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Land-use effects on genetic structure of a common grassland herb: A matter of scale

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Abstract

The most common management practices in European grasslands are grazing by livestock and mowing for silage and hay. Grazing and mowing differ in their potential effects on plant gene flow and resulting population–genetic structure. After assessing its breeding system, we investigated the effect of land use on the population–genetic structure in the common grassland plant *Veronica chamaedrys* using 63 study populations on meadows, mown pastures and pastures in three regions in Germany, the so-called Biodiversity Exploratories. We determined plant density and analysed the genetic diversity, differentiation and small-scale genetic structure using amplified fragment length polymorphism (AFLP) markers. The breeding system of *V. chamaedrys* turned out as self-incompatible and outcrossing. Its genetic diversity did not differ among land-use types. This may be attributed to large population sizes and the strong dispersal ability of the species, which maintains genetically diverse populations not prone to genetic drift. Genetic differentiation among populations was low (overall $F_{ST} = 0.075$) but significant among the three regions. Land use had only weak effects on population differentiation in only one region. However, land use affected small-scale genetic structure suggesting that gene flow within plots was more restricted on meadows than on mown and unmown pastures. Our study shows that land use influences genetic structure mainly at the small scale within populations, despite high gene flow.

Zusammenfassung

Die am häufigsten praktizierten Formen der Landnutzung in europäischen Grünländern sind Beweidung durch Vieh und Mahd zur Produktion von Silage und Heu. Obwohl Beweidung und Mahd sich unterschiedlich auf die Populationsstruktur von Pflanzen auswirken können, indem sie Genfluss verändern, sind vergleichende Studien bisher selten.

Wir untersuchten den Effekt der Landnutzung auf die populationsgenetische Struktur der häufigen Grünlandpflanzenart *Veronica chamaedrys* auf 63 Untersuchungsflächen in Wiesen, Mähweiden und Weiden in drei Regionen Deutschlands, die Teil der Biodiversitäts-Exploratorien sind. Wir ermittelten die Abundanz der Art auf allen Untersuchungsflächen und analysierten die genetische Diversität, Differenzierung und kleinräumige genetische Struktur mittels molekularer Marker (AFLP).

Unsere Analyse des Befruchtungssystems ergab, dass *V. chamaedrys* selbstinkompatibel und auskreuzend ist. Die genetische Diversität unterschied sich nicht zwischen den Landnutzungstypen. Dies kann auf hohe Populationsgrößen und die starke Ausbreitungsfähigkeit der Art zurückzuführen sein, wodurch genetisch diverse Populationen erhalten werden, die

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kaum durch genetische Drift beeinflusst werden. Die genetische Differenzierung zwischen den Populationen war gering (Gesamt-$F_{ST} = 0.075$) aber signifikant zwischen den drei Regionen. Die Landnutzung hatte einen nur schwachen Effekt auf die Differenzierung der Populationen in nur einer der drei Regionen. Allerdings wurde die kleinräumige genetische Struktur von der Landnutzung beeinträchtigt, was darauf hindeutet, dass die kleinskalige Pollen- und Samenausbreitung auf Wiesen stärker eingeschränkt war als auf Mähweiden und Weiden.

Unsere Studie zeigt, dass die Landnutzung die genetische Struktur hauptsächlich auf kleinräumiger Skala innerhalb von Populationen beeinflusst, obwohl hoher Genfluss stattfindet.

Keywords: Biodiversity exploratories; Mowing; Grazing; AFLP; Veronica; Breeding system; Pollination experiment; Pollen–ovule ratio; Isolation by distance; Spatial autocorrelation

Introduction

Anthropogenic land use has shaped the European landscape. Especially the variety of historical land-use types in European grasslands has resulted in high biodiversity (Poschlod, Bakker, & Kahmen 2005). Grassland plant diversity is affected by land use both at the community level, e.g. species richness (Schläpfer, Zoller, & Körner 1998) and the within species level, where population density or flowering phenology may respond to management (Reisch & Poschlod 2009). Consequently, land use can lead to isolation and fragmentation of populations potentially resulting in genetic differentiation and genetic erosion in subdivided populations. Thus, genetic differentiation of populations can reflect both natural selection and the amount of historic gene flow between them and thereby enable inferences about microevolutionary processes.

