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Anthropogenic and natural disturbances increase local genetic diversity in an early spring geophyte (*Ficaria verna* Huds)

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Abstract

The tetraploid *Ficaria verna* is a common spring geophyte in central Europe and is considered invasive in the USA and Canada. It is considered an almost seed-sterile taxon, relying on vegetative reproduction by underground tubers and aerial bulbils. Recent studies have revealed high levels of population genetic diversity in *F. verna*, raising the question of how genetic diversity is maintained and which factors may be responsible for the observed patterns. Polymorphic nuclear microsatellite markers were established to define multilocus genotypes (MLGs), to analyze fine-scale spatial genetic structure (SGS) using grid and cross-sampling schemes, and to quantify genetic diversity within and between nine populations with different disturbance regimes in central Germany. In total, 115 MLGs were identified among a total of 347 samples. The G/N ratio varied between 0.16 and 0.70 among populations, and in each population several unique MLGs occurred. Genotypes were highly intermingled within populations, suggesting a "guerrilla" dispersal strategy. Significant SGS (negative regression slope of kinship coefficients against inter-individual distances) was found in four out of nine populations in fine-scale cross-sampling (up to 4 m) and in only one population in grid sampling (up to 14.6 m). No single MLG was found in more than one population, while many alleles were shared between populations. Within-population clonal and allelic diversity increased with greater exposure to both anthropogenic and natural disturbances. Regular gap openings, facilitated propagule establishment, and propagule dispersal by water and mowing machines are likely important factors explaining the positive effects of disturbance on local genetic diversity of *F. verna*.

KEYWORDS

disturbance, invasive species, nuclear microsatellites, reproductive strategy, spatial genetic structure

1 | INTRODUCTION

Genetic variation is considered an essential prerequisite for adaptation to changing environmental conditions and for the long-term persistence of any population or species (Hoffmann et al., 2017; Kardos et al., 2021; Lande & Shannon, 1996; Reed & Frankham, 2003). In plants, genetic diversity is affected by various endogenous and environmental factors (Ellegren & Galtier, 2016; Smith et al., 2020). Understanding which of these factors are most important and how they interact in a given species is crucial for predicting how species will respond to environmental change and for providing management guidelines for endangered and invasive species (e.g., Hughes et al., 2008; Martel et al., 2021; Segelbacher et al., 2022; Stange et al., 2021; Willi et al., 2022).

A primary determinant of how genetic diversity is structured in plants is the mode of reproduction (Hamrick & Godt, 1997; Loveless & Hamrick, 1984). Flowering plants exhibit a wide range of strategies to propagate, including both sexual and asexual reproduction. Asexual reproduction can take place either vegetatively, that is, by the formation of clonal reproductive units such as rhizomes, stolons, and bulbils, or by apomixis (the formation of seeds without fertilization) (Klimeš et al., 1997; Klimešová & Klimeš, 2008; Richards, 1997; Zona & Howard, 2022). Clonal propagation allows plants to rapidly increase population sizes through horizontal growth with low energy expenditure (Yang & Kim, 2016), which reduces the probability of extinction of clonal genotypes (Klimeš et al., 1997), but can lead to the development of monoclonal populations over long periods of time (Honnay & Bossuyt, 2005). At least in theory, clonal reproduction is also expected to lead to increased heterozygosity, assuming new mutations are maintained in a heterozygous state (Balloux et al., 2003; Ellegren & Galtier, 2016). However, mutations that reduce fertility can lead to sexual dysfunction and eventual loss of sex (Barrett, 2015). On the other hand, clonal populations may be highly vulnerable to harsh habitat disturbances and parasite and disease infestations due to a lack of genetic variability and/or adaptive potential (Lei, 2010). In addition, vegetative propagules such as bulbils tend to be less persistent than seeds, and long-distance dispersal is much less likely for most vegetative propagules as compared to seeds (Boedeltje et al., 2008; Yang & Kim, 2016; but see Ronsheim, 1994). Therefore, obligate vegetative propagation is considered disadvantageous and therefore rare in plants (Caetano-Anollés, 1999; Kondrashov, 1994; Silvertown, 2008). In contrast, sexual reproduction enables the production of genetically diverse offspring through recombination and admixture of genetic entities, which increases the adaptability of a population to its environment and thus provides a long-term evolutionary advantage (Yang & Kim, 2016). However, the production of flowers and seeds requires high energy and resource inputs, so the formation of generative structures is often limited by the availability of light, water, nutrients, and other site-specific conditions (Lei, 2010; Vandepitte et al., 2010; Wilk et al., 2009). To compensate for the disadvantages of each mode of reproduction, a high proportion of flowering plants exhibit both types of reproduction (Yang & Kim, 2016; Zhang & Zhang, 2007).

Clonality also has a strong effect on spatial genetic structure (SGS), because in clonal plants SGS results from the combined effects of pollen and seed dispersal and clonal growth. Consequently, clonality can lead either to substantial SGS, because the local production of clonal progeny can increase the extent to which plants are surrounded by genetically similar plants, or to more irregularly distributed genets when ramets are widely spread (Alberto et al., 2005; Arnaud-Haond et al., 2007; Holt et al., 2020; Loh et al., 2015; Vallejo-Marín et al., 2010).

Genetic diversity of a species at the population level is also affected by site-specific environmental conditions. In this context, disturbance is discussed as an important determinant of local genetic diversity (Almeida-Rocha et al., 2020; Banks et al., 2013; Eriksson, 1993). Disturbances can occur at very different scales and are generally accepted as a major driver of biodiversity at both species and population levels (Banks et al., 2013; Davies et al., 2016). The way in which disturbances affect genetic diversity is subject to several interacting effects. Depending on their type, intensity, frequency, and duration, disturbances can have very different effects on the genetic diversity of a species or population (Almeida-Rocha et al., 2020; Rusterholz et al., 2009, 2021; Silvertown, 2008). Less intense, regular, and locally limited disturbances often lead to an increase in genetic diversity (Banks et al., 2013), as they may leave belowground regenerative organs intact and thus allow resprouting (Klimešová et al., 2016; Klimešová & Herben, 2015), and gaps created by disturbance events allow for seedling establishment (Bullock, 2000; Clark et al., 2007). On the other hand, severe disturbances usually lead to the loss of genetic diversity or even to the extinction of entire populations (Banks et al., 2013), and populations reestablish by dispersing through seeds to disturbed habitats, either in time or in space (Klimešová et al., 2016). When considering SGS, rates of recruitment following disturbances and the presence of habitat-specific dispersal vectors are expected to have significant effects on SGS patterns (Duffy et al., 2020; Holt et al., 2020; Loh et al., 2015; Vekemans & Hardy, 2004).

