgate	20	G1, G2	2	0.10	0.02	
13[v]	OR	Umatilla	Walla Walla River	20	G1	1	0.05	0.00	
14[b]	WA	Chelan	Lake Chelan 1	20	G4	1	0.05	0.00	
15[b]	WA	Walla Walla	Biscuit Ridge	20	G5	1	0.05	0.00	
16[b]	WA	Walla Walla	Blacksnake	20	G5	1	0.05	0.00	
17[b]*	WA	Chelan	Lake Chelan 2	7	G3	1	n/a	0.00	

[1]Number of plants sampled. [2]Number of unique AFLP genotypes. [3]Proportion of loci polymorphic at the > 5% level. [4]L mean pappus length of 15 seeds (in mm). [v]*Crupina vulgaris* var. *vulgaris*, [b]*Crupina vulgaris* var. *brachypappa*. *Seeds from this collection were collected in 2001, sent to a laboratory for storage, then grown for DNA sampling; thus, this may not represent a true population in that the seed could have come from one or many plants.

CA, USA) 3130 Genetic Analyzer and any individuals that did not produce a typical electropherogram pattern (i.e. noise > 20 relative fluorescence units (rfu) or failed to produce sufficient number of peaks) were omitted. We repeated AFLPs for all unique genotypes and to estimate AFLP error rate, we performed repeats of 56 samples (17% of all samples) starting with CTAB extracted material, scored them blindly and calculated the number and percentage of mismatches between the original and repeat AFLP datasets. NTSYS-PC ver. 2.2 software (Rohlf 2005) was used to calculate the Dice pairwise similarity coefficient between AFLP genotypes. The Dice coefficient ranges from 0.0 to 1.0, with values of 1.0 indicating that individuals are genetically identical. To visually assess similarity of genotypes, we used the UPGMA clustering method on Dice similarity coefficients as implemented in the SAHN module of NTSYS to create a dendrogram of the genotypes. To determine level of diversity in a population, we calculated G/N as number of unique genotypes found, divided by the number of plants genotyped. To determine how distinct genotypes were within a population, we manually calculated PLP (Proportion of Loci Polymorphic at the > 5% level) by counting how many of the loci varied within a population. To determine population structure and amount of differentiation amongst and within taxonomic varieties and populations, we performed distance based AMOVA (analysis of molecular variance) and resulting Φ values (analogous to F values) on the binary AFLP data, using the GenAlEx add-in for Excel (Peakall and Smouse 2012) with 95% confidence intervals generated from 999 permutations. To determine taxonomic variety, we measured average pappus length for 15 samples per

population (for nine populations listed in Table 1) as in Couderc-LeVaillant (1993). We used a Student's t-test to determine if pappus length means of the two putative taxonomic varieties were significantly different.

Results

We found 47 repeatable and reliable loci using the two AFLP primer pairs listed above. We did 56 repeats for both primer pairs (i.e. 56 × 47 = 2632 cells compared) and found 0 errors. All repeated AFLPs for unique genotypes were identical to the original. Across all plants, the PLP was 100% (i.e. this indicates that all 47 loci varied across the 326 plants we analysed).

For the 326 plants, seven AFLP genotypes were identified and designated G1–G7 (Suppl. material 1; AFLP Data tab). G/N in populations varied from 0.5 (all plants are one genotype) to 0.15 (three genotypes in a population). PLP per population ranged from 0–0.04 (Table 1). Pairwise Dice similarity of the seven genotypes ranged from 0.20–0.98. Similarity values between genotypes of the two varieties ranged from 0.20–0.33 (Table 2).

Mean pappus length from populations containing G1, G2, G6 and G7 was 7.9 mm (S.D. 0.73; n = 135 seeds measured) and was similar to measurements of *C. v.* var. *vulgaris* (mean = 7.98 mm ± 0.19 mm) performed by Couderc-LeVaillant (1993). Mean pappus length from populations containing genotypes G3 and G5 (G4 not measured) was 5.3 mm (S.D. 0.74; n = 30 seeds measured) and was similar to measurements of *C. v.* var. *brachypappa* (mean = 5.14 ± 0.1 mm) performed by Couderc-LeVaillant (1993). Our means for pappus length from the two taxonomic varieties were significantly different based on the results of a Student's t-test ($t = 17.5955$, $df = 163$, $P < 0.0001$; data not shown).

