Population genetics of *Trichophorum planifolium* structured by inbreeding, not distribution limits

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Abstract

Premise

Peripheral populations are understood to hold special conservation significance as repositories of divergent genetic diversity. They may also be at increased risk of extirpation, due to a combination of small size, isolation, and marginal habitat conditions. However, empirical studies testing these expectations yield mixed results. We evaluated the relationship between genetic diversity, geographical range limits, spatial isolation, and environmental conditions. This work provides important insight into the conservation of peripheral populations, and contributes to our understanding of the factors shaping range limits.

Methods

We used population samples, herbarium records, and climate data to survey genetic diversity, geographic distribution, and environmental niche characteristics of *Trichophorum planifolium* across its range in eastern North America. Our analysis included distinct measures of geographic peripherality, spatial isolation, and niche marginality, allowing us to assess the influence of each of these factors on genetic diversity.

Results

Our data show that populations of *T. planifolium* have remarkably low diversity, with the majority of populations having fewer than 3 distinct multi-locus genotypes. While there was significant spatial structure, we found no relationship between genetic diversity and peripherality, isolation, or marginality.

Conclusions

In *T. planifolium*, population genetic diversity is primarily a reflection of local processes. Its reproductive biology favours inbreeding, and genetic diversity is paritioned almost entirely among populations. This highlights the importance of life history characteristics when evaluating biogeographic patterns.

Keywords

abundant center hypothesis; conservation genetics; microsatellites; *Trichophorum planifolium*; peripheral populations; range limits; species distribution models

Introduction

No limit, aim for 8000 words.

In high-latitude countries, geographically peripheral populations form a significant proportion of species-at-risk (Yakimowski and Eckert, 2007; Gibson et al., 2009). For example, in Canada, approximately 90% of threatened or endangered plant species are locally rare representatives of species that reach the northern extent of their range in Canada, but are common further south (Yakimowski and Eckert, 2007). Concern over the investment of conservation resources into species that are locally rare but globally common has led to debate over whether, or when, peripheral populations merit national concern (e.g., Hunter and Hutchinson, 1994; Hampe and Petit, 2005; Gibson et al., 2009).

Peripheral populations are typically small and geographically isolated due to increased habitat variability and reduced resource availability at the range margin (i.e., the abundant center hypothesis, Brown, 1984). Consequently, they may be at greater risk of extirpation due to genetic (i.e., inbreeding depression) as well as stochastic demographic, environmental and catastrophic factors (Soulé and Mills, 1998; Brook et al., 2002). These risks may be exacerbated by anthropogenic threats such as habitat loss and fragmentation (Young et al., 1996). Nevertheless, peripheral populations may be important sources of adaptive and evolutionary potential for the species (Safriel et al., 1994; Leppig and White, 2006; Eckert et al., 2008), harbouring unique genetic diversity and/or adaptations to conditions at the range edge (Lesica and Allendorf, 1995; Bunnell et al., 2004).

However, not all peripheral populations are genetically differentiated from core populations (Lesica and Allendorf, 1995; Leppig and White, 2006). In a review of 134 genetic studies in plants, (Eckert et al., 2008) found that only 70.3% of studies identified increased genetic differentiation towards the range margin. These differences may be attributable to species-specific characteristics (Gibson et al., 2009). In plants, life-history characteristics (i.e., breeding system and dispersal mechanisms) are a major determinant of population genetic structure, because they are related to gene flow (Loveless and Hamrick, 1984; Frankel et al., 1995). In this regard, knowledge of the level and spatial distribution of intraspecific genetic diversity can add valuable information to guide conservation efforts for peripheral populations, especially when the reproductive biology and ecology of the species is not well understood.

Lesica and Allendorf (1995) proposed a framework for evaluating the conservation value of peripheral populations, identifying population isolation and divergent environmental conditions as main criteria. In practice, neutral genetic markers, such as microsatellites, can be used to identify populations that have experienced restricted gene flow (i.e., are genetically isolated) (Crandall et al., 2000; Moritz, 2002). However, microsatellite markers cannot provide information on adaptive divergence of populations (Holderegger et al., 2006). Therefore, to capture variation in selection pressures across the species' range, environmental differences among populations should be assessed (Allendorf et al., 2013).

These issues have received renewed attention recently, in light of new approaches to assess the factors generally influencing range limits (Cross and Eckert, 2020). There is a growing recognition of the need to distinguish between related spatial and ecological processes contributing to the distribution limits of species (Lee-Yaw et al., 2018; Willi and Van Buskirk, 2019). Three key concepts are frequently treated as loosely interchangeable: geographic peripherality, geographic isolation, and ecological marginality. However, the increasing availability of high-resolution distribution and environmental data provides an opportunity to examine the distinct influences of these processes on species range limits.