Here, we investigated the site-specific local diversity and SGS in an abundant spring geophyte of central Europe, the lesser cendaline (*Ficaria verna* Huds.).

The species grows in patch-like populations throughout central Europe (Jalas & Suominen, 1989). Although it generally prefers moist conditions, it is found in a wide range of habitats, including forest understory, pastures and meadows, along streams, and in variously disturbed urban habitats (Hegi, 1912; Oberdorfer, 2001; Taylor & Markham, 1978; Zonneveld, 2015). The infrageneric systematics of the genus *Ficaria* is still under debate (Sell, 1994; Veldkamp, 2015; Zonneveld, 2015). Throughout most of central Europe only the tetraploid, bulbil-forming lineage is found, which should be correctly named *Ficaria verna* Huds. subsp. *verna* (hereafter *F. verna*), formerly also *F. verna* subsp. *bulbilifera* Á. Löve & D. Löve (Veldkamp, 2015). Due to its invasive behavior, especially in the northern USA and southern Canada, the species has received considerable attention also outside its natural range (Axtell et al., 2010; Kermack & Rauschert, 2019; Masters & Emery, 2015; Mattingly et al., 2023; Post et al., 2009).

Ficaria verna is capable of both sexual and asexual reproduction. Although the species flowers regularly, few viable seeds are produced (Kocot et al., 2022; Metcalfe, 1939). No evidence for the production of viable seeds by autonomous apomixis has been found in *F. verna* (Popelka, Trávníček, et al., 2019; but see Metcalfe, 1939). Vegetative reproduction by dispersal of bulbils and root tubers has been postulated as the dominant mode of propagation in central Europe (Mudrack, 1934; Verheyen & Hermy, 2004). Mudrack (1934) even postulated that *F. verna* only produces seeds in open habitats, whereas in shaded sites the species is exclusively clonal. Jung et al. (2008) observed that intermediate levels of disturbance favor sexual reproduction in *F. verna*. Bulbils are mostly dispersed by rainwater (Mudrack, 1934), but rare cases of endozoochoric dispersal have been reported (Heinken et al., 2013; Taylor & Markham, 1978). Seeds are locally dispersed by ants (Jung et al., 2008).

Despite a predominantly vegetative reproduction, recent genetic studies of *F. verna* have revealed unexpectedly high levels of genetic variability (Mattingly et al., 2023; Popelka, Sochor, & Duchoslav, 2019; Reisch & Scheitler, 2009), raising the question of how clonal diversity is maintained in this species. We established polymorphic nuclear simple sequence repeat (ncSSR) markers for *F. verna* to define multi-locus genotypes (MLGs), to visualize the clonal architecture of individual sites, to estimate SGS, and to quantify clonal and allelic diversity within sites with different disturbance regimes in central Germany. This allowed us to (1) gain insight into the reproductive strategy of *F. verna* and (2) assess how site-specific disturbances affect the local genetic diversity of the species.

2 | MATERIALS AND METHODS

2.1 | Development of nuclear microsatellite markers

Whole-genome shotgun sequences (Illumina HiSeq) of a single tetraploid *F. verna* individual from the Czech Republic (locality details: distr. Prostějov, village of Plumlov, mesic grassland in the valley of the Kleštínek stream, 1.2 km south-south-east of the Plumlov castle; 290 m.a.s.l.; 49°27′16″ N, 17°01′19″ E), was kindly generated by Dr. B. Hüttel (Max Planck Genome Center, Cologne). In order to generate locus-specific primers for ncSSR loci, all sequences were merged and assembled into contigs using Geneious R10.0.5. The resulting contigs were filtered for microsatellite motifs using the Phobos 3.3.11 plug-in (Mayer, Christoph, 2006–2010, http://www.rub.de/ecoevo/cm/cm_phobos.htm) and manually checked for sufficiently long flanking regions. Subsequently, microsatellite containing fragments were blasted against the whole set of Illumina sequences to eliminate loci that obviously occurred as multi-copy regions in the genome. For selected SSR-containing fragments, primer sequences were generated using Primer3 (Rozen & Skaletsky, 2000). Out of a total of 20 primer pairs tested, nine gave scorable PCR products (Table 1). All primer sequences are available at GenBank (accession numbers OR147801 to OR147809). Illumina sequences are available on request from the first author.

2.2 | Population genetic analysis in *F. verna*

2.2.1 | Sampling strategy

Samples were collected in April/May 2019 at nine sites near Kassel (Northern Hesse, Germany) (Table 2). The sampling sites differed in terms of disturbance regime. Anthropogenic disturbance was defined as low at sites that are located away from roads/trails and/or are situated in the understory of forests, where human access is unlikely (R, S, G, V). Sites that were located less than 5 m from highly frequented trails, but obviously not regularly visited by humans, were defined as having moderate anthropogenic disturbance (H, K, A). High anthropogenic disturbance was assumed for two sites located next to a highly frequented path in a park (P) and in a playground (B) and prone to regular trampling by people and dogs. Mowing takes place at sites K, P, and B, all of which are located in well-accessible open public areas. As some of our sampling sites are located along streams, we further differentiated sites according to their exposure to

TABLE 1 Locus characteristics of nuclear microsatellite (ncSSR) markers for *Ficaria verna*.

Locus	Primer sequences (5′–3′)	Repeat motif	No. of alleles	Size range (bp)	Missing data	GenBank accession No.
Fv65054[a]	GCCTCAACCTTAGCCAACTG AGGAGCGACATTGAATCTGG	$(TCT)_{16}$	10	140–167	0/22	OR147801
Fv76978[a]	GCTCCAACTAATCAGCAAGAC GAAACAAACACTTCGAATCGC	$(TTC)_{13}$	10	150–186	0/22	OR147802
Fv51356	ACCTACTTTCCTTTTCACCCC TCATGCTACTATTGTGGTCAC	$(CTA)_{23}$	16	175–250	0/22	OR147803
Fv66758	TCAACCAAATATGAGCCGATTG ACGATGACTTATGCACTGGG	$(ATC)_{16}$	14	125–179	0/22	OR147804
Fv33625[a]	GCGACAACCATTTTCCAGTC TGTTCAGCCACTCATACCAC	$(GGA)_{16}$	13	188–248	1/22	OR147805
Fv39461	TGGGCTTAGATTTACTACTTG CTCGTTCTGCTTCTTCTTGC	$(CTT)_{19}$	8	131–158	3/22	OR147806
Fv95546[a]	GGCTACATCCCTTTATCCAATTC TGTGTGTCCGAGATGTGTTC	$(GAA)_{16}$	7	243–267	4/22	OR147807
Fv67949[a]	CCATCTACCTTAAAACTTGACCC GCTAGAACGAAGAGTGTTGC	$(TTC)_{26}$	19	159–237	0/22	OR147808
Fv7750[a]	CGCCTTGGAAGTTAAGCATC ACGTCACAATCCCCGATAAAG	$(TTC)_{20}$	14	343–403	4/22	OR147809