The most common current grassland-management practices are grazing by livestock and mowing for silage or hay. Generally, mowing is a more homogenous disturbance regime than grazing (Kölliker, Stadelmann, Reidy, & Nosberger 1998; Wallis De Vries, Bakker, & Van Wieren 1998). The greater heterogeneity of microhabitats on pastures is largely formed by random grazing and deposition of dung, trampling and avoidance of unpalatable species (Schläpfer et al. 1998; Kleijn & Steinger 2002). Very few studies addressed land-use effects on genetic differentiation among populations, and no consistent pattern was found for different species regarding land-use effects on genetic variation within populations (Zopfi 1998; Kleijn & Steinger, 2002; Rudmann-Maurer, Weyand, Fischer, & Stöcklin 2007; Reisch & Poschlod 2009; Smith et al. 2009). Genetic variation among and within populations can strongly differ among plant species depending on their life history traits, especially those affecting gene flow by seed and pollen dispersal. Common species with outcrossing breeding system are predicted to be threatened by population-genetic effects of fragmentation only in the long run because they have more genetic variability to lose compared to rare and selfing species (e.g. Aguilar, Quesada, Ashworth, Herrerias-Diego, & Lobo 2008). However, high levels of gene flow can compensate the effects of fragmentation to a certain extent and differentiation is usually lower in outcrossing compared to selfing species. Hence, if genetic differentiation is observed in outcrossing species, even stronger effects may be anticipated in species with lower levels of gene flow.

We selected *Veronica chamaedrys* L. (Germander Speedwell, Plantaginaceae) as study species, a common and putatively outcrossing grassland herb, and tested whether it has an outcrossing breeding system. As typical for outbreeding species, we expected genetic diversity within and among populations to be largely independent from population density and land use in this species due to high levels of gene flow relative to genetic drift (Nybom 2004). However, as mowing and grazing can differently affect seed and pollen dispersal within populations they may affect the small-scale spatial genetic structure (SGS). Besides gene dispersal, SGS depends also on local genetic drift, the level of self-compatibility, clonal propagation, plant density and pollination (Van Rossum & Triest 2006). SGS of natural populations has mostly been studied to determine propagation in general (Vekemans & Hardy 2004; and references therein). However, it has rarely been used to compare the effects of mowing and grazing, but Kleijn and Steinger (2002) indeed observed a more pronounced clumping of genets and SGS on pastures compared to meadows. Thus, SGS may be crucial for microevolutionary processes, especially in outcrossing species where the relatedness among neighbouring plants is closely associated with offspring fitness (Heywood 1991).

We investigated the effect of land use on population density and population–genetic structure at different spatial scales of *V. chamaedrys*. Our study was conducted on 134 study plots within the Biodiversity-Exploratories Project comprising three regions across Germany with three types of managed grassland: meadows, mown pastures and pastures (Fischer et al. 2010). We used amplified fragment length polymorphisms (AFLP) to characterise genetic variation within and among a subsample of 63 populations. We (i) determined the breeding system of *V. chamaedrys*. To gain insight into the effects of mowing and grazing we (ii) investigated genetic diversity, population differentiation and SGS and asked (iii) how land use affects population density and genetic structure on different spatial scales.

Materials and methods

Species

V. chamaedrys L. ssp. *chamaedrys* (Plantaginaceae) is a perennial herb propagating both sexually and clonally by short rhizomes. The subspecies is tetraploid ($2n = 4x = 32$) and diploid subspecies have not been reported for the study regions (Bardy, Albach, Schneeweiss, Fischer, & Schönswetter 2010). It flowers from May to July and is visited and pollinated mainly by Diptera, namely Syrphidae and Bombyliidae (Weiner, Werner, Linsenmair, & Blüthgen 2011). Seeds are small (0.2 mg) and lack special dispersal devices. *V. chamaedrys* has been suggested to be outcrossing due to flower anatomy (Knuth 1898), genome size (Albach & Greilhuber 2004) and anther size (Kampny & Dengler 1997).