Trichophorum planifolium (Sprengel) Palla, commonly known as Few-flowered Club-rush or Bashful Bulrush, is a species that is rare at its northern limit in Canada, but is common further south. It is a perennial woodland species in the family Cyperaceae, typically occurring on mesic to dry, often rocky, slopes in association with hardwoods, especially Oaks (*Quercus* spp.) (Crins, 2002). The range of *T. planifolium* extends from Massachusetts west to Ontario and south to Virginia and Kentucky, with disjunct populations occurring in Missouri and southern Illinois (Figure 1).

In general, *T. planifolium* is considered common within the central portion of its range, but rare along the periphery (Smith and Rothfels, 2007). The only extant locality in Canada is in southern Ontario, in

the nature sanctuaries at Royal Botanical Gardens in Hamilton, Ontario. *Trichophorum planifolium* was previously known from one other location in Ontario, in Rouge Valley near Toronto, but no plants have been located at this site since 2005 (Smith and Rothfels, 2007). Due to its limited Canadian range and an apparent decline in population size, *T. planifolium* is listed as Endangered under the Canadian Species at Risk Act (SARA, COSEWIC, 2000).

The species is caespitose, growing in dense, low tufts (~ 40 cm) from short rhizomes. It is wind-pollinated, possessing long exerted styles and stamens and lacking both a showy perianth and nectaries (Crins, 1989; Iwanycki et al., 2010). Seeds mature in mid-summer and disperse in late July to August (Iwanycki et al., 2010). A mechanism for seed dispersal has not been confirmed (Smith and Rothfels, 2007), but it has been suggested that the matting of leaves and stems during the fruiting period may limit most seed dispersal to within a meter of the parent plants, leading to the formation of colonies of closely related sibling groups (Crins, 1989). It has been suggested that low recruitment observed at the Hamilton site may be due to limited intrapopulational genetic diversity (Smith and Rothfels, 2007).

The main objective of our research was to assess genetic variability within the Canadian population, and to quantify the relationship between the geographic and ecological distribution of populations, and their relative genetic diversity and divergence. Specifically, we address the questions: does genetic diversity decline along spatial or environmental gradients (Eckert et al., 2008); or does gene flow from the core of a species' distribution maintain comparative levels of genetic diversity across the range of the species (Kirkpatrick and Barton, 1997; Sexton et al., 2016)? The results of our work provide crucial insight for ongoing conservation management of this endangered species.

MATERIALS AND METHODS

Population Sampling

In spring 2014, we sampled 29 populations of *T. planifolium* across its geographic distribution (Figure 1, Table 1). An additional population sample was previously obtained in 2011 in Kentucky. At each site, we sampled individuals haphazardly, with at least 1 m between individuals, and a maximum distance of 100 m between the most distant samples. In total, 919 tissue samples were obtained, with a minimum of four and a maximum of 50 samples per population (mean = 31.7). Our protocol was modified for one very small population (PACR), where one sample was taken at a distance of 150 m to increase sample size to seven. A single herbarium voucher was collected per population, except for the locations in Ontario, New Hampshire, and West Virginia, where the species is Endangered and collection of whole plants is prohibited. Vouchers have been deposited at DAO (abbreviations follow Thiers, 2020).

Genetic Analysis

After collection, samples were immediately placed on silica gel and stored at room temperature prior to molecular analysis. A random subsample of 20 samples per population were selected for genetic analysis. For populations with fewer than 20 samples, all individuals were used.

For each individual, 20 mg of dried leaf tissue was ground with 3 mm-diameter stainless steel beads in a 2 mL Eppendorf tube at 30 Hz for 2 min using a TissueLyser II (Qiagen, Venlo, Limburg, Netherlands). Total genomic DNA was extracted from ground tissue using the Nucleospin Plant II Kit (Machery-Nagel, Düren, Germany). The quality and concentration of DNA obtained was verified using a Nanodrop 2000 UV-Vis Spectrophotometer (ThermoScientific, Waltham, Massachusetts, U.S.A.).

We developed eleven nuclear microsatellite markers (see Nowell et al., 2015 for details) to assess the population genetics of *T. planifolium*. Amplification of microsatellite fragments were performed in 8 µl reaction volumes containing 0.416 µL primer mix (10 mM; 10:1 untagged to tagged primer), 0.192 µL dye-labelled CAG Tag (10 mM; 6-FAM or VIC, Life Technologies, Carlsbad, California, U.S.A.), 0.24 µL DMSO, 4 µL 2X Phusion

Table 1: Populations sampled. The first two letters of the Population Code indicate the US state or Canadian province. N: total samples per population (samples with all 11 loci scored in parentheses). Ap: private alleles. Ar: mean allelic richness per locus. mlg: multilocus genotypes. eMLG: multilocus genotypes in rarefied samples of 10 individuals. Hs: gene diversity. Ho: observed heterozygosity.