Note: Number of alleles, allele size ranges, and the number of individuals with missing data were calculated for population B (Bremelbach, $n = 22$), which was the genetically most diverse population.
[a]Loci used for genotyping in this study.

potential flooding, for example, after heavy rainfall, as an indicator of natural disturbance (Table 2).

To assess site-specific genetic diversity and SGS, a total of 35–42 samples were collected at each site, using two different sampling schemes: grid and cross-like. For grid sampling, up to 24 samples were collected in a grid pattern (plots of 4 × 7 m), with each sample 2 m apart (Figures 1 and S1, left). For the cross-like scheme, we followed the strategy of Reisch and Scheitler (2009). Starting from the center, samples were collected at distances of 5, 25, 50, 100, and 200 cm in each direction, resulting in up to 21 samples per cross (Figures 1 and S1, right). At each collection point, the ramet closest to the midpoint was taken. Where there was no ramet less than 3 cm from the midpoint, no sample was taken (indicated by a cross in Figures 1 and S1). Voucher specimens of each one to three individuals per site were deposited in the Herbarium of the University of Kassel.

2.3 | Laboratory protocols for SSR genotyping

For population genetic analyses, a total of 347 samples were genotyped at six of the nine newly established ncSSR loci. DNA was isolated following standard CTAB protocols (Weising et al., 2005). For amplification of the six target loci, each locus was amplified by three-primer PCR employing a fluorophore-tagged M13-primer (Schuelke, 2000), to allow detection of fragments on an automated sequencer (ABI Prism® 310, Applied Biosystems). As fluorophores, 6-FAM, TAMRA, and ROX were used. PCR amplifications were set up in final volumes of 10 µL, containing 1× PCR buffer (Bioline), dNTP mix (0.2 mM each, ROTH), primer concentrations of 0.4 µM and 0.1 µM according to Schuelke (2000), 0.2 µg/µL BSA (ThermoScientific), and 0.025 U/µL Taq DNA Polymerase (Bioline). After initial denaturation (94°C, 5 min), samples were submitted to 35 cycles of a touchdown PCR protocol consisting of 45 s at 94°C (denaturation), 30 s at 65–54°C (annealing), and 45 s at 72°C (elongation) using a Biometra thermocycler. The initial annealing temperature of 65°C was decreased by 1°C per cycle until 54°C was reached. PCR products were diluted with 40 volumes of water. For analysis on the ABI 310 sequencer, PCR products were pooled into two groups of each three loci (Fv65054, Fv33625, Fv7750 and Fv76978, Fv95546, Fv67949), using 1 µL of each diluted PCR product in 10 µL formamide. As a size standard, GeneScan™ 500 LIZ orange (ThermoFisher) was used. Evaluation of the data was performed with the software GeneScan ver. 3.7

TABLE 2 Characteristics of the nine study populations, including information on the disturbance regime, sample size, and parameters describing the population-specific genetic diversity (allelic diversity: average number of alleles per locus [x ± s = mean ± standard deviation averaged over loci], clonal diversity: number of multi-locus genotypes [MLGs], Shannon and Simpson index and genets per ramets [G/N] ratio).

Site (population)	Anthropogenic disturbance	Flooding	Sample size (grid/total)	No. of alleles (x ± s)		No. of MLGs (grid/total)	Shannon (H′) (grid/total)	Simpson (D) (grid/total)	G/N (grid/total)
				(grid)	(total)				
(R) Rinnbachtal	Low	No	23/38	5.83 ± 2.14	6.67 ± 2.50	4/6	0.53/0.68	0.75/0.71	0.173/0.158
(S) Hessenschanze	Low	No	22/35	7.00 ± 2.97	7.00 ± 2.97	7/7	1.51/1.26	0.25/0.36	0.318/0.200
(G) Geilebach	Low	Yes	23/41	9.00 ± 2.28	10.83 ± 3.31	10/13	1.78/1.89	0.22/0.22	0.435/0.317
(V) Espenau	Low	Yes	24/40	7.12 ± 0.98	7.67 ± 1.63	10/13	2.00/1.96	0.13/0.19	0.417/0.325
(H) Harleshausen	Intermediate	No	24/40	7.50 ± 2.07	8.17 ± 2.14	7/8	1.19/0.58	0.38/0.76	0.292/0.200
(K) Karlsaue	Intermediate	No	21/35	7.67 ± 2.34	8.50 ± 2.81	6/8	1.15/1.14	0.44/0.51	0.286/0.229
(A) Ahnetal	Intermediate	Yes	24/41	10.17 ± 2.04	11.00 ± 2.00	11/17	1.85/1.89	0.22/0.29	0.458/0.415
(P) Ahnepark	High	No	23/39	8.50 ± 2.17	9.50 ± 2.66	15/20	2.51/2.60	0.06/0.08	0.652/0.513
(B) Bremelbach	High	No	23/38	12.17 ± 4.17	15.33 ± 5.61	16/23	2.79/2.91	0.03/0.04	0.696/0.605

Note: All values are provided for grid samples only (grid) and grid plus cross-scheme samples (total).

(Applied Biosystems). Final determination of fragment lengths was made by eye.

2.4 | Statistical data analysis

Parameters of genetic diversity were estimated with R and RStudio (R Core Team, 2022; RStudio Team, 2020), using the R package polysat (Clark & Jasieniuk, 2011), specifically adapted for the analysis of nuclear microsatellite data in polyploids. Due to allele dosage and scoring ambiguities, assignment of genotypes in polyploids is not as straightforward as in diploids. Because allele dosage in heterozygous polyploids cannot be inferred reliably from traditional ncSSR data (Dufresne et al., 2014), we treated allele data as dominant markers during analysis (presence/absence of alleles). MLGs were defined based on a matrix of pairwise distances between all sampled individuals using the Lynch.distance function. The Lynch distance is a simple measure of dissimilarity based only on band sharing and does not take into account allele lengths or putative mutation rates between alleles (Lynch, 1990). In *Ficaria*, the allele lengths of nuclear microsatellite loci were found to be highly variable, spanning considerable size ranges (Table 1). We therefore refrained from any speculation on underlying mutation patterns or rates and did not use measurements that take allele size into account (e.g., Bruvo distance). In order not to overestimate possible scoring errors that might have occurred due to the generally very high variability of the loci and the tetraploid nature of the species, we chose a comparatively high (and thus conservative) threshold of 0.25 (Figure S2) to define two allele patterns as different MLGs.