Based on the UPGMA, genotypes G1, G2, G6 and G7 clustered separately from G3, G4 and G5 (Fig. 1b). Similarity within *C. v.* var. *vulgaris* ranged from 0.96–0.98, while similarity within *C. v.* var. *brachypappa* ranged from 0.67–0.73 (Table 2, Fig. 1c (UPGMA)). In the AMOVA analysis, 91.6% (Φ_{RT}) of the genetic variation was amongst taxonomic varieties, 8.2% (Φ_{PR}) was amongst populations in those varieties and 0.14% (Φ_{PT}) was found within populations ($P = 0.001$ for all values).

Table 2. Pairwise genetic Dice similarity values amongst the seven AFLP genotypes of *Crupina vulgaris* in the western USA. Shaded cells are *Crupina* var. *brachypappa*; non-shaded cells are *Crupina* var. *vulgaris*.

	G1	G2	G3	G4	G5	G6	G7
G1	-						
G2	0.98	-					
G3	0.20	0.20	-				
G4	0.33	0.32	0.73	-			
G5	0.22	0.21	0.74	0.67	-		
G6	0.98	0.96	0.20	0.32	0.26	-	
G7	0.98	0.96	0.24	0.32	0.26	0.96	-

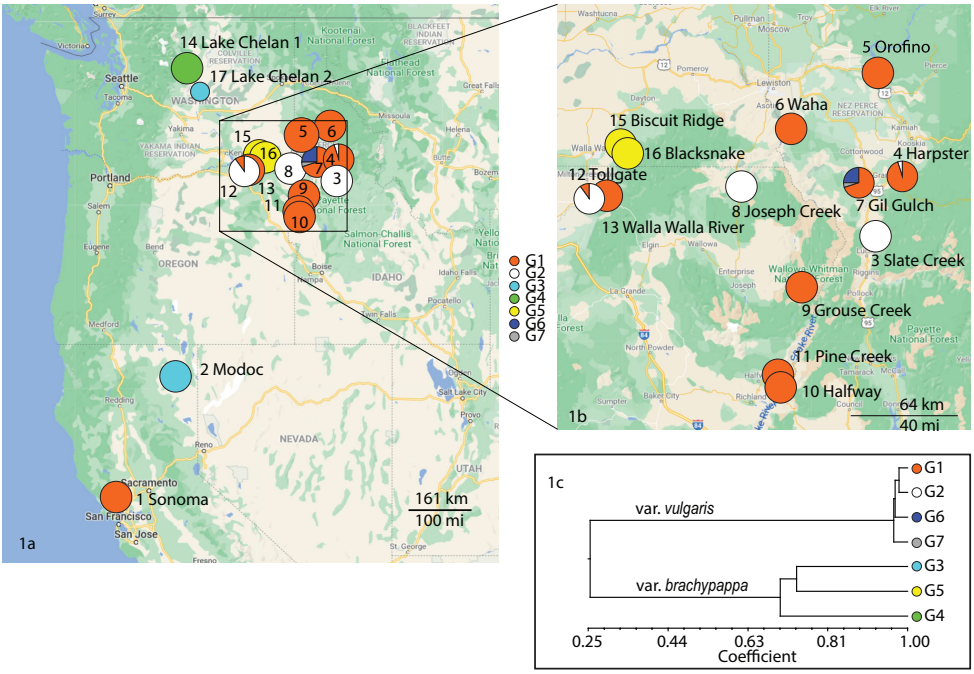

Figure 1. a Map of 17 populations of *Crupina vulgaris* and their AFLP genotypes in the western USA **b** expanded map of north-eastern Oregon, south-western Washington and western Idaho **c** UPGMA (unweighted pair group method with arithmetic mean) of the seven AFLP genotypes showing their similarity.