population	Latitude	Longitude	N	Ap	Ar	mlg	emlg	Hs	Ho	Fis	Fst
CTTP	41.3714	-73.4527	17(16)	0	1.00	1	1.00	0.00	0.00		0.89
KYLE	37.5906	-83.7815	5(2)	1	1.07	3		0.05	0.02	0.60	0.84
MABM	42.3017	-72.5295	19(14)	1	1.12	5	3.63	0.07	0.00	0.93	0.81
MAMT	42.4471	-72.5389	19(19)	0	1.06	3	2.05	0.03	0.03	0.13	0.85
MAST	42.0585	-72.1300	20(20)	0	1.00	1	1.00	0.00	0.00		0.89
MDDM	39.5678	-78.9119	20(19)	0	1.32	7	4.53	0.18	0.02	0.87	0.76
MOBS	36.9475	-90.9931	41(17)	2	1.31	17	6.53	0.16	0.01	0.93	0.70
MOBU	37.5356	-91.2116	14(11)	1	1.45	6	5.34	0.25	0.01	0.97	0.60
MOHC	37.0226	-92.1677	14(12)	0	1.11	4	3.43	0.06	0.01	0.90	0.83
MOOC	37.6643	-90.8998	17(12)	0	1.03	3	2.18	0.02	0.01	0.65	0.86
MOSH	37.1996	-91.3258	20(15)	0	1.14	5	4.21	0.07	0.01	0.84	0.78
NHER	42.7643	-71.3613	19(14)	1	1.27	4	3.51	0.15	0.03	0.79	0.68
NJKP	40.7612	-74.7158	17(16)	0	1.14	3	2.18	0.07	0.01	0.93	0.82
NYMP	43.0301	-77.5737	19(19)	0	1.02	2	1.53	0.01	0.00	1.00	0.90
NYTS	42.1176	-73.4972	20(15)	0	1.29	3	2.89	0.17	0.00	1.00	0.67
OHBH	39.6205	-82.4048	13(13)	0	1.00	1	1.00	0.00	0.00		0.80
OHHH	39.4381	-82.5306	20(20)	0	1.20	7	5.32	0.11	0.00	0.96	0.71
OHPR	38.6525	-83.1637	18(17)	1	1.04	3	2.11	0.02	0.00	1.00	0.77
OHSC	39.0583	-83.3886	17(15)	0	1.00	1	1.00	0.00	0.00		0.87
ONRB	43.2656	-79.9210	27(22)	1	1.01	2	1.37	0.01	0.00	1.00	0.87
PABE	41.0438	-77.6540	15(7)	0	1.11	4	3.31	0.06	0.00	1.00	0.82
PACR	40.3703	-79.2275	6(5)	0	1.06	2		0.04	0.00	1.00	0.82
PAGP	40.0746	-76.9091	18(13)	0	1.34	7	4.71	0.19	0.05	0.76	0.68
PASG	40.0976	-76.9484	9(8)	0	1.04	2		0.02	0.00	1.00	0.76
VAAC	37.8035	-78.2493	17(14)	0	1.09	3	2.59	0.05	0.00	1.00	0.81
VAHB	37.1885	-80.4192	18(14)	0	1.00	1	1.00	0.00	0.00		0.90
VASC	36.8198	-79.0665	3(1)	0	1.09	2		0.09	0.00	1.00	0.75
WVFM	38.6642	-79.2404	19(18)	2	1.30	9	6.08	0.16	0.01	0.97	0.67
WVMO	38.5188	-79.3705	20(18)	1	1.30	7	4.92	0.16	0.00	1.00	0.72
WVSS	39.2222	-77.8200	4(2)	0	1.07	2		0.05	0.00	1.00	0.85
Total			505(408)			115	9.03	0.08	0.01	0.91	0.80

