

Studies

Variation in reproductive mode across the latitudinal range of invasive Russian knapweed

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Abstract

For invading species, reproduction is a critical determinant of population establishment as well as spread into new areas. When species have multiple modes of reproduction, the prevalence of different modes can influence management decisions. We used genetic markers to determine the prevalent method of recruitment for invasive Russian knapweed (*Acroptilon repens*). This species forms patches and can spread by both rhizomic growth and seed from outcrossing. We found no shared genotypes between 41 western North American populations, indicating at the macroscale, Russian knapweed is spreading via seed to distant locations. We also examined drivers of reproductive mode by comparing clonality with large-scale environmental factors across the invasion. We found a correlation between latitude and clonal versus seed reproduction, with clonality higher in northern latitude populations. This trend was associated most parsimoniously with decreasing maximum annual temperature and 30-year average of available growing degree days, and increasing soil organic carbon content. These results have management implications: if not properly temporally implemented, grazing or herbicide applications that create open spaces for recruitment may increase the likelihood of Russian knapweed patch persistence through seed, and recently released galling biological control agents in North America may be less effective in northern latitudes where Russian knapweed spread by seed is less prevalent.

Keywords: Clonal; invasion; latitude; plant; reproduction; Russian knapweed.

Introduction

The abilities to spread locally and disperse across long distances are important characteristics of successful invasive plant species (Lockwood *et al.* 2005; Nathan 2006). Locally, plants exploit an open niche within or adjacent to conspecific plants through recruitment of seedlings or vegetative ramets. Many perennial invasive plant species can utilize both sexual (seed from outcrossing) and clonal methods of spread (Pyšek 1997; Liu *et al.* 2006) but it is often unclear which reproductive mode prevails (Eckert 2002) or if reproductive mode varies across the invasion, and what factors determine reproductive mode at ecologically relevant spatial or temporal scales. Successful local reproduction from sexual or clonal methods entails complex and distinct hurdles for each process (Eriksson 1993; Watkinson and Powell 1993). The variance of sexual and clonal reproduction within a species will affect demography and the evolutionary potential of populations (Dorken and Eckert 2001) and possibly their invasive success. Understanding how invasive plants reproduce and spread enables better management strategies targeting plant or demographic processes responsible for reproduction (Ramula *et al.* 2008; Ward *et al.* 2008; Gowton *et al.* 2016).

Clonal reproduction is predicted to be beneficial for evolutionarily short periods of time and under stable environments (Zhang and Zhang 2007; Douhovnikoff and Dodd 2015), though many clonally reproducing plants are long-lived and common in heterogeneous environments (Benson and Hartnett 2006; Song *et al.* 2013). Sexual reproduction is predicted to be beneficial for providing genetic variation for adaptation to different environments, usually involves smaller propagules that can travel further, and can allow for long-term dormancy until conditions are better for seedling establishment (Eriksson 1993).

Within a species, whether local spread and persistence results primarily from sexual or clonal reproduction may depend on environmental conditions such as temperature, latitude, and resource availability and soil type, demographic factors such as seed quality, plant age, resource allocation (above- vs. below-ground growth) and density-dependent processes operating over multiple spatial and temporal scales (Sun *et al.* 2001; De Frenne *et al.* 2013 and citations within). Seed recruitment is assumed to be restricted when the growing season is shorter and colder, as happens at higher elevations and latitudes (Eriksson 1997), and thus clonal reproduction should be advantageous under these conditions. Clonality is

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also common among plant species that occur in nutrient-poor habitats and under poor light conditions (Van Groenendael *et al.* 1996). Reduction in seed quality, related to underlying environmental gradients, may diminish the relative contribution of seed production to recruitment (De Frenne *et al.* 2013). Additionally, environmentally mediated variation in the frequency of clonal die-off and plant density can influence seedling recruitment success, determining the spatial structure of genotypes in a patch (Eriksson 1993; Radosavljević *et al.* 2020). Disturbance regimes such as grazing can vary across sites, with diverse implications for the relative prevalence of clonal reproduction (Chen *et al.* 2015; Seeber *et al.* 2015; Lehdal *et al.* 2016; Johnson *et al.* 2020; but see Gowton *et al.* 2016).

Plant age and spatial structure also influence the frequency of seedling recruitment, and therefore the genetic diversity of a population (Eriksson 1993). Size of clones is often equated with age, but environmental conditions also affect relative size hierarchies of ramets among populations of the same species (Jacquemyn *et al.* 2005). Reproductive mode can also vary locally, with plants from seed only establishing outside large existing genets (e.g. Gaskin and Littlefield 2017). Type of vegetative propagule, such as shoots arising from rhizomes, stolons, bulbils, tillering and fragmentation, influences the spatial and temporal patterns that develop. Dynamics of clonal turnover can also be influenced by a balance of self-versus other density and available gaps for seedling recruitment (Auge and Brandl 1997; but see Bishop and Davy 1985). Plants that typically reproduce clonally either have episodic recruitment, so that many clones are similarly aged which may limit diversity, or some continual recruitment from seed, leading to relatively higher genetic diversity (Watkinson and Powell 1993).

Russian knapweed (*Acroptilon repens* = *Rhaponticum repens* (family Asteraceae)) is a perennial herb native to Central Asia that has invaded North America. It was first introduced to North America in the early 1900s as a seed contaminant of alfalfa and sugar beet (Watson 1980). Russian knapweed is now found in all but the most eastern states and provinces of USA and Canada (USDA, NRCS 2022), primarily in the semi-arid portion of western North America, but at a wide range of latitudes (at least 31–50°N; EDDMapS 2021). Seeds are able to germinate under a wide range of temperatures (0.5–35 °C; Watson 1980). Infestations are estimated at 557 000 ha in 1998 (Whitson 1999), with spread in the western USA estimated at up to 14 % per year (Duncan 2005). It can create new shoots from rhizome growth and also reproduces by seed (Morrison *et al.* 1995). At northern latitudes of the USA, patches are typically monotypic, spreading via rhizomic growth (Gaskin and Littlefield 2017).

The Russian knapweed invasion in North America is relatively recent (~100 years) and most ongoing long-distance spread is likely via human activity such as movement of contaminated hay (Robbins *et al.* 1951; Jacobs and Denny 2006). Given this relatively fast human-induced spread across long distances, we hypothesize that the predominant mode of local spread of the invasion will be the same as in an earlier study of patches in Montana, USA. But, if reproductive methods vary across space, invasive populations may require different management strategies (e.g. Gaskin *et al.* 2011; Gowton *et al.* 2016). In this study we investigate the spatial population genetic structure of this invasive weed to determine its predominant reproductive mode (i.e. seedlings vs.

ramets), and question if clonality varies across the invasion; if so, what coarse-scale environmental factors are associated with site-level reproductive modes?

Materials and Methods

The species is entomophilous and an obligate outcrosser (Harrod and Taylor 1995; Young and Clements 2003). In Colorado, Goslee *et al.* (2003) determined that the species is most prevalent on fine-textured soil in warmer and drier regions. Russian knapweed produces up to 1200 mature seeds per shoot (Ivanova 1966). Though germination is generally considered to be low (Selleck 1964), optimal temperature and light regime (20 °C/30 °C, 8 h dark/16 h light) and seed coat disruption may increase germination rates (Larson and Kiemnec 2005; Alebrahim *et al.* 2011). Dense patches may have 100–300 shoots per m² and can spread to an area of 12 m² within 2 years (Frazier 1944).