Breeding system analysis

We determined the breeding system of *V. chamaedrys* with pollination experiments using 10 plants from several populations in Hainich-Dün. Inflorescences were bagged from bud to fruit stage to prevent free pollination. Only flowers that unfolded after bagging were used and pollinated with pollen of the same flower ($N = 29$), of a different flower of the same plant ($N = 30$), of a different plant ($N = 29$) or were left untreated as control ($N = 17$). We calculated self-incompatibility from fruit set according to Pound, Wallwork, Potts, and Sedgley (2002). As an additional indication of the breeding system (Cruden 1977; but see Michalski & Durka 2009), we determined the pollen-to-ovule ratio of *V. chamaedrys* and 11 congeneric species with known breeding system, sampled in Germany, mostly in the biodiversity exploratories.

Population structure: sites and sampling

Our study was conducted within the biodiversity exploratories: Schorfheide-Chorin (SC), Hainich-Dün (HD) and Schwäbische Alb (SA) in north, central and southwest Germany, respectively (Fischer et al. 2010) which are 300–600 km apart from each other. Each exploratory spans an area of 600–1600 km^2 in which 50 study plots (50 m × 50 m) were established on three types of managed grassland: meadows (mown 1–3 times per year), pastures (grazed by sheep, horse or cattle) and mown pastures (both mown and grazed each year). Most of the studied grasslands have persisted for at least 25 years.

During summer 2008 we sampled leaves of *V. chamaedrys* on 134 plots. Plots were partitioned into 16 squares (12.5 m × 12.5 m) and 16 individuals were sampled per plot, if possible, keeping a distance of at least 5 m to minimize re-sampling of clonal genotypes. Sample coordinates were recorded and population density was assessed as the number of sampled individuals per plot (≤16 per 0.25 ha), which was partitioned into 5 ranks (0–4, 5–8, 9–12, 13–15, ≥16).

AFLP analysis

We only considered populations where at least 7 individuals were sampled and randomly chose up to 12 individuals per population for AFLP analysis which resulted in 698 samples from 63 populations (SC = 122, HD = 261, SA = 315; see Appendix A: Table 1). AFLPs were produced as described in Appendix A. GENEMAPPER v.3.7 (ABI) software was used for manual binning and automated scoring which resulted in peak height data of 279 preliminary AFLP loci for 4 primer combinations. Reproducibility was assessed with a total of 82 (11.7%) replicate samples, either replicate DNA extractions ($N = 13$) or replicate genotyping of the same DNA extraction ($N = 69$). For each preliminary AFLP locus we specified an individual minimum peak-height threshold, calculated locus-specific error rates and discarded loci with a reproducibility <90%, resulting in an overall error rate of 1.9%. Finally 189 AFLP loci were retained for further analyses of which 179 (95%) were polymorphic. Considering the error rate and allowing for two differences within a genotype, three pairs of samples from two populations in SA may represent clones. Deleting these samples from the data set did not change the overall results. We performed a band based analysis of AFLP data, as allele frequencies of dominant markers cannot be unequivocally estimated in polyploids.

We looked for potentially adaptive loci that depart from a neutral model, putatively due to natural selection, using BayeScan v.2.0 (Foll & Gaggiotti 2008) with a threshold of >3 for the Bayes Factor.

Population structure among and within exploratories

We inferred the overall genetic structure using a Bayesian model-based clustering method with BAPS v.5.2 (Corander, Waldmann, & Sillanpää 2003) without *a priori* information about individual origin. We conducted individual-level spatial and non-spatial mixture analyses with maximum number of gene pools set to $K = 15$. A similar analysis was performed with STRUCTURE v.2.3.3 (Pritchard, Stephens, & Donnelly 2000) that yielded the same results with respect to the number of clusters (results not shown).

We quantified the degree of differentiation among and within the gene pools identified by BAPS with an analysis of molecular variance (AMOVA) with ARLEQUIN v.3.1 (Excoffier, Laval, & Schneider 2005). We tested for genetic differentiation among land-use types with an AMOVA for each exploratory.

Genetic differentiation among populations within exploratories was assessed by Wright's F_{ST} (Lynch & Milligan 1994) in AFLP-SURV v.1.0 (Vekemans 2002). We checked for isolation by distance by correlating pairwise

$F_{ST}/(1-F_{ST})$ values (Rousset 1997) with geographic distance and applied a Mantel test in FSTAT v.2.9.3.2 (Goudet 2001). The influence of land use on the isolation-by-distance pattern was assessed with Mantel tests in R v.2.9.2 (R Development Core Team 2009) correlating the matrices of either pairwise $F_{ST}/(1-F_{ST})$, geographic distance or land-use similarity. In the land-use similarity matrix, we coded different land-use types as '1' and identical land-use types as '0'.