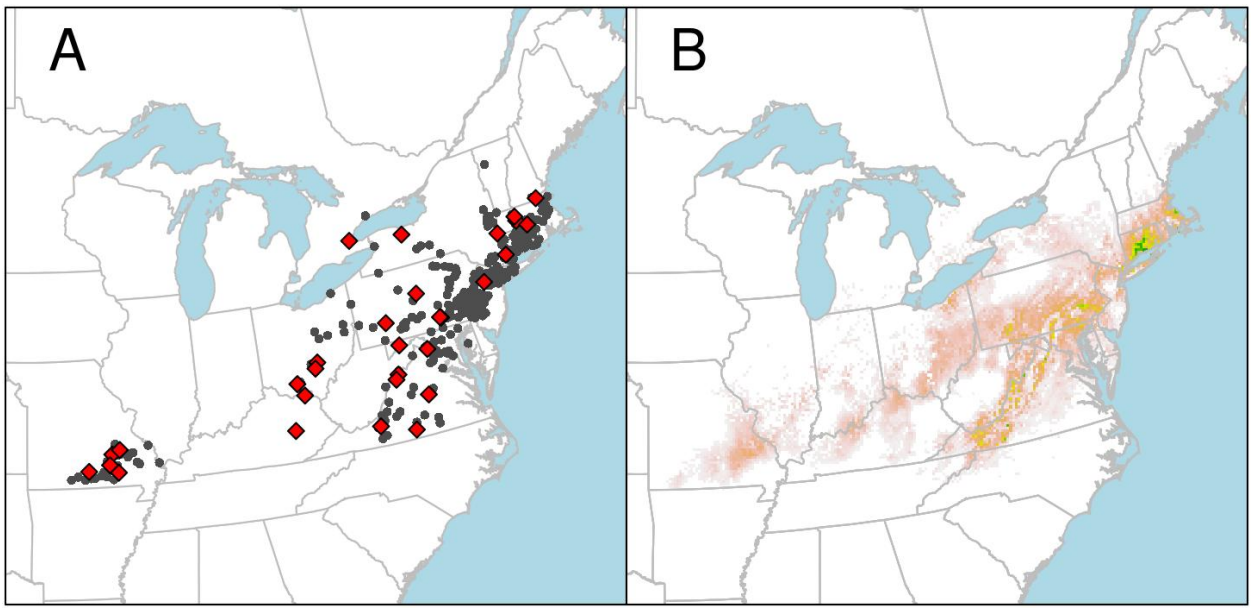

Figure 1: *Trichophorum planifolium* distribution. A. Populations Sampled: Grey points are GBIF records used to generate the species distribution model and geographic isolation and marginality indices. Red diamonds are populations sampled for the genetic survey. B. Maxent species distribution model. Highest suitability is indicated in green, declining through yellow, orange and grey.

High-Fidelity Master Mix with HF Buffer (New England Biolabs, Ipswich, Massachusetts, U.S.A.), 2.152 µL ddH2O and 1 µL DNA (10 ng/µL) using a T-100 Thermal Cycler (Bio-Rad, Hercules, California, U.S.A.). Cycling conditions followed Touchdown-TD PCR (Korbie and Mattick, 2008) as in Nowell et al. (2015). PCR products were visualized on a 1% agarose gel stained with GelRed (Biotium, Hayward, California, U.S.A.) and viewed with a High Performance Transilluminator (UVP, Upland, California, U.S.A.) with a 100 bp DNA ladder (New England Biolabs) to confirm the presence and size of amplification products and absence of contamination prior to genotyping.

Amplification products were subsequently pooled into groups of four and visualized by capillary electrophoresis using a 3130xL Genetic Analyzer (Life Technologies) with the GeneScan 500 LIZ Size Standard (Life Technologies). Individual samples were genotyped using Genemapper v.5 software (Life Technologies) and verified with manual scoring.

Due to amplification failures, we were unable to score all samples for all 11 markers. We retained only those samples for which we could score at least 8 loci. We enumerated private alleles, calculated allelic richness (El Mousadik and Petit, 1996; Adamack and Gruber, 2014), gene diversity, observed heterozygosity and the fixation index F_{IS} (Nei, 1987; Goudet and Jombart, 2015) for each population. Tests for departures from Hardy-Weinberg equilibrium (HWE) were performed with the R package 'pegas' ver. 0.13 (Paradis, 2010).

We counted the number of distinct multi-locus genotypes (MLG) for each population, using the methods provided by Kamvar et al. (2015). When samples with incomplete genotypes could not be unambiguously assigned to a single MLG, we randomly assigned them to a matching MLG in same population, if present. To account for differences in sample size, we also calculated rarefied MLG counts for each population, as a standardized measure of genetic richness. The rarefaction estimates were calculated for subsamples of 10 individuals per population (referred to as eMLG hereafter, Oksanen et al., 2019). Populations with fewer than 10 invidivduals scored were excluded from rarefaction, and all analyses based on eMLG.

We also assessed genetic divergence, using an index based on population pairwise-F_{ST} values. We calculated our divergence index (F_D) as the mean of pairwise-F_{ST} values for a given population, when compared with all other populations.

Preliminary review of the data revealed excessive levels of homozygosity, and no locus was in Hardy-Weinburg equilibrium. As discussed below, this indicates high levels of selfing and/or inbreeding among close relatives. As such, analyses based on assumptions of Hardy-Weinburg equilibrium, linkage equilibrium and panmictic populations, such as STRUCTURE (Pritchard et al., 2000), are inappropriate for our data. Accordingly, we used multivariate ordinations to visualize genetic structure among populations (Jombart et al., 2008). Similarly, we did not test for the presence of null alleles, as we are not aware of a test that can distinguish between excessive homozygosity due to the presence of null alleles, and excessive homozygosity to due to high levels of inbreeding (e.g., Brookfield, 1996).