We collected young, disease-free leaf material from 487 plants from 41 sites (populations) in the western USA and Canada (Fig. 1; see Supporting Information—Table S1). The smallest distance between our populations was 87 km. In our experience, Russian knapweed is usually found as discrete, dense patches in a landscape, and rarely as isolated stems. We collected plants across one or multiple Russian knapweed patches in a population, with distance between visually discrete patches at 5–10 m. In each patch we collected tissue from a shoot every 5 m along a transect. If the patch was not big enough to collect 12 samples (i.e. patch < 60 m long), we moved to the next nearest patch and continued sampling, keeping track of which patch a sample came from. We sampled from an average of 11.9 plants per population. The collected leaves were immediately stored in silica gel at ambient temperature.

We extracted genomic DNA from approximately 20 mg of silica-dried leaf material using a modified Cetyltrimethylammonium Bromide (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 + CAA/EcoRI + ACC and MseI + CAC/EcoRI + ACC). We generated all AFLP data on an Applied Biosystems (ABI, Foster City, CA, USA) 3130 Genetic Analyser, and omitted any individuals that did not produce a typical electropherogram pattern (i.e. noise > 20 relative fluorescence units or failure to produce sufficient number of peaks).

We used Dice similarity coefficients (SIMQUAL program in NTSYS-pc ver. 2.1 software; Rolf 1992) to identify AFLP genotypes (<98.8 % similarity = distinct genotype; see error calculations below). To estimate AFLP error rate we performed repeats of 48 samples (9.8 % of total) starting with CTAB-extracted material, scored them blindly and calculated the number and percentage of mismatched alleles between the original and repeat AFLP data sets.

To compare genetic diversity we calculated proportion of loci that are polymorphic at the ≥5 % level (PLP) and Nei's (1978) gene diversity (H_j ; equivalent to expected heterozygosity H_E) in AFLP-SURV 1.0 (Vekemans *et al.* 2002). We calculated significant differences for H_j using 95 % confidence

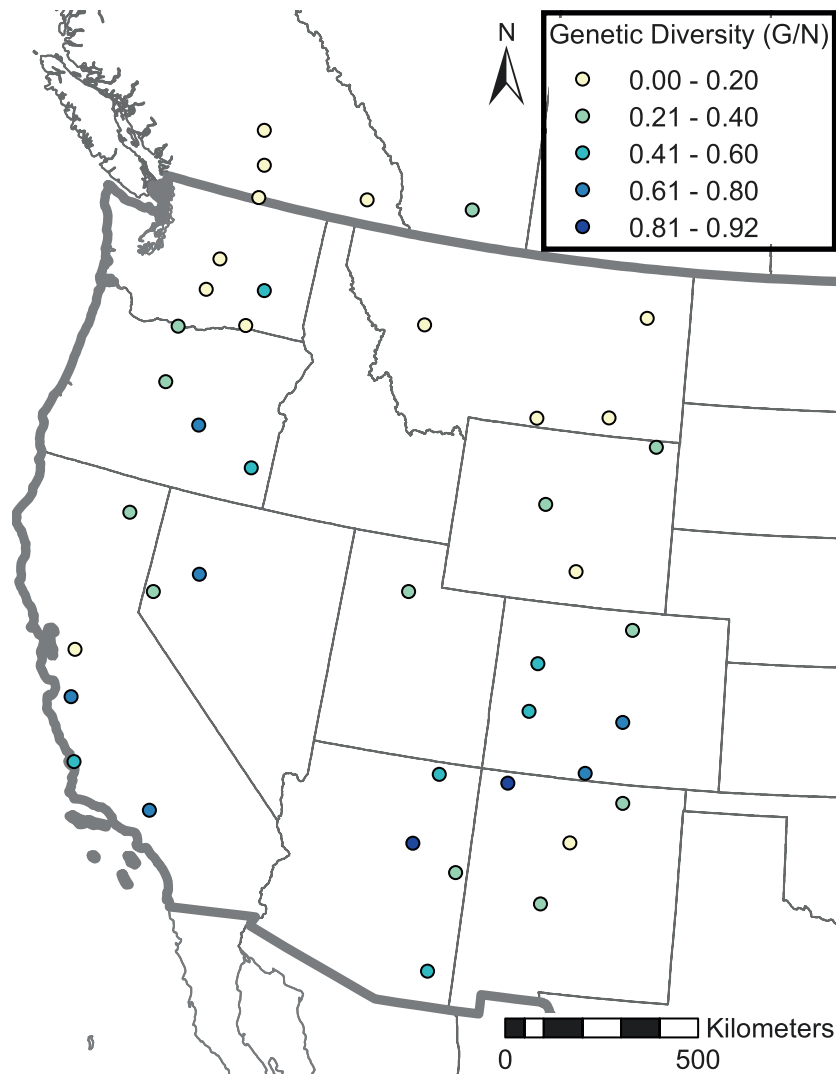


Figure 1. Location of Russian knapweed populations sampled from western North America, with genetic diversity of each population calculated as G/N (number of genotypes found/number of plants sampled).

intervals derived from standard errors. We used a Shapiro-Wilk test (since our data were not normal) to see if mean G (number of genotypes)/ N (number of plants genotyped) per population varied. Simpson's diversity index (D) corrected for finite sample size (Pielou 1969) was calculated with $D = 1 - \sum n_i(n_i - 1)/N(N - 1)$ for $i = 1$ to G , where n_i is the number of ramets that share genotype i . Values of D can range from 0 to 1, with higher values corresponding to greater genetic diversity, and were used to calculate genotypic evenness (E) (Fager 1972), with $E = (D - D_{\min})/(D_{\max} - D_{\min})$, where $D_{\min} = (G - 1)/(2N - G)/N(N - 1)$ and $D_{\max} = N(G - 1)/G(N - 1)$. To analyse genetic population structure we investigated genetic differentiation (F_{ST}). We compared F_{ST} in AFLP-SURV as above, with 95 % confidence intervals around F_{ST} values generated from 1000 permutations. To determine the number of genetic clusters (K) represented in the genotypes, we diploidized AFLP results (Falush *et al.* 2007) with presence of a fragment coded as (1/-9) and absence of a fragment coded as (0/0), no population or geographic location information was included, admixture was assumed as possible, allele frequencies were considered to be independent and a 50 000 run burn-in (α stabilized at approximately 1000 runs) and 100 000 run

length were used. We performed clustering and assignment tests using the software STRUCTURE v 2.3.3 (Pritchard *et al.* 2000; Falush *et al.* 2003; Falush *et al.* 2007). We tested for number of genetic clusters ($K = 1-15$) with 20 repetitions for each value of K with the criterion delta K of Evanno *et al.* (2005) in the software STRUCTURE HARVESTER web v0.6.92 (Earl 2012) and also with Puechmaille (2016) supervised estimators using STRUCTURESELECTOR (Li and Liu 2018), which vary in estimation of K , to test for consensus in K values. To visually assess population structure we performed principal coordinates analysis on Dice similarity coefficients using the DCESTER and EIGEN programs of NTSYS.