Population structure within populations

We determined population-level genetic diversity as proportion of polymorphic loci *PLP* at the 5% level and as expected heterozygosity H_e, using AFLP-SURV, with fragment frequency equalling allele frequency. Additionally, we calculated band richness B_r, the mean number of phenotypes expected per locus rarefied to $N=7$ with AFLPDIV v.1.1 (Coart, Van Glabeke, Petit, Van Bockstaele, & Roldán-Ruiz 2005), thus correcting for different sample sizes.

We assessed the effect of land use on the within-population small-scale spatial genetic structure (SGS) with spatial genetic autocorrelation analyses. First, individuals were either pooled per exploratory or per land-use type and the autocorrelation coefficient r was calculated for 7 distance classes covering the plot size testing for significance with 10,000 permutations in GENALEX v.6.2 (Peakall & Smouse 2006). We tested for differences among exploratories and land-use types with a non-parametric heterogeneity test that yields an overall test statistic ω and individual class statistics t^2 (Smouse, Peakall, & Gonzales 2008). Second, we quantified the SGS with the *Sp* statistic (Vekemans & Hardy 2004) based on the kinship coefficient in SPAGEDI v.1.3 (Hardy & Vekemans 2002).

Statistics

We compared population density among exploratories and land-use types with a Kruskal–Wallis rank-sum test and two nonparametric multiple comparisons (Behrens–Fisher test and Steel test). Genetic diversity and allele frequencies of potentially adaptive loci were compared among exploratories or land-use types by ANOVA and Tukey's post hoc test. If not indicated otherwise, analyses were performed in R.

Results

Breeding system

Out of 29 flowers that received pollen from another plant 22 produced fruits. Only one fruit was produced in 39 self-pollinated flowers and no fruit was formed without hand pollination. This corresponds to a degree of self-incompatibility of 97.4%. Compared with congeneric species the pollen–ovule ratio of *V. chamaedrys* ranked very high with 984 (±373 SD) and corresponded to self-incompatible, outcrossing species (Table 1).

Genetic population structure

Analysis of population structure in BAPS revealed three different gene pools (log likelihood non-spatial analysis: −3,533; spatial analysis: −35,621). In both analyses, the three gene pools were equivalent to the exploratories with a stronger differentiation of Schwäbische Alb (SA) from both Schorfheide-Chorin (SC) and Hainich-Dün (HD) than the differentiation between SC and HD.

Considering all 63 populations, genetic differentiation was low (overall $F_{ST}=0.075$, $P<0.001$), with genetic variation partitioned to 6% among exploratories and 3% among populations within exploratories (AMOVA, Table 3). Separate analyses per exploratory similarly revealed 3% variation among populations (Table 3). In all exploratories, populations within land-use types were weakly differentiated (2–3%, AMOVA, Table 3). However, in SC, there was a low but significant differentiation (0.8%) among land-use types (Table 3) which was mainly due to differentiation between meadows and mown pastures (2%, AMOVA $P<0.05$).

The BayeScan-analysis revealed three potentially adaptive loci (ACT-CTC_124, ACT-CTC_192, ACA-CAC_277) in the analysis including all exploratories. Here, in two loci (124, 277) the allele frequencies of SA differed significantly from SC and HD, while locus 192 differed only between SC and SA. However, in the analysis of single exploratories, all loci followed the neutral expectation, indicating that selection did not play a significant role in population differentiation.

We found an isolation-by-distance pattern in SC but not in HD and SA (Fig. 1). In HD, a weak isolation-by-distance pattern was present among populations of the same land-use type (Fig. 1). Here, land-use similarity explained 1.6% of variation in pairwise $F_{ST}/(1-F_{ST})$ between populations ($r=-0.124$, $P<0.05$), whereas geographic distance did not explain any variation ($r=0.033$, $P>0.5$).

The plant density of *V. chamaedrys* was low with an overall average of 28.4 individuals per hectare. Plant density was significantly higher in SA compared to HD and SC ($\chi^2=25.41$, $P<0.001$, Table 2) and was not related to land use ($\chi^2=1.45$, $P>0.1$).