Geographic and Ecological Analysis

We assessed spatial structure using spatial Principal Components Analysis [i.e., sPCA; Jombart et al. (2008); Thioulouse et al. (2018)]. We defined the spatial neighbourhood of a sample as all other samples within 650 km, with the strength of each link weighted inversely by distance (via function `spdep::dnearneigh` with the upper distance bound set to 650). We chose 650 km to ensure there were no disconnected subgroups (i.e., there was at least one link between the Missouri populations and the eastern populations). We tested for statistically significant spatial autocorrelation using a randomization test (function `global.rtest` in R package `adegenet`).

Additionally, we used eMLG and F_D to assess the relationship between genetic diversity and three indexes representing geographic and ecological distribution: Isolation (the distance between a population and its nearest neighbours), Peripherality (the relative spatial location of a population relative to the core distribution of the species), and Suitability (the relative ecological suitability of a population's location).

We generated all three of these indices using specimen records downloaded from GBIF.org (2019) to characterize the geographic distribution of *T. planifolium*. To control for collection bias (see Radosavljevic and Anderson, 2014), we used the `spThin` package (Aiello-Lammens et al., 2015) to create a thinned data set, in which retained samples were at least 10 km from all other samples.

We calculated the isolation index as the mean geographic distance between a sampled population and its five nearest neighbours in the thinned GBIF data set. We decided to use five nearest neighbours, rather than the single nearest neighbour, as this would score isolated clusters of 2-4 populations as having greater isolation than a similar sized clusters that occur in close proximity to other populations.

We calculated the peripherality index as the Mahalanobis distance between each sample and the centroid of the GBIF data. We used the Mahalanobis distance, rather than the Euclidean distance, as the former accounts for the density & distribution (variance and covariance) of points, as well as their absolute distances (Legendre and Legendre, 1998; see Lee-Yaw et al., 2018 for a similar approach).

The suitability index was generated using a Maxent distribution model (Phillips et al., 2017). We limited the study extent to 31N-50N degrees latitude, 66W-98W degrees longitude; this area was chosen to include a 500 km buffer around a minimum convex polygon containing all GBIF records for *T. planifolium*. Background training data was limited to level 1 Ecoregions (Omernik and Griffith, 2014) in which *T. planifolium* is present within this extent. The following environmental layers were considered: 19 bioclimatic variables (WorldClim: Fick and Hijmans, 2017, www.worldclim.org), aridity (Trabucco and Zomer, 2019), and terrain ruggedness. Terrain ruggedness was calculated from SRTM DEM data (Jarvis et al., 2008) with 3-arc second (approximately 90m) horizontal resolution. We used the Terrain Ruggedness Index in QGIS (QGIS.org, 2020). The resulting raster was upscaled to match the 30 arc-second (~1km) resolution of the other layers, using the "average" resampling method. To reduce collinearity among variables, We selected a subset of these layers such that the variance inflation factor was less than 10 (function `vif` in the R package `usdm`: Naimi et al., 2014). The final set of variables included aridity, WorldClim Bio2 (mean diurnal temperature range), Bio6 (minimum temperature of coldest month), Bio13 (precipitation of wettest month), and Terrain Ruggedness. Maxent model parameters were tuned with the `ENMevaluate` function from the package `ENMeval` (Muscarella et al., 2014).

We tested the relationships between genetic diversity (eMLG) and genetic divergence (F_D), and Isolation,

Peripherality, and Suitability using linear regression. The index values were log-transformed to improve normality.

All analyses were performed in R version 4.0 (R Core Team, 2020), unless otherwise specified.

RESULTS

We scored a total of 505 individual genotypes for at least 8 loci, and completed all 11 loci for 408 individuals (Table 1). 46 alleles were detected (mean 4.2 per locus, range 2–9), and a total of 115 unique multi-locus genotypes (MLG) were described. All but three MLG were confined to a single population. One MLG was shared by two Missouri populations; one was shared by two Ohio populations; and one MLG was found in three populations, one each in Connecticut, Maryland, and eastern New York.

465 (92.1%) individuals were homozygous for all alleles, with only 40 individuals heterozygous at one or more loci. Of the 25 populations with at least 10 individuals genotyped, the mean eMLG (i.e., genotypes per 10 individuals) was 3.1 (range 1–6.5, Figure 2). The Ontario population had an eMLG of 1.37, corresponding to the 20th percentile of the distribution. The mean F_D was 0.79 (range 0.59–0.9, Figure 2); F_D for the Ontario population was 0.87, corresponding to the 80th percentile of the distribution.