To test for isolation by distance we determined the correlation between population pairwise fixation (F_{ST}) and geographic distance using a Mantel test in NTSYS (MXcomp function) with 1000 replications. To obtain genetic differentiation (F_{ST}) between populations we first calculated F_{IS} estimates using I4A (inbreeding for AFLP; Chybicki *et al.* 2011) using a mean of F_{IS} results from α and β values of 0.1, 0.5, 1.0 and 5.0. The F_{IS} value was used in AFLP-surv to determine F_{ST} between pairs of populations and estimates of allele

frequencies were computed with the fourth option: 'Bayesian method with non-uniform prior distribution of allele frequencies'. We calculated geographic distances between populations using Geographic Distance Matrix Generator v1.2.3 (Ersts 2019). We log-transformed F_{ST} and geographic distances between populations for the Mantel test because they were each not normal in a Shapiro–Wilk test.

Latitude and longitude coordinates for each site were imported into an ArcGIS 10.8.1 (ESRI 2018) database and mapped using ArcMap. Grid layers containing climate, productivity and soil variables were added to the map, and grid values for each variable were extracted for each sampling location. Grid layers for climatic variables, including long-term averages (1981–2010) in annual precipitation and maximum and minimum temperature (4 km resolution), were obtained from the PRISM Climate Group (2020). A grid layer (2.5 km resolution) containing 30-year (1981–2010) average accumulated growing degree days (GDD; base 50 °F) for the year (1 January to 31 December) was obtained from the USA National Phenology Network (2017). Net annual primary productivity (NPP) from NASA's MODIS products suite (MOD17A3HGF Version 006; 500 m resolution) was downloaded from Running and Zhao (2019). Annual NPP data were extracted for 2000–18, and averaged over this interval for each site. Gridded predictions (100 m resolution) of soil properties, including percent organic carbon, total nitrogen, bulk density, pH, and percent sand and clay were downloaded from Ramcharan *et al.* (2017). We extracted data for each soil property at four of the soil depths included in the data set (0, 5, 15 and 30 cm) and averaged them to obtain a single estimate of each variable per site.

To examine how clonal prevalence related to environmental factors, we evaluated associations with clonality (measured as G/N ; [number of genotypes found/number of plants genotyped]) among large-scale ecological variables that have been linked in the literature with clonality and clonal trait variation. These consisted of spatially and biologically associated variables (mean annual maximum and minimum temperatures [MaxT, MinT], mean annual precipitation [Precip], elevation [Elev], GDD, site productivity [NPP]) as well as soil factors (soil N [N], soil organic carbon [SOC], sand and clay content, bulk density [BulkD]).

Because G/N displayed a distinct latitudinal trend (Fig. 2; Pearson correlation: $r^2 = -0.49$, $P > 0.01$), similar to other studies that found clonality increased with decreasing latitude (e.g. Ye *et al.* 2014), and latitude was significantly correlated with other cross-correlated variables (see below and Fig. 2), we focused attention on variables significantly correlated with latitude, and then reduced the variable set one step further based on the correlation matrix among the full variable set and with G/N . We used variation partitioning (Borcard *et al.* 1992) to evaluate the relative contribution of spatial components to variation in G/N versus that of biologically associated growth and soil components. If one or both of these groups contributed substantially and separately to variation in G/N , we then compared binomial regression models relating G/N to different combinations of identified variables (R (R Core Team 2020) package MuMIn, function *dredge*) to determine the best model, based on AICc, and the importance of each variable (package MuMIn, function *importance*, i.e. Burnham and Anderson 2002) within the compared set. We used non-metric multidimensional scaling (distance metric

= Gower) to display site dissimilarities described by the full variable set (i.e. sites farther apart in ordination space are less similar), and fitted vectors to the ordination to show how increase in latitude and 'important' variables across multivariate space was related to the distribution of site G/N .

Results

We found 137 repeatable polymorphic loci using our two AFLP primer pairs (AFLP data in Supporting Information—Table S2). Our AFLP error rate calculated from repeats was 1.2 %, which is equivalent to 1.58 miscalled loci per plant sample. Using this error rate (i.e. plants that are >98.8 % similar are assumed to be the same genotype), we identified 180 genotypes in 487 plants ($G/N = 0.37$).

Eight out of 41 (20 %) populations contained only one AFLP genotype, and G per population varied from 1 to 11 (Fig. 3), with a mean of 4.4. G/N (Fig. 1) for each population varied from 0.08 (all one genotype) to 0.92 (almost each shoot was a different genotype); mean = 0.37. There were no shared genotypes among populations. Genotypic evenness in populations varied from 0 (all shoots from one clone) to 0.99 (each shoot a different genotype) (Fig. 3). Percentage of loci that were polymorphic (PLP) ranged from 0 to 49.6 % per population with a mean of 23.1 %. Expected heterozygosity (H_e) ranged from 0.006 to 0.181 per population with a mean of 0.09 (Fig. 3). Genetic similarity (Dice similarity coefficient) between all plants ranged from 28 to 100 % (data not shown). The highest genetic similarity between plants from different populations was 80 % (populations 39 and 10). Within populations, genetic similarity varied from 42 to 100 %. Population differentiation (F_{ST}) was 0.55 with a standard error of 0.08. F_{ST} between pairs of populations varied from 0.13 to 0.97. Isolation by distance was weak (log population pairwise F_{ST} vs. log geographic distance); i.e. there was little correlation between genetic and geographic distances ($r = 0.08639$; $P[\text{random } Z \geq \text{observed } Z] = 0.0200$). According to structure analyses, there are no reasonable clusters of AFLP genotypes using $K = 1$ –15 (delta K values very low; see Supporting Information—Figs S1–S3).

G/N was correlated with maximum temperature ($r^2 = 0.36$, $P = 0.03$) and SOC ($r^2 = -0.43$, $P = 0.01$) in addition to latitude (above, Fig. 2). Six variables (three spatial: elevation and maximum and minimum temperatures; three biological: SOC, bulk density and GDD) were significantly correlated with latitude. Our final variable subset therefore included Maximum Temp (MaxT) and Elevation (Elev) (spatial variables) and SOC and GDD (biological variables). The two dropped variables were correlated with more than one other factor included in the final subset. Although variables across the two groups were correlated, they were not correlated within groups (e.g. MaxT and GDD were highly negatively correlated but SOC and GDD were not). The spatial variables (MaxT and Elev, $R^2_{\text{adjusted}}: 0.14$) and biological variables (GDD and SOC, $R^2_{\text{adjusted}}: 0.13$) separately contributed to the combined total variation explained ($R^2_{\text{adjusted}}: 0.28$, 4 df); their overlap explained only a small fraction of the variation ($R^2_{\text{adjusted}}: 0.01$). Therefore, we retained all four variables for the regression model. The best fit from the model comparison contained three of these: MaxT, GDD, and SOC (AICc: 176.6; log likelihood: -83.64; weight: 0.77, Likelihood Ratio Test (LRT) versus intercept: $\chi^2 = 41.2$, $P > 0.01$, 3 df). The