Allelic diversity of individual sites (populations) was estimated as the number of alleles averaged over loci, clonal diversity was characterized as the number of MLGs per site, the Shannon index (H′) and Simpson index (D), as made available through the R package polysat, and by providing the G/N ratio (G = genets, defined here as unique MLGs, and N = ramets, total number of ramets sampled) (Table 2). For each population, diversity values were calculated firstly for grid samples only, and secondly for all samples from each site (grid plus cross-transect samples).

To test for the presence and strength of SGS within individual sites, we performed spatial autocorrelation analysis using Spagedi 1.5d (Hardy & Vekemans, 2002). Pairwise average multi-locus kinship coefficients (Loiselle et al., 1995) were computed and averaged within six distance classes for both cross-sampling (max. distances of 0.36, 0.56, 1.1, 1.5, 2.1, and 4.0 m) and grid sampling (max. distances of 2.0, 4.0, 5.7, 7.3,

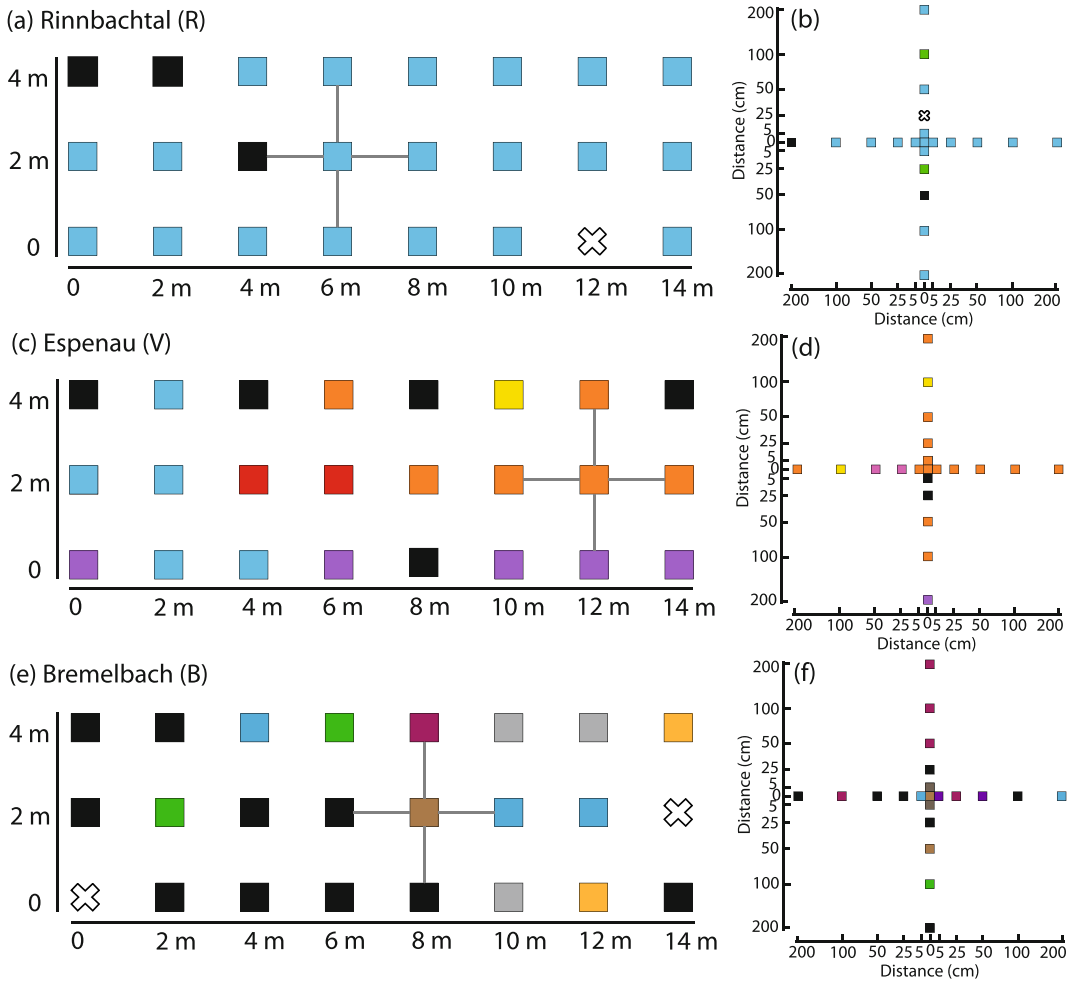

FIGURE 1 Distribution of multi-locus genotypes (MLGs) in three sites of *Ficaria verna* in central Germany (a, c, e: grid sampling, b, d, f: cross-scheme sampling). The position of the cross-scheme sampling within grids is indicated by lines in (a, c, e). Each color represents one specific MLG, black squares represent unique MLGs that were detected in a single ramet only, white crosses indicate positions where no sample was taken. Note that no MLGs were shared between populations; identical colors in different populations therefore do not code for identical MLGs. Data are provided for three out of nine study populations: Rinnbachtal (R, low anthropogenic disturbance, no flooding; a, b), Espenau (V, intermediate anthropogenic disturbance, flooding possible; c, d), and Bremelbach (B, high anthropogenic disturbance, no flooding; e, f). See Figure S1 for data of all nine study populations.

10.0, and 14.6 m). Intervals were chosen so that all distance classes had a minimum of 30 pairs of individuals. For the observed kinship coefficients, 95% confidence intervals were obtained from 5000 permutations. Additionally, kinship coefficients were regressed on the linear distance in order to obtain estimates of the regression slope b. Significantly negative b, as tested by 5000 permutations, was interpreted as the presence of SGS (increasing genetic dissimilarity with increasing distance, Table 3).

Principal coordinates analysis based on inter-individual Lynch distances, as implemented in polysat, was used to visualize population differentiation, using ggplot2 (Wickham, 2016) for plotting.