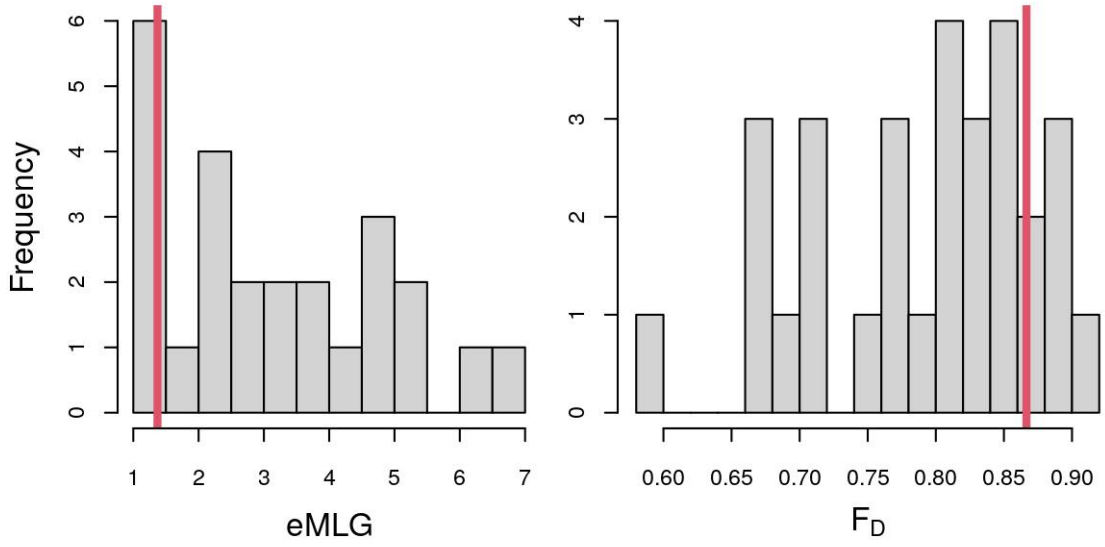

Figure 2: Distribution of rarified multi-locus genotypes (eMLG) and population mean pair-wise Fst values (F_D). The red line indicates the population in Ontario, Canada.

There was significant spatial structure in the genetic data, as determined by a Monte Carlo test of Morin's I (function `adespatial::global.rtest`, $p < 0.001$). Plotting the first axis of the sPCA on a geographic map reveals this structure is largely due to divergence of the Missouri populations from the eastern locations (Figure 3). However, the Ontario population and one of the Ohio populations are genetically more similar to the Missouri populations than to their nearer neighbours.

We found no evidence supporting a relationship between genetic diversity or genetic divergence and geographic location (isolation or peripherality) or ecological condition (suitability; Table 2).