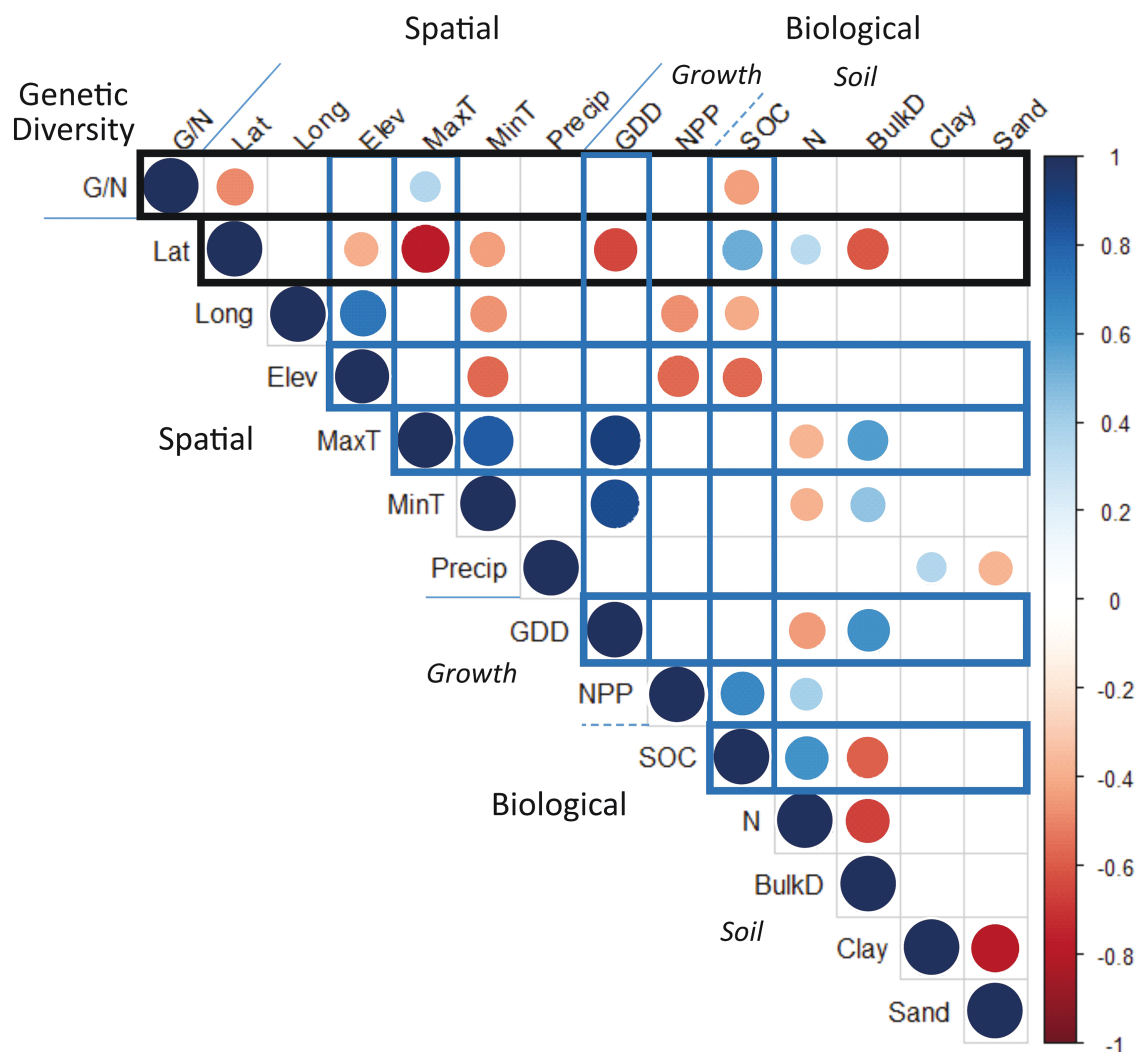


Figure 2. Correlation among environmental and spatial variables in relation to genetic diversity (G/N: number of genotypes found/number of plants genotyped) and latitude (Lat) (outlined in black). Variables were designated either Spatial or Biological (which includes both biotic [growth] factors and abiotic [soil] factors), and a subset of two variables uncorrelated within classes (outlined in blue) were used to examine the portion of total variation in G/N explained by each class and the importance of each included variable in relationship to G/N. The presence of a circle indicates a statistically significant correlation between the intersecting variables. Circle sizes are scaled relative to correlation strength.

next best model in the list included Elev, but the weight was substantially lower (0.22) and AICc more than 2 units higher (179.1; see [Supporting Information—Table S3](#)). Elev similarly had a much lower importance value (0.22) compared to the other three variables (0.99–1). Thus, clonal prevalence, quantified as G/N, increased at higher latitudes (Lat); this trend was associated most parsimoniously with decreasing maximum annual temperature (MaxT) and 30-year average of available GDD, and increasing SOC content ([Fig. 4](#)). There was also a significant but very weak correlation between latitude and patch number ($r^2 = 0.07$, $P = 0.02$), which is indicative of patch size in our study.

Discussion

We found no shared genotypes between populations; thus, at a macroscale, Russian knapweed is spreading via seed. We found G/N to range from 0.03 to 0.92, suggesting a high level of variation in reproductive mode across populations. Russian knapweed can persist and locally expand entirely

via clonal reproduction, by seed or a combination of both ([Fig. 3](#)). [Ruiz \(1999\)](#), using RAPDs (randomly amplified polymorphic DNAs), found shared genotypes across western USA Russian knapweed populations, but that study was working with less variable markers (13 RAPD patterns found in 353 plants; $G/N = 0.03$). An earlier AFLP study of six patches in three populations in Montana, USA ([Gaskin and Littlefield 2017](#)) found that most patches were monotypic, with an occasional different genotype on the edge of the patch, and no genotypes shared across patches. This pattern did not hold true across wider geographic range in this study; we found that as we sampled further south, populations relied less on clonal reproduction for persistence and local spread.

Genetic diversity hints at patterns and limitations on recruitment. Numerous studies address the trade-offs between vegetative and sexual reproduction in clonal plants, and how they drive the genetic diversity of plant patches (e.g. [Eriksson 1993](#); [Watkinson and Powell 1993](#)). Generally, these discussions are framed in the context of the frequency of seedling recruitment versus conditions