Genetic diversity and small-scale structure within populations

Measures of genetic diversity within populations are shown in Table 2 (see Appendix A: Table 1). Among the three exploratories, populations in HD had the highest genetic diversity and the ones in SA the lowest (ANOVA $P<0.05$, Table 2). Land use did not influence the measures of genetic diversity within populations (ANOVA $P>0.1$).

Table 1. Pollen–ratio; SC, self-compatible; SI, self-incompatible.

Species	*N*	P/O-ratio (SD)	Pollen grains per flower (SD)	Ovules per flower (SD)	Reference	Compatibility[a]	Breeding system[a]
V. peregrina		6.7 (0.4)			Cruden (1977)	SC	Selfing
V. arvensis		24 (5.2)			Cruden (1977)	SC	Selfing
V. arvensis	34	62 (18)	1101 (333)	18 (2)	[c]	SC	Selfing
V. serpyllifolia	30	29 (15)	1703 (908)	59 (10)	[c]	SC	Mixed mating?
V. serpyllifolia		32			Cruden (1977)	SC	Mixed mating?
V. polita	5	45 (23)	1050 (658)	22 (3)	[c]	SC	Selfing
V. dillenii	5	83 (16)	1690 (482)	20 (3)	[c]		Unknown
V. officinalis	3	103 (47)	2070 (834)	21 (4)	[c]	SI or SC[b]	Outcrossing
V. hederifolia	10	156 (48)	625 (192)	4 (0)	[c]	SC	Facultatively selfing
V. persica	9	168 (49)	2606 (706)	16 (2)	[c]	SC	Facultatively selfing
V. filiformis	8	289 (173)	3558 (1577)	14 (4)	[c]	SI	Outcrossing
V. montana	2	322 (88)	3220 (877)	10 (0)	[c]		Unknown
V. chamaedrys	48	984 (373)	15,198 (5315)	16 (2)	[c]	SI[c]	Outcrossing[c]
V. teucrium	2	1872 (30)	29,970 (3125)	16 (1)	[c]		Unknown

[a] BIOLFLOR database (Klotz, Kühn, & Durka 2002).
[b] Knuth (1898).
[c] This study.

Table 2. Mean population values of *Veronica chamaedrys* in the exploratories; population density in all sampled plots; *N*, number of samples analysed per population; H_e, expected heterozygosity; *PLP*, percentage polymorphic loci; *Br*, band richness based on 7 samples and 189 loci.

Exploratory	Population density [ha^{-1}]	Number of populations		Genetic diversity			
		Present	Analysed	*N*	H_e	*PLP*	*Br*
Schorfheide-Chorin	14.13^{a}	19	11	11.1	0.16^{ab}	44.0^{ab}	1.38^{ab}
Hainich-Dün	28.34^{a}	37	24	10.9	0.16^{a}	45.2^{a}	1.39^{a}
Schwäbische Alb	42.91^{b}	39	28	11.3	0.15^{b}	42.3^{b}	1.36^{b}

Values with the superscript letter are not significantly different at $P = 0.05$.

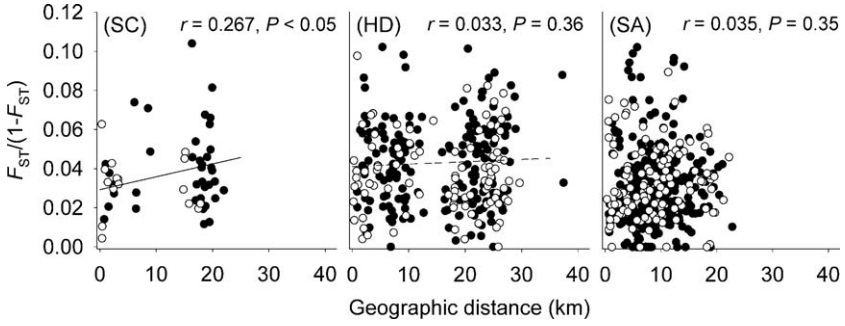

Fig. 1. Patterns of isolation by distance of *Veronica chamaedrys* populations in the three exploratories (SC, Schorfheide-Chorin; HD, Hainich-Dün; SA, Schwäbische Alb). Results of overall Mantel tests are given. Solid circles indicate pairs of populations with different land use; open circles indicate similar land use. Significant correlations are indicated by regression lines (solid for overall results, dashed for similar land use).