3 | RESULTS

3.1 | Establishment of ncSSR loci

Nine ncSSR loci yielded scorable PCR products. These loci were characterized using 22 grid samples from site B, which was the most genetically diverse population analyzed. The number of alleles per locus ranged from 7 to 19 in this population with sizes of up to 75 bp (Table 1). The comparatively high levels of missing data (especially at loci Fv39461, Fv95546, and Fv7750) suggest that null alleles or uneven allele amplification may have affected the PCR results. The high level of genetic variability at ncSSR loci in

TABLE 3 Tests for the presence of fine-scale spatial genetic structure (SGS) in nine *Ficaria verna* populations, as determined by the significant slope of the linear regression line (b lin) and by determining the significance of distance class-specific Loiselle coefficients.

Site (population)	b lin	Significance (*p*-value)	Interpretation	Significant Loiselle coefficients (Lc) per distance class (*p*-value)
(a) Cross-sampling				
R	−0.0051	0.443	No relationship between genetic similarity and spatial distance	None
S	−0.0231	**0.016**	**Increasing genetic dissimilarity with increasing distance**	Distance class 6 (Lc lower than expected, **0.033**)
G	−0.0183	0.053	No relationship between genetic similarity and spatial distance	Distance class 2 (Lc lower than expected, **0.042**)
V	−0.0003	0.822	No relationship between genetic similarity and spatial distance	Distance class 3 (Lc lower than expected, **0.042**)
H	0.0044	0.395	No relationship between genetic similarity and spatial distance	Distance class 1 (Lc lower, **0.0006**), 2 (Lc higher than expected, **0.047**)
K	−0.0298	**0.039**	**Increasing genetic dissimilarity with increasing distance**	None
A	−0.0107	0.161	No relationship between genetic similarity and spatial distance	None
P	−0.0163	**0.027**	**Increasing genetic dissimilarity with increasing distance**	Distance class 1 (Lc higher than expected, **0.001**)
B	−0.0096	**0.033**	**Increasing genetic dissimilarity with increasing distance**	Distance class 2 (Lc higher than expected, **0.016**)
(b) Grid sampling				
R	−0.0006	0.543	No relationship between genetic similarity and spatial distance	Distance class 3 (Lc lower than expected, **0.014**)
S	−0.0061	0.161	No relationship between genetic similarity and spatial distance	Distance class 5 (Lc lower than expected, **0.032**)
G	0.0016	0.668	No relationship between genetic similarity and spatial distance	None
V	−0.0064	**0.044**	**Increasing genetic dissimilarity with increasing distance**	Distance class 1 (Lc higher than expected, **0.032**)
H	−0.0028	0.145	No relationship between genetic similarity and spatial distance	None
K	0.0031	0.434	No relationship between genetic similarity and spatial distance	None
A	0.0008	0.866	No relationship between genetic similarity and spatial distance	None
P	−0.0000	0.851	No relationship between genetic similarity and spatial distance	None
B	−0.0015	0.397	No relationship between genetic similarity and spatial distance	None

Note: All *p*-values were obtained by two-sided permutation tests, *p*-values of <0.05 are shown in bold. Data are provided separately for (a) cross-sampling and (b) grid sampling. Abbreviations for sites as in Table 2.

F. verna suggests that mutations in the flanking region and at primer binding sites may be a problem in this species. This could limit the applicability of the markers, especially in studies that consider allele frequencies.

3.2 | Clonal architecture of individual *F. verna* sites

Based on the above parameter settings (Lynch distance, threshold 0.25), 115 MLGs were identified from a total of

347 samples. At least six different MLGs were found in each population (four when considering only the grid samples). At the most variable site (B) a total of 23 MLGs were identified across 38 sampled ramets. While some sites (R, S, H) were dominated by only one or two MLGs, at other sites several MLGs occurred at comparatively similar frequencies (V, P, B) (Figures 1 and S1).

The G/N ratio varied from 0.17 and 0.16 at the least variable site (R) to 0.70 and 0.61 at the most variable site (B) for the grid and total samples, respectively (Table 2).

The spatial distribution of MLGs in grids and crosses is shown in Figures 1 and S1. Regardless of the total number of MLGs present at each site, a considerable number of unique MLGs, that were represented by only a single ramet, occurred in each plot (ranging from four unique MLGs at sites R and S up to 16 and 15 unique MLGs at the most diverse sites, P and B).

3.3 | Fine-scale SGS

Analysis of fine-scale SGS revealed significantly increasing dissimilarity with increasing distance for four of nine populations (S, K, P, B) in the cross-sampling scheme (max. distances of 4 m), but only for one population (V) in the grid sampling scheme (max. distances of 14.6 m) (Table 3, Figure S3). Significantly positive or negative Loiselle coefficients within distance classes did not follow any obvious pattern, nor did they show any correlation with the presence/absence of general SGS (Table 3), so we refrain from interpreting these values as meaningful in the context of our study. Furthermore, the results of the SGS analysis did not reveal any correlation between the presence/absence of fine-scale SGS and the type or intensity of site-specific disturbance regimes.

3.4 | Within-site genetic diversity of *F. verna* and its relationship to disturbance regimes

Genetic diversity within sites can be quantified using either allele or genotype frequencies. A total of 111 alleles were found at six loci at the nine sites of *F. verna*, 32 of which occurred only at a single site (private alleles). Only at sites P and R no private alleles were found. However, most private alleles occurred at very low frequencies (<0.10). Only four private alleles occurred with frequencies of >0.20 at the respective sites (site A: allele 234/locus Fv95546 with frequency 0.212, site G: allele 349/locus Fv7750 with frequency 0.421, site K: allele 171/locus Fv67949 and allele 406/locus Fv7750 with frequencies 0.333 and 0.342, respectively). Average allele numbers across loci ranged from 5.8 in population R to 12.2 in population B for grid samples and from 6.7 to 15.3 for grid plus cross-transect samples (Table 2). The number of MLGs was lowest at site R and highest at site B, as reflected in the widely varying estimates of Shannon and Simpson indices (Table 2). When comparing the within-site genetic diversity of the nine sites, it becomes clear that genetic diversity increases with greater exposure to disturbance. Both anthropogenic and natural disturbances (as exemplified here by temporary flooding) correlate with levels of clonal and allelic diversity in *F. verna*. Sites with low to moderate levels of anthropogenic disturbance and no exposure to temporary flooding (R, S, H, K) showed the lowest levels of genetic diversity, whereas sites with low or moderate levels of anthropogenic disturbance and exposure to flooding (G, V, A) generally showed higher levels of genetic diversity (Table 2, Figure 2). By far the highest estimates of genotype diversity were found at sites with extremely high levels of anthropogenic disturbance (P, B) (both sites are not exposed to flooding).