P	G	N	G/N	Latitude	Longitude												Patches	PLP	Hj
35	1	12	0.08	50.633	-119.935	o	o	o	o	o	o	o	o	o	o	o	1	0	0.007
34	2	12	0.17	49.831	-119.622	o	o	o	o	o	o	o	o	o	o	o	1	6	0.038
31	3	12	0.25	49.815	-112.076	o	o	o	o	o	o	o	o	o	o	o	2	22	0.119
32	1	12	0.08	49.584	-115.800	o	o	o	o	o	o	o	o	o	o	o	1	0	0.008
33	2	12	0.17	49.053	-119.527	o	o	o	o	o	o	o	o	o	o	o	1	1	0.012
41	1	12	0.08	47.828	-105.513	o	o	o	o	o	o	o	o	o	o	o	1	0	0.007
4	2	12	0.17	47.427	-120.300	o	o	o	o	o	o	o	o	o	o	o	3	16	0.097
3	6	12	0.50	46.963	-118.572	o	o	o	o	o	o	o	o	o	o	o	4	35	0.130
40	2	12	0.17	46.948	-113.017	o	o	o	o	o	o	o	o	o	o	o	2	14	0.033
36	1	12	0.08	46.656	-120.497	o	o	o	o	o	o	o	o	o	o	o	1	0	0.007
5	1	12	0.08	46.061	-118.907	o	o	o	o	o	o	o	o	o	o	o	2	0	0.006
37	4	12	0.33	45.656	-121.090	o	o	o	o	o	o	o	o	o	o	o	1	7	0.029
2	1	12	0.08	45.419	-106.440	o	o	o	o	o	o	o	o	o	o	o	1	0	0.007
1	1	12	0.08	45.203	-108.809	o	o	o	o	o	o	o	o	o	o	o	1	0	0.008
30	4	12	0.33	44.851	-104.791	o	o	o	o	o	o	o	o	o	o	o	1	34	0.109
38	4	12	0.33	44.334	-120.999	o	o	o	o	o	o	o	o	o	o	o	1	25	0.087
39	9	12	0.75	43.544	-119.615	o	o	o	o	o	o	o	o	o	o	o	1	29	0.121
21	3	10	0.30	43.247	-108.161	o	o	o	o	o	o	o	o	o	o	o	2	23	0.122
6	7	12	0.58	42.866	-117.648	o	o	o	o	o	o	o	o	o	o	o	4	45	0.171
29	2	12	0.17	41.777	-106.946	o	o	o	o	o	o	o	o	o	o	o	2	20	0.070
7	4	12	0.33	41.183	-121.028	o	o	o	o	o	o	o	o	o	o	o	2	50	0.181
20	4	12	0.33	40.774	-112.027	o	o	o	o	o	o	o	o	o	o	o	1	19	0.080
17	3	12	0.25	40.566	-105.000	o	o	o	o	o	o	o	o	o	o	o	3	26	0.132
18	8	12	0.67	40.189	-118.464	o	o	o	o	o	o	o	o	o	o	o	1	42	0.157
28	7	12	0.58	39.557	-107.776	o	o	o	o	o	o	o	o	o	o	o	1	35	0.151
19	4	12	0.33	39.544	-119.702	o	o	o	o	o	o	o	o	o	o	o	1	25	0.107
27	5	12	0.42	38.440	-107.866	o	o	o	o	o	o	o	o	o	o	o	3	40	0.155
16	9	12	0.75	38.419	-105.038	o	o	o	o	o	o	o	o	o	o	o	3	40	0.158
8	2	12	0.17	37.787	-121.539	o	o	o	o	o	o	o	o	o	o	o	3	22	0.044
15	8	12	0.67	37.160	-105.992	o	o	o	o	o	o	o	o	o	o	o	3	39	0.166
14	11	12	0.92	36.728	-108.222	o	o	o	o	o	o	o	o	o	o	o	1	43	0.160
9	9	12	0.75	36.713	-121.278	o	o	o	o	o	o	o	o	o	o	o	5	39	0.140
13	6	12	0.50	36.710	-110.249	o	o	o	o	o	o	o	o	o	o	o	5	45	0.156
22	4	12	0.33	36.553	-104.804	o	o	o	o	o	o	o	o	o	o	o	2	23	0.094
23	1	11	0.09	35.519	-106.218	o	o	o	o	o	o	o	o	o	o	o	1	0	0.007
10	7	12	0.58	35.275	-120.710	o	o	o	o	o	o	o	o	o	o	o	2	22	0.107
12	10	12	0.83	35.039	-110.700	o	o	o	o	o	o	o	o	o	o	o	2	38	0.136
11	9	12	0.75	34.609	-118.271	o	o	o	o	o	o	o	o	o	o	o	2	47	0.178
26	3	10	0.30	34.508	-109.368	o	o	o	o	o	o	o	o	o	o	o	2	45	0.158
24	3	12	0.25	34.043	-106.868	o	o	o	o	o	o	o	o	o	o	o	1	7	0.025
25	6	12	0.50	32.154	-109.756	o	o	o	o	o	o	o	o	o	o	o	2	22	0.084

Figure 3. Russian knapweed population information including population number (*P*), number of unique genotypes (*G*), number of plants sampled (*N*), number of genotypes found/number of plants genotyped (*G/M*), latitude and longitude of populations, a coloured chart showing spatial pattern of unique genotypes along linear transects within a population. Note that no genotypes were shared among any populations. The small circle (o) indicates a genotyped plant, white space indicates separation between plant patches and any shared colour of genotype in this figure solely indicates spatial location of identical genotypes within a population. Plants were collected every 5 m. For example, in population 35 all plants genotyped are from one patch and all were identical genotype. In population 4 there were three patches of Russian knapweed and two genotypes in the population (represented by purple and grey colours); the middle patch has both genotypes, while the first and third patches contain one genotype each. Populations are in order of decreasing latitude in this table. Also included are number of visual patches sampled in each population (Patches), percent of loci polymorphic (PLP) and gene diversity (*H_j*).

governing the rapidity of clonal turnover. Seed availability presents opportunities for new genet establishment, but clonal density and die-off often determine spatial structure of the patch (Benot *et al.* 2013). Thus, conditions

and interactions governing the frequency of seed–seedling versus vegetative recruitment may change with plant stage and patch age as well as across space (Eriksson 1993; De Kroon *et al.* 2005; Bittebiere *et al.* 2020).

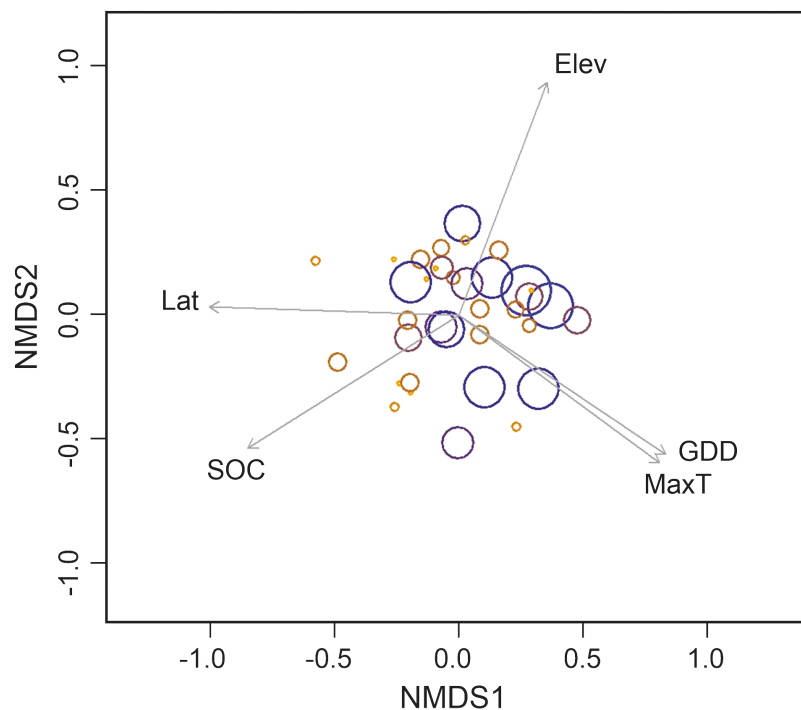


Figure 4. Variation in population diversity measured as number of genotypes found/number of plants genotyped (G/N : points scaled relative to G/N and indicated by colour ramp from orange [$G/N = 0.1$] to blue [$G/N = 1.0$]) of sites separated in ordination space based on all environmental variables measured. Plotted vectors indicate the direction of increase for the subset of variables included in subsequent analyses.