Analysis of small-scale spatial genetic structure (SGS) indicated a significant, though not very pronounced, within-population structure (Fig. 2A). This pattern did not differ among exploratories (heterogeneity test $\omega < 18.2$, $P > 0.07$). Thus, low values of the *Sp* statistic were obtained ranging from 0.0021 in HD and SA to 0.0028 in SC. However, SGS was affected by land use. Populations in meadows (M) showed a stronger SGS than populations in pastures (P) or mown pastures (MP) ($\omega_{M/MP} = 21.7$, $\omega_{M/P} = 37.2$, $P < 0.05$, Fig. 2B), due to higher similarity among individuals within the distance classes up to 30 m.

Discussion

Variation among exploratories

V. chamaedrys was significantly differentiated among exploratories, which may be due to several non-exclusive processes. First, limited gene flow, following an isolation-by-distance model, could not fully counteract genetic drift across larger geographic distance, even in this widespread and common species (Hutchison & Templeton 1999). Second, historic colonisation processes may be involved. It remains to be

Table 3. Summary of analysis of molecular variance (AMOVA) in *Veronica chamaedrys* according to exploratories (SC, Schorfheide-Chorin; HD, Hainich-Dün; SA, Schwäbische Alb) and land-use types.

Exploratory	Overall			SC			HD			SA		
Source of variation	df	% var.	*P*	df	% var.	*P*	df	% var.	*P*	df	% var.	*P*
Among exploratories	2	6.1	***									
Among populations within exploratories	60	2.7	***	10	2.7	***	23	3.2	***	27	2.7	***
Within populations	635	91.2	***	111	97.3	***	237	96.9	***	287	97.4	***
Among land-use types				2	0.8	*	2	0.2	n.s.	2	−0.2	n.s.
Among populations within land-use types				8	2.2	***	21	3.0	***	25	2.8	***
Within populations				111	97.0	***	237	96.8	***	287	97.4	***

* $P < 0.05$.
*** $P < 0.001$.

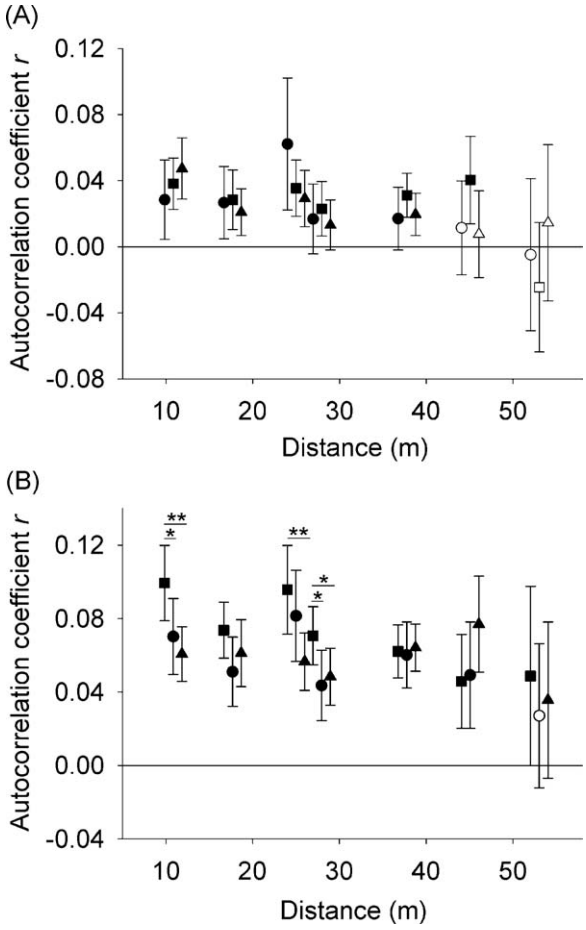

Fig. 2. Genetic similarity between *Veronica chamaedrys* individuals, expressed as autocorrelation coefficient *r*, over the 50 m spatial scale of a study plot (note that symbols are displaced for better visibility). Individuals are significantly more similar than expected by chance when symbols are filled. Mean values (±95% CI) are given for populations of (A) exploratory: Schorfheide-Chorin (◯), Hainich-Dün (□) and Schwäbische Alb (△) and (B) land-use type: meadow (□), mown pasture (◯) and pasture (△). Asterisks indicate significant differences ($P < 0.05$) between categories according to t^2 single class test criteria.