3.5 | Differentiation of sites

Differentiation between sites was at its maximum when referring to MLGs. All MLGs were site-specific, that is, no single genotype was found at more than one site, despite geographical distances of less than 9 km between sites. In contrast, the distribution of alleles between sites was rather homogeneous, with many alleles being shared between sites. Accordingly, principal coordinate analysis based on Lynch distances revealed no clear differentiation between sites (Figure 3).

4 | DISCUSSION

4.1 | Reproductive strategy of *F. verna* in central Germany

Despite sampling at a very local scale, we found high levels of allelic and clonal diversity in *F. verna*, as evidenced by high allele numbers, large ranges of allele lengths, and generally high numbers of MLGs at individual sampling sites (Table 2). Our data thus support previous observations that the species is genetically diverse (Mattingly et al., 2023; Popelka, Sochor, & Duchoslav, 2019; Reisch & Scheitler, 2009) and provide evidence that *F. verna* is capable of maintaining high levels of genetic diversity despite its predominantly clonal growth (Kocot et al., 2022; Popelka, Trávníček, et al., 2019; Verheyen & Hermy, 2004). *Ficaria verna* has several characteristics

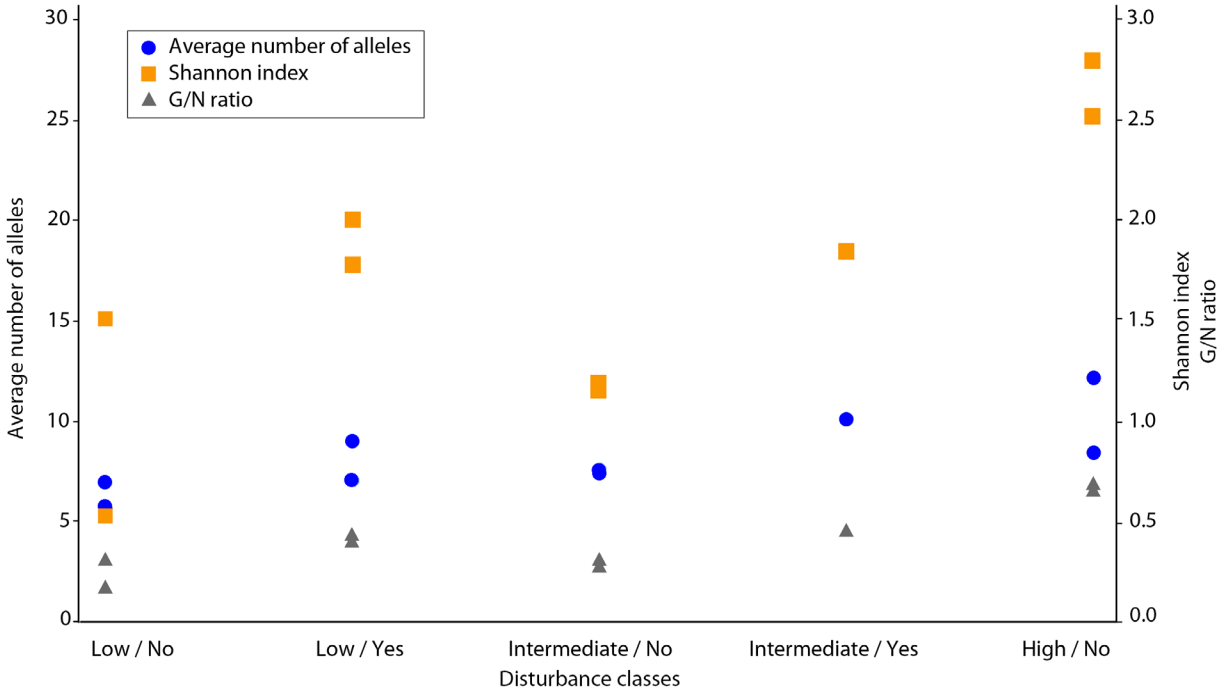

FIGURE 2 Illustration of the positive effect of disturbances on allelic and clonal diversity in *Ficaria verna*. Five disturbance classes are defined based on the occurrence of anthropogenic disturbance (low, intermediate, high) and the occurrence of flooding (no, yes). Allelic diversity is exemplified by the average number of alleles (blue dots), clonal diversity is illustrated by values of the Shannon index (orange squares) and the G/N ratio (gray triangles). Diversity parameters generally increase with increasing anthropogenic disturbance and are higher at sites with additional exposure to flooding as compared to sites without flooding.