G/N reflects the adult cohort, and incompletely reflects the cycle of recruitment limitations or contrasting investment in different reproductive modes. In plant populations, post-emergence factors limiting growth beyond the seedling stage may result in an adult population structure that looks very different. For instance, low seed availability (seed limitation) may exacerbate mortality costs associated with adult recruitment, resulting in low adult plant abundance (i.e. Rand *et al.* 2020). Alternatively, greater limitation at the seedling stage may relax density-dependent self-thinning and increase adult success (Garren and Strauss 2009). Seedling recruitment may be favoured on patch edges not occupied by self-ramets. Radosavljević *et al.* (2020) showed that in one species, *Salvia brachyodon* (Lamiaceae), plant patch size negatively correlates with genetic richness and young plants were mostly found at the outer edge of, not within, large existing genets. Gaskin and Littlefield (2017) sampled distinct small (<10 m) and large (>10 m) patches, and found both to be almost entirely monotypic, but that both size classes had at least one unique genotype (at two sites) located on the patch perimeter. To collect leaf material from Russian knapweed plants 5 m apart in this study, we had to sometimes move across visual patches, and there was a trend towards increasing G/N with increasing number of patches sampled (Fig. 5). This trend suggests local conditions are likely important for structuring spatial dynamics of size hierarchies among clones. If the number of patches sampled within sites was strongly correlated with latitude, then the latitudinal relationship with diversity we detected may have merely reflected a higher likelihood of sampling more unique individuals due to diminishing patch sizes with decreasing latitude. However, we found only a weak correlation between patch number and latitude, and high diversity was still more frequent in low latitude sites even when few (1–2) patches were sampled. Therefore, patterns are

consistent with a signature of increased seedling recruitment at low latitudes.

Traits-based studies have associated clonal reproduction with higher latitudes, whether due to greater climate unpredictability (Ye *et al.* 2014) or decreasing strength of biotic interactions (Schemske *et al.* 2009; Lehndal and Ågren 2015) at increasing latitude. However, these trends are seldom quantified among populations of a single species. Our latitudinal gradient data did not include our four northernmost populations that are in Canada, for which we were unable to obtain soil data, but we retained them for all other analyses. Likely, the addition of these populations would have strengthened the detected trends, as all were highly clonal (Fig. 3).

Mechanisms governing demographic rates can vary across space, and local associations with site-level factors may not be consistent drivers at large scales. For instance, genetic diversity was positively correlated with SOC, a factor positively correlated with net productivity. High SOC is associated with multiple variables related to greater productivity, such as high precipitation, water retention and microbial activity (Sparling 1992). However, vegetation type may be more important in governing SOC than direct effects of precipitation (Jobbágy and Jackson 2000), and low SOC may increase the importance of soil texture to water retention (Rawls *et al.* 2003). In this study, negative latitudinal gradients in temperature and GDD were also associated with relatively higher SOC. This relationship could ameliorate some stressors related to increased latitude. Northern latitude populations may have shorter growth windows and high seasonality, but greater soil quality and stability associated with SOC combined with high site productivity should favour ramet success over seedlings. Lower temperatures and fewer GDD suggest a shorter window for seedling recruitment, giving an advantage to patch size increase via ramets. Population theory suggests

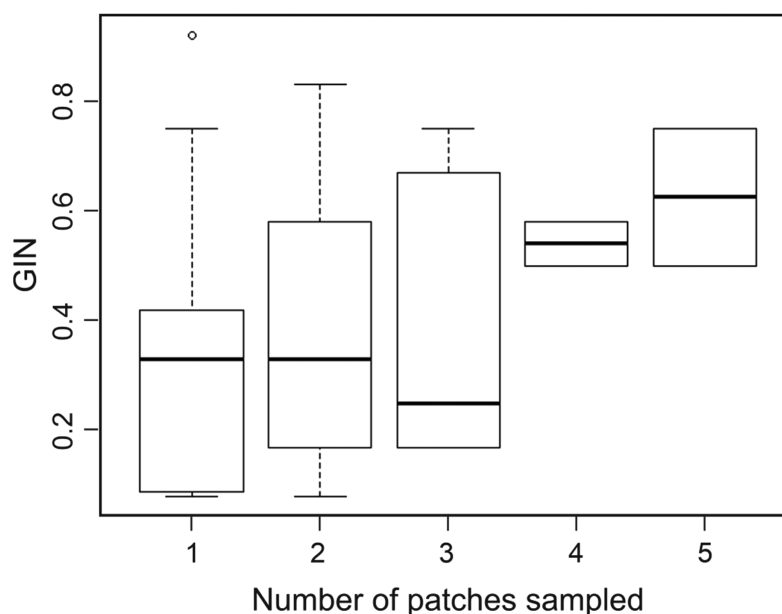


Figure 5. Box plot of Russian knapweed number of genotypes found/number of plants genotyped (G/N) in a population versus number of visual patches of Russian knapweed sampled in a population.

conditions driving the frequency and success of seedling recruitment have an outsized importance to genetic diversity and population structure even when quantitative increase in ramet numbers is low (Watkinson and Powell 1993). Interactions among environmental gradients may therefore drive divergent recruitment, persistence or management outcomes in different regions of the knapweed invasion (i.e. Ramula *et al.* 2008).

Clonal reproduction can be valuable to local-scale spread and persistence in Russian knapweed. Across the landscape, we can expect the relative frequency of seedling recruitment to change, and this association has implications for management decisions. We have shown that at higher (northern) latitudes there tend to be extensive monotypic patches, suggesting infrequent seedling recruitment. Thus, management disturbances such as grazing or herbicide application that open spaces for recruitment may increase the likelihood of patch persistence through seeds, if not properly temporally implemented. There are also two permitted biological control agents released in North America; both galling insects that stunt growth and reduce seed production (Djamankulova *et al.* 2008; Winston *et al.* 2014). These agents may be less effective in northern latitudes where spread by seed is less abundant. Plants may tolerate herbivore attack through reliance on clonal propagation for spread and persistence, though other biotic and abiotic factors are also likely affecting the insect success across latitudes (e.g. Buckley *et al.* 2005).

Many other factors (e.g. herbivory, local pollinator success, fine-scale soil qualities, timing of precipitation events, etc.) that likely influence trade-offs between clonal and seed reproduction rates were not tested. Our first step was to consider large-scale factors for which data already exist. We only analysed genotypes without reference to any functional genes, and there may be diverse genetic factors influencing rate of vegetative versus sexual reproduction such as functional genes, epigenetics and phenotypic plasticity. For example, Liu *et al.* (2006) found latitudinal clines for sexual reproduction with correlations to mean annual

temperature, GDD, tidal range and SOC. However, variation mostly disappeared in a common garden experiment, indicating that variation was largely due to phenotypic plasticity. Lehdal and Ågren (2015) investigated plant response to insect damage in a common garden experiment and found that plant resistance decreased with latitude of origin, while tolerance increased. This pattern arose in response to herbivores emerging later relative to plant phenology in the north compared to the south, and driving a decrease in selection pressure from herbivores with increasing latitude. A common garden approach would be a beneficial next step in researching Russian knapweed reproductive mode. Such information would increase our knowledge of how genetic and environmental factors are driving reproductive dynamics, and the extent to which demographic factors might drive variation in management success.

Supporting Information

The following additional information is available in the online version of this article—

Table S1. Collection site information.

Table S2. AFLP data.

Table S3. Model comparison of G/N and large-scale variables.

Figure S1. Delta K values graph.

Figure S2. Mean of estimated $\ln$ probability graph.

Figure S3. STRUCTURESELECTOR graphs.

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None declared.

Contributions by Authors

J.F.G., T.A.R. and N.M.W. analyzed the data. All authors wrote the manuscript and agreed to the published version of the manuscript.

Conflict of Interest

None declared.

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Data Availability

AFLP data used in this publication are available in the [Supporting Information](#).