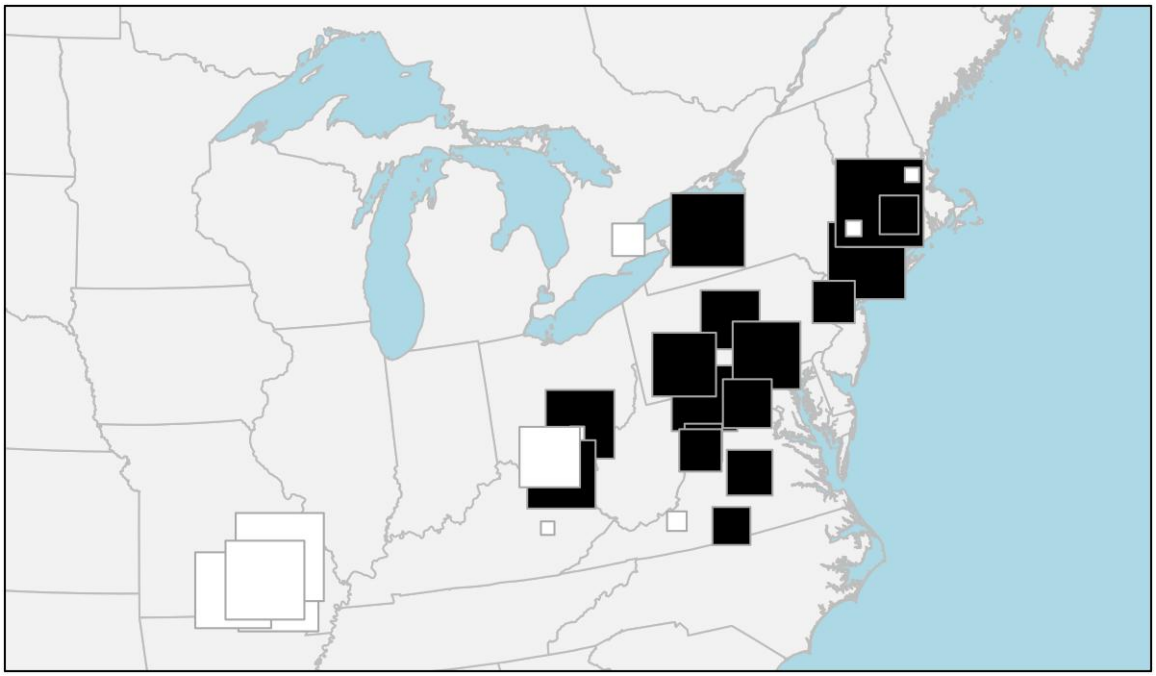

Figure 3: *Trichophorum planifolium* sPCA. Colour and size of points indicate the position of each population on sPCA axis 1: size indicates absolute distance from the origin, and colour indicates positive or negative values. i.e., large black squares have large negative values, large white values have large positive PCA values, with smaller squares indicating lower absolute values.

Table 2: Biogeographic Tests. Each line reports linear regression parameters F-statistic, adjusted R-squared value and P-value. Dependent variables: eMLG, rarefied richness of multi-locus genotypes; Fst, mean pairwise Fst values for each population. Independent variables: indexes of isolation, peripherality (see text), and habitat suitability from SDM.

	Dependent	Independent	F	R	p
2	eMLG ~	isolation	0.53	-0.02	0.47
3		marginality	0.23	-0.03	0.64
4		suitability	0.06	-0.04	0.82
5	Fst ~	isolation	0.47	-0.02	0.50
6		marginality	0.42	-0.02	0.52
7		suitability	0.54	-0.02	0.47

DISCUSSION

We found no evidence to support the hypothesis that genetic diversity declines with geographic peripherality, ecological marginality, or spatial isolation. However, the highly structured populations (F_ST = 0.8) and significant east-west spatial structure suggest that this lack of pattern is not due to 'swamping' gene flow from the core of the distribution homogenizing genetic diversity in peripheral populations. If that were the case, we would expect to see lower among-population variation (F_ST), and relatively higher within-population variation (F_IS).

This contrasts with other recent studies that have found significant relationships between genetic diversity and spatial or environmental gradients (Sexton et al., 2016; Lee-Yaw et al., 2018). Differences in life history and reproductive biology may explain the difference. Lee-Yaw et al. (2018) found that genetic diversity declined towards the geographic range limits of *Arabidopsis lyrata* ssp. *lyrata*, and also with distance from the species' environmental niche. In addition, their data shows that environmental conditions declined towards the range periphery. This supports the abundant center hypothesis (Brown, 1984).

On the other hand, Sexton et al. (2016) found no relationship between genetic diversity and spatial structure in *Mimulus laciniatus*. Instead, their work documented Isolation by Environment (Sexton et al., 2014), where genetic diversity is structured along environmental gradients, presumably a consequence of natural selection or non-random mating.

Neither pattern appears in our data. The most striking observation in our study is the extremely low variation present within populations (mean FIS 0.91, 1% heterozygosity), and high level of differentiation among populations (Fst = 0.80). *Trichophorum planifolium* is apparently a wind-pollinated, outcrossing species, but these values are higher even than typical for selfing species (i.e., the mean Fst for selfing species with similar life history traits is 0.4–0.57, following Hamrick and Godt, 1996; Nybom, 2004).

This supports Crins' (1989) hypothesis that the low stature of *T. planifolium*, and the complex terrain it occupies, limits outcrossing. Crins also noted that *T. planifolium* is protandrous, presumably an adaptation to lessen the frequency of self-pollination. However, Friedman and Barrett (2009) showed that the spatial and temporal separation of male and female flowers in woodland sedges (monecious *Carex*) are largely ineffective at preventing geitonogamy. They studied seven *Carex* species, many of which co-occur with *T. planifolium* in woodland habitats (pers. obs) and have similar caespitose habit. They found only one species had an outcrossing rate greater than 0.1 (three species had no polymorphic allozymes, which may reflect a long history of inbreeding).

Lacking any obvious adaptations for long-distance seed dispersal, gene flow via seed dispersal is likely similarly limited in *T. planifolium*. It is well documented that plant seeds often travel only one or a few metres (Cain et al., 2000). Seed dispersal in *T. planifolium* occurs in late July and August, during which time the plants' leaves become matted. Crins (1989) suggested that this would facilitate deposition of the seeds immediately adjacent to parent plants (i.e., seeds are gravity dispersed), resulting in small spatial clumps of closely related tussocks. Like self-pollination, mating among closely related individuals limits recombination and leads to increased homogeneity of genotypes in the population (Loveless and Hamrick, 1984).