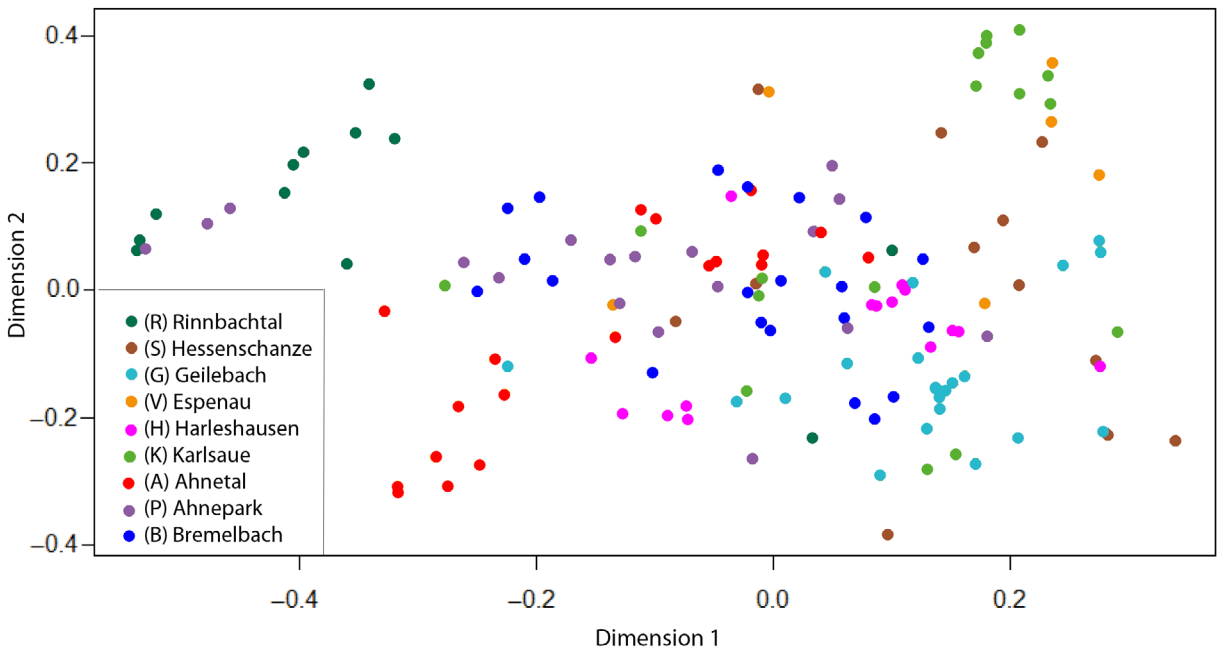

FIGURE 3 Principal coordinates analysis of nine populations of *Ficaria verna* in central Germany, based on Lynch distances. Each color represents one population.

that are beneficial for maintaining genetic diversity. The species is a very abundant spring geophyte and grows in large populations (Taylor & Markham, 1978; Verheyen & Hermy, 2004), which reduces allele loss due to genetic drift (Ellegren & Galtier, 2016). In a certain way, clonality itself has a positive effect on allele maintenance, because

large clone sizes reduce the risk of alleles being lost through random processes (Eriksson, 1993). Similarly, polyploids are considered to be less prone to the effects of genetic drift due to the higher number of effective alleles per individual and population (Meirmans et al., 2018; Moody et al., 1993). However, whether genetic diversity in *F. verna* is mainly maintained by the long-term persistence of existing variation or whether sexual reproduction occurs more frequently than generally assumed remains to be discussed. The observed clonal architecture of individual *F. verna* sites (Figures 1 and S1) corresponds well to that predicted for species that reproduce predominantly through secondarily dispersed structures such as bulbils (i.e., the "guerilla strategy," see Barrett, 2015; Vallejo-Marín et al., 2010). Intermingling of different genets was observed regardless of how many MLGs dominated a site (Figures 1 and S1). Analysis of fine-scale SGS showed that SGS was present in about half of the populations at distances less than 4 m (as examined by cross-sampling), while SGS was absent at greater distances. The only exception was found at site V, where two of the more frequent MLGs in the grid sampling (orange and blue in Figure 1) occurred less intermingled than MLGs at other sites, resulting in the significantly positive Loiselle coefficient in the first distance class and the presence of SGS (Table 3). Fine-scale genetic structure and clonal aggregation at very small distances are likely to result from reproduction via clonal propagules produced close to the parent plant and not easily dispersed, as is the case with root tubers (Herben & Klimešová, 2020; Vallejo-Marín et al., 2010), the second most important propagule for vegetative reproduction in *F. verna* (Grime et al., 1988). Similarly, bulbils (Klimešová et al., 2017), when formed in the leaf axils of lower stem leaves (Popelka, Trávníček, et al., 2019), may only be very locally dispersed. However, the comparatively rare and weak pattern of SGS suggests that the spread of vegetative units in *F. verna* is generally effective already in the range of a few meters, as exemplified by grid sampling in this study.

The occurrence of several unique MLGs at all study sites further suggests that successful recruitment of genetically variant propagules occurs on a rather regular basis, even in established *F. verna* populations. At least at some sites, unique MLGs carried several alleles that were not otherwise found in the site-dominating MLGs, suggesting that unique MLGs were not exclusively derived from sexual reproduction of nearby parental plants, but were likely descendants (either vegetatively or generatively formed) of plants outside the study sites. The comparatively loose growth pattern of *F. verna* (Taylor & Markham, 1978), which leaves bare patches of soil between individual plants, is likely to facilitate the establishment of propagules in this species. *Ficaria verna* thus appears to maintain within-site clonal diversity mainly through continuous propagule establishment, even in well-established populations, as also suggested by Reisch and Scheitler (2009). A similar strategy has been reported for other clonal species (e.g., *Saxifraga cernua*, Gabrielsen & Brochmann, 1998; *Globba racemosa*, Zhou et al., 2008; *Allium oleraceum*, Duchoslav & Staňková, 2015; *Aechmea nudicaulis*, Loh et al., 2015), and has led to the conclusion that clonal species are able to maintain high levels of genetic diversity even when sexual reproduction is rare, as long as recruitment of genetically variant individuals within established clones occurs continuously (Honnay & Jacquemyn, 2008; Loh et al., 2015; Vallejo-Marín et al., 2010; Widén et al., 1994).

In many clonal plant species, the ratio of vegetative to sexual reproduction depends on site-specific conditions. Under suboptimal conditions, many forest understory plants do not reproduce sexually (Kanno & Seiwa, 2004; Kawano et al., 1990), and this has also been postulated for *F. verna* (Kermack & Rauschert, 2019; Mudrack, 1934). For sexual reproduction to occur in clonal plants, the availability of light, water, and nutrients is considered to be one of the most important factors (Lei, 2010). In addition, intra-specific competition, which is more likely to occur at high densities (Morschhauser et al., 2009), may reduce sexual reproduction. This may explain why ramets at the periphery of clones sometimes produce more flowers than those in the center (Vallejo-Marín et al., 2010). Our own observations on *F. verna* are consistent with these assumptions. There was considerable variation in flower density between sites. Plants in forested sites flowered less frequently than plants growing in open habitats such as meadows or urban parks. Consequently, the relative contribution of sexually and asexually produced propagules to the next generation and the extent to which genetic admixture between existing MLGs occurs may vary considerably between sites in *F. verna* (Mattingly et al., 2023).

An important finding of our study was that differentiation between sites was maximal in terms of MLGs (i.e., despite geographical proximity, no single MLG was found at more than one sampling site), while populations were only moderately differentiated in terms of allelic diversity (Figure 3). While clonal MLGs are exclusively dispersed by vegetative organs, alleles are transported by both sexual (pollen and seeds) and asexual propagules. We conclude that in *F. verna* gene flow at the regional scale occurs by pollen and/or seed dispersal, whereas dispersal of vegetative organs is restricted to the local scale. This is consistent with observations in other species (Alvarez et al., 2005; Eriksson, 1993) and with the observation that species with low sexual reproduction show greater overall population differentiation than species

with higher rates of sexual reproduction (Vandepitte et al., 2010; Wilk et al., 2009).