Literature Cited

- Alebrahim MT, Rouhi HR, Serajchi M, Majd R, Ghorbani R. 2011. Study of dormancy-breaking and optimum temperature for germination of Russian knapweed (*Acroptilon repens* L.). *International Journal of AgriScience* 1:19–25.
- Auge H, Brandl R. 1997. Seedling recruitment in the invasive clonal shrub, *Mahonia aquifolium* Pursh (Nutt). *Oecologia* 110:205–211.
- Benot M-L, Bittebiere A-K, Ernoul A, Clement B, Mony C. 2013. Fine-scale spatial patterns in grassland communities depend on species clonal dispersal ability and interactions with neighbours. *Journal of Ecology* 101:626–636.
- Benson EJ, Hartnett DC. 2006. The role of seed and vegetative reproduction in plant recruitment and demography in tallgrass prairie. *Plant Ecology* 187:163–178.
- Bishop GF, Davy AJ. 1985. Density and the commitment of apical meristems to clonal growth and reproduction in *Hieracium pilosella*. *Oecologia* 66:417–422.
- Bittebiere A-K, Benot M-L, Mony C. 2020. Clonality as a key but overlooked driver of biotic interactions in plants. *Perspectives in Plant Ecology, Evolution and Systematics* 43:125510.
- Borcard D, Legendre P, Drapeau P. 1992. Partialling out the spatial component of ecological variation. *Ecology* 73:1045–1055.
- Buckley YM, Rees M, Sheppard AW, Smyth MJ. 2005. Stable coexistence of an invasive plant and biocontrol agent: a parameterized coupled plant-herbivore model. *Journal of Applied Ecology* 42:70–79.
- Burnham KP, Anderson DR. 2002. *Model selection and multimodel inference*. New York: Springer.
- Chen X-s, Li Y-f, Xie Y-h, Deng Z-m, Li X, Li F, Hou Z. 2015. Trade-off between allocation to reproductive ramets and rhizome buds in *Carex brevicuspis* populations along a small-scale elevational gradient. *Scientific Reports* 5:12688.
- Chybicki IJ, Oleksa A, Burczyk J. 2011. Increased inbreeding and strong kinship structure in *Taxus baccata* estimated from both AFLP and SSR data. *Heredity* 107:589–600.
- De Frenne P, Graae BJ, Rodríguez-Sánchez F, Kolb A, Chabrierie O, Decocq G, De Kort H, De Schrijver A, Diekmann M, Eriksson O, Gruwez R. 2013. Latitudinal gradients as natural laboratories to infer species' responses to temperature. *Journal of Ecology* 101:784–795.
- De Kroon H, Huber H, Stuefer JF, Van Groenendael JM. 2005. A modular concept of phenotypic plasticity in plants. *New Phytologist* 166:73–82.
- Djamankulova G, Khamraev A, Schaffner U. 2008. Impact of two shoot-galling biological control candidates on Russian knapweed, *Acroptilon repens*. *Biological Control* 46:101–106.
- Dorken ME, Eckert CG. 2001. Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). *Journal of Ecology* 89:339–350.
- Douhovnikoff V, Dodd RS. 2015. Epigenetics: a potential mechanism for clonal plant success. *Plant Ecology* 216:227–233.
- Duncan CL. 2005. Russian knapweed. In: Duncan CL, Clark JK, eds. *Invasive plants of range and wildlands and their environmental, economic, and societal impacts*. Lawrence, KS: Weed Science Society of America, 8–15.
- Earl DA. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4:359–361.
- Eckert CG. 2002. The loss of sex in clonal plants. In: Huber H, ed. *Ecology and evolutionary biology of clonal plants*. Dordrecht, The Netherlands: Springer, 279–298.
- EDDMapS. 2021. Early detection & distribution mapping system. The University of Georgia—Center for Invasive Species and Ecosystem Health. <http://www.eddmaps.org> (01 November 2021).
- Eriksson O. 1993. Dynamics of genets in clonal plants. *Trends in Ecology and Evolution* 8:313–316.
- Eriksson O. 1997. Clonal life histories and the evolution of seed recruitment. In: De Kroon H, Van Groenendael J, eds. *The ecology and evolution of clonal plants*. Leide: Backhuys, 211–226.
- Ersts PJ. 2019. Geographic distance matrix generator (version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation. https://biodiversityinformatics.amnh.org/open_source/gdmg/ (01 November 2021).
- ESRI. 2018. *ArcGIS Desktop 10.8.1*. Redlands, CA: Environmental Systems Research Institute, Inc.
- Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology* 14:2611–2620.
- Fager EW. 1972. Diversity: A sampling study. *The American Naturalist* 106:293–310.
- Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164:1567–1587.
- Falush D, Stephens M, Pritchard JK. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes* 7:574–578.
- Frazier JC. 1944. Nature and rate of development of root system of *Centaurea picris*. *Botanical Gazette* 105:345–351.
- Garren JM, Strauss SY. 2009. Population-level compensation by an invasive thistle thwarts biological control from seed predators. *Ecological Applications* 19:709–721.
- Gaskin JF, Bon MC, Cock MJ, Cristofaro M, De Biase A, De Clerck-Floate R, Ellison CA, Hinz HL, Hufbauer RA, Julien MH, Sforza R. 2011. Applying molecular-based approaches to classical biological control of weeds. *Biological Control* 58:1–21.
- Gaskin JF, Kazmer DJ. 2009. Introgression between invasive saltcedars (*Tamarix chinensis* and *T. ramosissima*) in the USA. *Biological Invasions* 11:1121–1130.
- Gaskin JF, Littlefield JL. 2017. Invasive Russian knapweed (*Acroptilon repens*) creates large patches almost entirely by rhizomic growth. *Invasive Plant Science and Management* 10:119–124.
- Goslee SC, Beck KG, Peters DPC. 2003. Distribution of Russian knapweed in Colorado: climate and environmental factors. *Journal of Range Management* 56:206–212.
- Gowton C, Budsock A, Matlaga D. 2016. Influence of disturbance on Japanese knotweed (*Fallopia japonica*) stem and rhizome fragment recruitment success within riparian forest understory. *Natural Areas Journal* 36:259–267.
- Harrod RJ, Taylor RJ. 1995. Reproduction and pollination biology of *Centaurea* and *Acroptilon* species, with emphasis on *C. diffusa*. *Northwest Science* 69:97–105.
- Hillis DM, Mable BK, Larson A, Davis SK, Zimmer EA. 1996. *Molecular systematics*. Sunderland, MA: Sinauer.
- Ivanova TS. 1966. Biological control of mountain bluet (*Acroptilon picris* C. A. M.). *Izvestiya Akademii Nauk Tadzhikskoi SSR, Otdeleniye biologicheskikh Nauk* 2:51–63 (in Russian).
- Jacobs J, Denny K. 2006. *Ecology and management of Russian knapweed (Acroptilon repens (L.) DC)*. Invasive Species Technical Note No. MT-7. US Department of Agriculture, Natural Resources Conservation Service, 1–9.