studied whether differentiation among exploratories, namely between the two northern sites Schorfheide-Chorin (SC) and Hainich-Dün (HD) and the southern Schwäbische Alb (SA), is due to different phylogeographic origins from different refugia, e.g. as has been shown for the Balkan Peninsula (Bardy et al. 2010). Third, different environmental conditions may have lead to, or maintained differentiation by disruptive selection. In fact, three AFLP loci were more differentiated than expected from a neutral model, potentially indicating adaptive differentiation. However, the underlying selective forces cannot easily be disentangled, as the exploratories differ in numerous environmental variables, and, additionally, the presence of selection has to be proven experimentally.

Variation within exploratories and populations

We detected very low genetic differentiation among and within populations, which indicates that gene flow among populations was large enough to counteract effects of genetic drift (Hutchison & Templeton 1999). This is in line with our expectations for a common outcrossing perennial herb (Nybom 2004; Musche, Settele, & Durka 2008). However, our finding of isolation by distance in the exploratory with the lowest abundance of *V. chamaedrys* (SC), suggests that gene flow and drift can be affected on a regional scale when species become less common. Though population density of *V. chamaedrys* was found to be higher in SA compared to SC and HD, the highest genetic diversity was found in HD. This does not correspond to the generally positive relationship between population size and genetic diversity often found in self-incompatible species (Leimu, Mutikainen, Koricheva, & Fischer 2006). However, genetic diversity may also be independent of abundance in outcrossing plant species (Rudmann-Maurer et al. 2007; Musche et al. 2008). As clonal propagation was irrelevant at our sites, a trade-off between vegetative and sexual reproduction at the investigated scale can be ruled out. Instead, even in our low-density populations, the species may have been too abundant for genetic drift to be effective and hence it will not suffer from genetic erosion. In addition, our results may illustrate that high gene flow of outcrossing species can counteract effects of genetic drift either by high seed and pollen dispersal or large effective population sizes.

We found no significant differences between land-use types concerning population density or genetic diversity. This suggests that frequency and intensity of mowing and grazing did not affect the balance between gene flow and genetic drift. A similar pattern was reported for the population structure of the long-lived grass *Brachypodium pinnatum* that was not affected after 16 years of experimental mowing or burning (Schläpfer & Fischer 1998). Yet, our finding of a weak isolation by distance only among plots with the same land-use type in HD may indicate that patterns of gene flow are more similar within the same land-use type than among land-use types. However, land use had only little influence on large-scale genetic structure as genetic differentiation between land-use types was only found in SC. Previous studies have reported genetic differentiation between mown and grazed grasslands (Zopfi 1998; Kleijn & Steinger 2002; Reisch & Scheitler 2009). However, land-use effects may be small as in *Poa alpina*, where differentiation was more strongly affected by distance than by land use (Rudmann-Maurer et al. 2007).

Gene flow within populations was hardly restricted over the plot distance as revealed by low small-scale spatial genetic structure (SGS) and *Sp* statistic that correspond to outcrossing species (Vekemans & Hardy 2004). We found a significant difference of SGS among land-use types in all exploratories which suggests that land use generally affects genetic structure at the small scale. Here, the small-scale gene flow on meadows was more restricted than on mown pastures and