We conclude that short-distance dispersal of both generative (where available) and vegetative propagules is responsible for maintaining clonal diversity and mixing of MLGs at the local scale in *F. verna*, whereas dispersal of sexually produced pollen and/or seeds, even if infrequently produced (Kocot et al., 2022; Marsden-Jones & Turrill, 1952; Popelka, Trávníček, et al., 2019), is sufficient to ensure genetic admixture at the regional scale. Due to the generally high levels of local genetic diversity and the presence of unique MLGs at all sites of *F. verna*, we assume that seedling recruitment is at least occasional at all study sites, although the extent to which sexually produced propagules establish is likely to vary between sites. Due to large population sizes, clonal growth, and polyploidy, allele loss through genetic drift is likely to be of low importance (Ellegren & Galtier, 2016), so once alleles are established in a population, they have a good chance of persisting. Given that seed set is likely to be more efficient at sites with favorable environmental conditions, such sites may serve as a source of genetic diversity, while less favorable sites are likely to serve as a sink of genetic diversity.

4.2 | Effect of disturbance

We found clear evidence that disturbance increases allelic and clonal diversity in central German *F. verna* populations. However, while both sites exposed to temporary flooding and sites with high levels of anthropogenic disturbance exhibited much higher levels of genetic diversity than less disturbed sites (Table 2, Figure 2), the way in which disturbance affects local genetic diversity in *F. verna* is less clear.

Empirical evidence on how human disturbances affect local genetic diversity in plants varies between studies, reflecting a general trade-off between processes that reduce genetic diversity (e.g., by losing alleles through shoot extinction) and processes that increase genetic diversity. The latter can occur in two non-exclusive ways. Firstly, mechanical destruction of individual shoots as a result of disturbance (e.g., human trampling, mowing, grazing) can open gaps in clonal patches and thus pave the way for the establishment of genetically variant propagules (Reusch, 2006; Rusterholz et al., 2009), and secondly, disturbance can increase dispersal of propagules and hence gene flow within and between populations (Strykstra et al., 1997). For *Anemone nemorosa*, Rusterholz et al. (2009) found that human trampling led to increased mortality of individual shoots, reduced sexual potential, and thus an overall loss of genetic diversity. In contrast, Reusch (2006) showed that disturbance of a clonal marine species, *Zostera marina*, increased local genetic diversity, probably by facilitating seedling recruitment into gaps. When mowing is the main source of disturbance, timing and frequency of the intervention plays an important role and hence mowing may or may not affect genetic diversity (Billeter et al., 2002; Nakahama et al., 2016). Regular mowing can lead to reduced or even complete failure of fruit and seed set under conditions where sexual organs are cut off before maturity (Nakahama et al., 2016). However, if seeds or vegetative propagules are transported by mowing machines, mowing can increase gene flow within and between sites (Lehmair et al., 2020; Strykstra et al., 1997) and decrease SGS.

Our data showed that at all sites, even those where a single MLG was dominant, several shoots with unique MLGs occurred. This indicates that the establishment of genetically variant plants is continuous, even years after the establishment of a population. We conclude that the positive effect of human disturbance on the local genetic diversity of *F. verna* is most likely due to gap-opening processes and facilitated propagule establishment. Negative effects of human disturbance, such as mowing, on local genetic diversity are likely to be negligible due to the early flowering and fruiting time (Popelka, Trávníček, et al., 2019) and low height of the species. The two most genetically diverse sites in our study were highly frequented and regularly mowed urban sites (B, P), suggesting that mowing does not negatively affect genetic diversity, although the only other regularly mowed site (K) showed comparatively low levels of clonal diversity. Similarly, Reisch and Scheitler (2009) reported that *F. verna* populations in regularly mown meadows were generally more genetically diverse than those at forested sites. They concluded that mowing has a positive effect on the local genetic diversity of *F. verna*, probably through enhanced dispersal of bulbils. On the basis of our data, we suggest that mechanical transport of vegetative organs occurs only over short distances and mostly within populations.

Besides a positive effect of human disturbance on the local genetic diversity of *F. verna*, we found that sites located near to streams and temporarily flooded had higher clonal and allelic diversity. The positive effect of water on the genetic diversity of *F. verna* may be due to various effects. The increased availability of water and possibly nutrients at sites close to water could increase the ratio of sexually and vegetatively produced propagules (Lei, 2010; Vandepitte et al., 2010). Intermediate levels of disturbance have also been found to increase sexual reproduction by seeds in *F. verna* (Jung et al., 2008). In addition, seed dispersal by hydrochory may be greatly enhanced at these sites, allowing for increased gene flow between sites (Love et al., 2013; Mattingly et al., 2023). Finally,

temporary flooding may also lead to the opening of gaps within sites, facilitating the establishment of seeds and vegetative propagules.

In conclusion, we hypothesize that regular gap openings and facilitated propagule establishment as well as enhanced seed dispersal by water and possibly by mowing machines are the most important causes explaining the positive effects of anthropogenic and natural disturbances on the local genetic diversity of *F. verna*, and that these processes outweigh the negative effects of the loss of individual plants.

4.3 | Implications for future studies in *F. verna* with particular focus on invasive populations

The infrageneric systematics and taxonomy of *Ficaria* are still under debate (Drenckhahn et al., 2017; Veldkamp, 2015; Zonneveld, 2015). Evolutionary lineages within the genus are mostly distinguished based on ploidy level, genome size, and morphology (Sell, 1994; Zonneveld, 2015). To date, little is known about how the currently distinguished (sub)species relate to genetic lineages and to what extent hybridization between lineages occurs in *Ficaria* (Drenckhahn et al., 2017; Popelka, Sochor, & Duchoslav, 2019; Popelka, Trávníček, et al., 2019). However, a thorough understanding of infrageneric differentiation is not only of general interest but is also important in assessing the invasive potential of a species (Segelbacher et al., 2022; Ward et al., 2008). There is a general consensus that the management of invasive species should focus on reducing overall genetic diversity to reduce the species' adaptive potential and potential for further spread (Prentis et al., 2008; Smith et al., 2020). Non-native species are usually thought to have low levels of genetic diversity due to founder effects upon introduction, but where repeated introductions have occurred, non-native species may have high levels of genetic diversity and adaptability, increasing their invasive potential (Ward et al., 2008), as has been suggested for *F. verna* in the USA (Mattingly et al., 2023). A better understanding of how genetic diversity is maintained in the species and of the number of genetic lineages present in the invasive range may therefore help to establish effective management guidelines for this regionally invasive species.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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