- Jacquemyn H, Brys R, Honnay O, Hermy M, Roldán-Ruiz I. 2005. Local forest environment largely affects below-ground growth, clonal diversity and fine-scale spatial genetic structure in the temperate deciduous forest herb *Paris quadrifolia*. *Molecular Ecology* 14:4479–4488.
- Jóbbágy EG, Jackson RB. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications* 10:423–436.
- Johnson AJ, Orth RJ, Moore KA. 2020. The role of sexual reproduction in the maintenance of established *Zostera marina* meadows. *Journal of Ecology* 108:945–957.
- Larson LG, Kiemnec G. 2005. Germination of two noxious range weeds under water and salt stresses with variable light regimes. *Weed Technology* 19:197–200.
- Lehndal L, Ågren J. 2015. Latitudinal variation in resistance and tolerance to herbivory in the perennial herb *Lythrum salicaria* is related to intensity of herbivory and plant phenology. *Journal of Evolutionary Biology* 28:576–589.
- Lehndal L, Hambäck PA, Ericson L, Ågren J. 2016. Herbivory strongly influences among-population variation in reproductive output of *Lythrum salicaria* in its native range. *Oecologia* 180:1159–1171.
- Li Y-L, Liu JX. 2018. StructureSelector: a web-based software to select and visualize the optimal number of clusters using multiple methods. *Molecular Ecology Resources* 18:176–177.
- Liu J, Dong M, Miao S, Li Z, Song M, Wang R. 2006. Invasive alien plants in China: role of clonality and geographical origin. *Biological Invasions* 8:1461–1470.
- Lockwood JL, Cassey P, Blackburn T. 2005. The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution* 20:223–228.
- Morrison RG, Lownds NK, Sterling TM. 1995. Picloram uptake, translocation, and efficacy in relation to water status of Russian knapweed (*Acroptilon repens*). *Weed Science* 43:34–39.
- Nathan R. 2006. Long-distance dispersal of plants. *Science* 313:786–788.
- Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583–590.
- Pielou EC. 1969. *An introduction to mathematical ecology*. New York: Wiley Interscience.
- PRISM Climate Group. 2020. *Northwest alliance for computational science and engineering*. Oregon State University. <https://prism.oregonstate.edu/normals/> (25 June 2020).
- Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945–959.
- Puechmaile SJ. 2016. The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. *Molecular Ecology Resources* 16:608–627.
- Pyšek P. 1997. Clonality and plant invasions: can a trait make a difference. In: *The ecology and evolution of clonal plants*. Leiden, The Netherlands: Backhuys.
- R Core Team. 2020. *R: a language and environment for statistical computing*. Version 3.6.3. Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/> (01 November 2021).
- Radosavljević I, Antonić O, Hruševar D, Križan J, Satović Z, Turković D, Liber Z. 2020. The influence of a seedling recruitment strategy and a clonal architecture on a spatial genetic structure of a *Salvia brachyodon* (Lamiaceae) population. *Plants* 9:828.
- Ramcharan A, Hengl T, Nauman T, Brungard C, Waltman S, Wills S, Thompson J. 2017. *Soil properties and class 100m grids United States*. ScholarSphere, PennState University Libraries. <https://doi.org/10.18113/S1KW2H> (01 November 2021).
- Ramula S, Knight TM, Burns JH, Buckley YM. 2008. General guidelines for invasive plant management based on comparative demography of invasive and native plant populations. *Journal of Applied Ecology* 45:1124–1133.
- Rand TA, West NM, Russell FL, Louda SL. 2020. Post-dispersal factors influence recruitment patterns but do not override the importance of seed limitation in populations of a native thistle. *Oecologia* 193:143–153.
- Rawls WJ, Pachepsky YA, Ritchie JC, Sobecki TM, Bloodworth H. 2003. Effect of soil organic carbon on soil water retention. *Geoderma* 116:61–76.
- Robbins WW, Bellue MK, Ball WS. 1951. *Weeds of California*. Sacramento, CA: Printing Div. (Documents Sect.) Calif. State Dep. Agric., 547 pp.
- Ruiz RA. 1999. *Use of randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) to distinguish North American populations of Acroptilon repens (L.) DC*. The University of Texas-Pan American, Masters of Science dissertation.
- Rolf FJ. 1992. *NTSYS-PC: numerical taxonomy and multivariate analysis system*. Setauket, NY: Exeter Software.
- Running S, Zhao M. 2019. MOD17A3HGF MODIS/Terra Net Primary Production Gap-Filled Yearly L4 Global 500 m SIN Grid V006. NASA EOSDIS Land Processes DAAC. <https://doi.org/10.5067/MODIS/MOD17A3HGF.006> (28 July 2020).
- Schemske DW, Mittelbach GG, Cornell HV, Sobel JM, Roy K. 2009. Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution, and Systematics* 40:245–269.
- Seeber E, Miehe G, Hensen I, Yang Y, Wesche K. 2015. Mixed reproduction strategy and polyploidy facilitate dominance of *Kobresia pygmaea* on the Tibetan Plateau. *Journal of Plant Ecology* 9:87–99.
- Selleck G. 1964. A competition study of *Cardaria* spp. and *Centaurea repens*. In: *Proceedings of the 7th British Weed Control Conference*. 569–576.
- Song Y-B, Yu F-H, Keser LH, Dawson W, Fischer M, Dong M, van Kleunen M. 2013. United we stand, divided we fall: a meta-analysis of experiments on clonal integration and its relationship to invasiveness. *Oecologia* 171:317–327.
- Sparling GP. 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. *Soil Research* 30:195–207.
- Sun S, Gao X, Cai Y. 2001. Variations in sexual and asexual reproduction of *Scirpus mariqueter* along an elevational gradient. *Ecological Research* 16:263–274.
- USA National Phenology Network. 2017. Daily temperature accumulations, 30 year average—50 base temp 1981–2010, for region: USA. Tucson, AZ: USA-NPN. <http://dx.doi.org/10.5066/F7SN0723> (25 June 2020).
- USDA, NRCS. 2022. The PLANTS database. <http://plants.usda.gov> (May 2022).
- Van Groenendael JM, Klimeš L, Klimešová J, Hendriks RJJ. 1996. Comparative ecology of clonal plants. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* 351:1331–1339.
- Vekemans X, Beauwens T, Lemaire M, Roldán-Ruiz I. 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology* 11:139–151.
- Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M. 1995. AFLP—a new technique for DNA-fingerprinting. *Nucleic Acids Research* 23:4407–4414.
- Ward SM, Gaskin JF, Wilson LM. 2008. Ecological genetics of plant invasion: what do we know? *Invasive Plant Science and Management* 1:98–109.
- Watkinson AR, Powell JC. 1993. Seedling recruitment and the maintenance of clonal diversity in plant populations—a computer simulation of *Ranunculus repens*. *Journal of Ecology* 81:707–717.
- Watson AK. 1980. The biology of Canadian weeds. 43. *Acroptilon (Centaurea) repens* (L.) DC. *Canadian Journal of Plant Science* 60:993–1004.
- Whitson TD. 1999. Russian knapweed. In: Sheley RL, Petroff JK, eds. *Biology and management of noxious rangeland weeds*. Corvallis, OR: Oregon State University Press, 315–322.

- Winston RR, Randall CB, deClerck-Floate R, McClay A, Andreas J, Schwarzländer M. 2014. *Field guide for the biological control of weeds in the Northwest*. Morgantown, West Virginia: USDA and University Idaho Extension.
- Ye D, Hu Y, Song M, Pan X, Xie X, Liu G, Ye X, Dong M. 2014. Clonality-climate relationships along latitudinal gradient across China: adaptation of clonality to environments. *PLoS One* 9:e94009.
- Young JA, Clements CD. 2003. Rangeland monitoring and invasive weeds. *Arid Land Research and Management* 17:439–447.
- Zhang Y, Zhang D. 2007. Asexual and sexual reproductive strategies in clonal plants. *Frontiers of Biology in China* 2:256–262.