In combination, these life history characteristics may explain the low population genetic diversity documented here as the outcome of founder effects, followed by population expansion dominated by selfing. This is consistent with a general pattern noted in other sedges (*Carex*), which can be divided into two life history categories (Ford et al., 1998). "Group 1" are caespitose, with multiple hermaphroditic culms on each plant, with flowers in close proximity. This facilitates geitonogamy, resulting in low intra-population diversity, but high inter-population diversity Kull and Oja (2007).

In contrast, "Group 2" *Carex* are rhizomatous, with more space between culms from the same individual, and more physical mixing among individuals. This leads to greater intra-population diversity, and less differentiation among populations (Ford et al., 1998).

Trichphorum planifolium fits the description of *Carex* Group 1, aside from having bisexual flowers. This likely further exacerbates inbreeding, as it may engage in both autogamous and geitenogamous selfing. The low levels of heterozygosity we observed (Ho 0.00–0.03, Table 1) are comparable to values reported for other

sedges: *Trichophorum caespitosum*: Ho = 0.01 to 0.04 (Godt et al., 1996), *Carex loliaceae*: Ho = 0.00 to 0.12 (Kull and Oja, 2007) and *Carex magellanica* subsp. *irrigua*: Ho = 0.00 to 0.03 (Kull and Oja, 2010).

The main geographic structure in the genetic data reveals a broad division between Missouri and the eastern populations (Figure 1). This suggests current *T. planifolium* populations may have emerged from glacial refugia both in the Appalachian Mountains and on the Ozark plateau (Delcourt and Delcourt, 1991; Meyer, 1997; Soltis et al., 2006). However, if this is the case, it is interesting to note that populations representing both eastern and western genetic groups appear in close proximity in Ohio. The sPCA analysis also indicates the Ontario population is genetically more similar to the Missouri populations than the geographically closer eastern group.

Gene flow via pollen or seed between Missouri in the west, and Ohio and Ontario to the east, is unlikely, given the long distances between these locations (> 500 km). However, the SDM indicates pockets of suitable environment exist along the Ohio river in southern Indiana and northern Kentucky (Figure 1). This may have served as a post-glacial colonization route linking populations from the two refugia; there may even be overlooked populations of *T. planifolium* in this area, as this species is easily missed.

While our data do not show a general relationship between spatial or environmental conditions and genetic diversity, the lone remaining Canadian population is among the least diverse (eMLG; Figure 2). We have argued above that population dynamics in this species are characterized by bottlenecks followed by inbreeding. This is often associated with a reduction in fitness (Angeloni et al., 2011). However, this cost may be reduced in self-pollinating species (Holtsford and Ellstrand, 1990; Busch, 2005), and can be further mitigated if the inbreeding leads to purging of deleterious alleles (Crnokrak and Barrett, 2002).

We cannot address this issue directly with our data. Microsatellites are neutral markers, they do not provide direct evidence of adaptive diversity; they only provide insight into gene flow and reproductive strategies. We can say that *T. planifolium* populations with low diversity are relatively common and widespread (e.g., CT, MA, OH, VA), and several of them are quite large and apparently stable (personal observation). Furthermore, this pattern of limited intra-population genetic diversity is common in other woodland sedges. From this, we conclude that inbreeding depression is not likely to represent a conservation concern in this case.

The Canadian population is also one of the most divergent (F_D; Figure 2). This provides indirect evidence that this population is adapted to site-specific environmental conditions, and indicates that it is of high conservation value (as per Lesica and Allendorf, 1995). Indeed, protection of many populations of *T. planifolium* would be necessary to conserve species-level genetic diversity as it is chiefly partitioned among, rather than within, populations Koelling et al. (2011). These results also suggest that risks of outbreeding depression are high with outcrossing, and future restorative efforts (i.e., augmentation) for the Canadian population should be avoided or approached with caution (Frankham et al., 2011). It should also be considered that mixing of populations with differing genetic backgrounds could decrease the conservation value of the Canadian population (Barrett and Kohn, 1991).

CONCLUSION

In *T. planifolium*, population genetic diversity is primarily a reflection of local processes. Its reproductive biology favours inbreeding; genetic diversity is paritioned almost entirely among populations, with very little within populations. This highlights the importance of considering the influence of life history in shaping broader biogeographic patterns as the study of range limits advances.

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AUTHOR CONTRIBUTIONS

VJN and TWS conceived the study. VJN designed the field sampling, and VJN and TWS completed the sample collection. VJN and SW completed the lab experiments. VJN completed the analysis, with direction and supervision from TWS. VJN completed the first draft of the manuscript. TWS provided additional analyses and completed the final draft of the manuscript.

Data Availability

This manuscript and all associated data is available at the GitHub repository, https://github.com/plantarum/trich for inspection during peer review. This repository will be transfered to Zenodo for permanent, public archiving after completion of the peer review process.

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