Discussion

Earlier studies of the origins and invasion of common crupina (Roché et al. (2003) and Garnatje et al. (2002); the same data and analysis were published in both studies) used RAPDs (Randomly Amplified Polymorphic DNA) to identify differences between genotypes. Their RAPDs identified five genotypes in five locations, while our AFLP study identified seven genotypes in 17 locations. Our G1, G2, G6 and G7 ("*vulgaris*"-type) genotypes correlate with the "Oregon", "Sonoma" California and "Lawyer Canyon" Idaho (this location was not sampled by us, but likely falls between our populations 4 and 5) genotypes of the RAPDs study, while G3, G4 and G5 ("*brachypappa*"-type) correlate with the "Modoc" California and "Chelan" Washington RAPD genotypes. The RAPDs study by Garnatje et al. (2002) found different varieties and genotypes in different USA locations and suggested this as evidence of three or more successful introductions of common crupina into the USA. Our result of seven genotypes in the USA also supports this hypothesis of multiple introductions. The earlier study of five plants per population did not note any within population variation. In contrast, three of our 17 populations had multiple (2 or 3) closely-related genotypes and the rest were monotypic populations. Both RAPDs and AFLPs are typically highly variable at the population level for plants that outcross (Powell et al. 1996). This low level of within-population variation for the invasion suggests that seed

production occurs mostly through self-pollination (selfing). Common crupina is known to self-pollinate when conditions are not favourable for outcrossing (Couderc-LeVaillant 1984), without notable loss in fecundity (Roché 1996). The low level of diversity and predominant selfing in the invasion could facilitate management success, as lower genetic diversity can suggest fewer opportunities for future evolution of resistance or tolerance to herbivory (Núñez-Farfán et al. 2007) or herbicides (e.g. Baucom and Mauricio 2004).

There are many biotic and abiotic variables regulating efficacy of classical biological control agents of weeds (Waage and Greathead 1988; McFadyen 1998). Within a species, heritable differences in resistance or tolerance to herbivory or disease can exist (Strauss and Agrawal 1999). Differential susceptibility of common crupina to a fungal pathogen has been previously observed. Bruckart et al. (2006) demonstrated that plants from Modoc, CA (our G3, var. *brachypappa*) were resistant to the fungal rust pathogen *Puccinia crupinae*, while the other accessions (Idaho, Chelan WA, Santa Rosa CA and Oregon) were susceptible. In contrast, Bruckart et al. (2014) found no significant differences in susceptibility to the leaf-spotting fungus *R. crupinae* for the two common crupina varieties when evaluated against seven crupina populations. This evidence, combined with our results, suggests baseline genetic differences amongst populations are unlikely to encumber susceptibility to the release of the biocontrol agent, *R. crupinae*.

From our seed measurement data and data from earlier studies (Couderc-LeVaillant 1993; Garnatje et al. 2002; Roché et al. 2003), we note that the genetic data correlates with previously suggested taxonomic designations. This supports a geographical structuring of regions, with *C. v.* var. *brachypappa* found in Washington and north-eastern California and all other populations being *C. v.* var. *vulgaris*. Our extensive survey of the common crupina invasion will allow researchers to test potential agent efficacy for all known genotypes prior to release. Since we found strong population structuring and can accurately predict taxonomic variety for a given location, researchers and managers can, on a local level, better predict and survey for differential efficacy during initial biological control field releases, thereby increasing the likelihood of successful biocontrol establishment and impact. Even though there was no significant difference in susceptibility to the leaf-spotting fungus *R. crupinae* between the two crupina varieties tested in laboratory conditions (Bruckart et al. 2014), efficacy of attack on the different plant genotypes in the field will be an important part of future monitoring programmes during the impending *R. crupinae* release.

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Supplementary material 1

Population Data and AFLP Data

Authors: John F. Gaskin, Nisha Chapagain, Mark Schwarzländer, Matthew A. Tancos, Natalie M. West

Data type: excel document

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