pastures. In a previous study, the unpalatable grassland plant *Veratrum album* showed small-scale clumping of genotypes on pastures compared to meadows (Kleijn & Steinger 2002). While in *V. album* the clumping was due to clonal growth that was indirectly enhanced by grazing, the difference in small-scale autocorrelation of *V. chamaedrys*, extending up to 30 m, was not due to clonal propagation. This result indicates that mowing restricts pollen and seed dispersal more than grazing on this small scale. We suppose that gene flow is more restricted in meadows because the first mowing for hay making mainly takes place between May–June and therefore inhibits fruit set and thus reduces seed production. Additionally, livestock grazing can homogenize the spatial genetic structure via several processes like seed dispersal (Rudmann-Maurer et al. 2007; Smith et al. 2009) or the generation of safe sites for germination (Hellström, Huhta, Rautio, & Tuomi 2009). Another explanation could be an increase in pollinator visits in pastures which would lead to enhanced pollen dispersal (review in Hegland, Grytnes, & Totland 2009). The abundance of pollinators has been shown to be positively correlated with plant species diversity (e.g. Tscharntke, Klein, Kruess, Steffan-Dewenter, & Thies 2005). Thus, as plant species richness in the studied populations is higher on pastures than on mown pastures and meadows (Kloss, unpublished data), increased pollen dispersal on pastures is likely. Hence, SGS may be maintained by mowing through reduction of sexual propagation and by biparental inbreeding and thereby potentially decreasing population viability in the long run.

Conclusions

Our results confirm that common grassland species can maintain very similar levels of genetic diversity over a large spatial scale, facilitated by an outcrossing breeding system and efficient dispersal of pollen and seeds which largely precludes effects of genetic drift and genetic erosion. However, even in a common and outbreeding species differentiation among populations may increase either by reduced abundance, spatial isolation or different land-use types, which can lead to reduced gene flow. Furthermore, land use affected spatial structure at the small scale within populations which could alter microevolutionary processes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.baae.2011.06.001.

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Appendix A

Table 1. Characteristics of the studied populations of *Veronica chamaedrys* included in AFLP analysis: The first letters of the population name describes the exploratory (SC, Schorfheide-Chorin; HD, Hainich-Dün; SA, Schwäbische Alb); land-use type: M, meadow, MP, mown pasture, P, pasture; location coordinates in Gauss-Krüger (Potsdam) system; population density; *N*, number of samples analysed; genetic diversity: H_e, expected heterozygosity; *PLP*, percentage polymorphic loci; *Br*, band richness based on 7 samples.

Population	Land-use type	Location coordinates		Population density [ha^{-1}]	Genetic diversity			
		East	North		*N*	H_e	*PLP*	*Br*
SC13	MP	5420800	5871820	40	10	0.16	41.3	1.37
SC18	M	5424887	5890046	64	12	0.15	44.4	1.37
SC25	M	5407590	5887379	52	12	0.17	47.6	1.41
SC30	M	5421900	5891300	60	12	0.17	48.1	1.41
SC31	M	5422220	5891400	64	12	0.14	40.7	1.34
SC32	M	5422001	5891720	64	12	0.16	49.7	1.40
SC33	MP	5422400	5873220	36	9	0.15	40.2	1.37
SC39	MP	5421100	5872700	36	9	0.15	40.2	1.36
SC47	P	5421400	5873401	40	10	0.15	42.3	1.37
SC49	P	5423325	5871975	56	12	0.17	46.0	1.39
SC50	P	5416477	5888457	64	12	0.15	43.9	1.37
HD9	P	4386991	5677798	64	12	0.17	49.7	1.42
HD10	M	4391720	5683400	52	12	0.16	45.0	1.38
HD11	M	4392390	5684000	48	12	0.16	43.9	1.37
HD14	MP	4391000	5685520	64	12	0.16	43.4	1.37
HD17	P	4392900	5660600	32	8	0.19	48.1	1.46
HD18	P	4390200	5684380	64	12	0.16	49.2	1.40
HD19	P	4393100	5660900	64	12	0.16	46.0	1.39
HD20	P	4386299	5677498	32	8	0.16	43.4	1.41
HD21	P	4412899	5673599	52	12	0.16	46.0	1.39
HD27	M	4401802	5662197	64	12	0.17	45.5	1.40
HD28	M	4395199	5682299	40	10	0.17	47.1	1.41
HD29	M	4395300	5682097	64	12	0.16	45.0	1.38
HD30	M	4385478	5675378	44	11	0.16	43.4	1.38
HD31	MP	4375699	5672100	64	12	0.15	44.4	1.37
HD32	MP	4399720	5661020	32	8	0.17	42.9	1.41
HD35	MP	4389100	5677299	64	12	0.16	45.0	1.38
HD41	P	4386015	5677500	48	12	0.16	47.6	1.40
HD42	P	4392500	5660490	64	12	0.16	46.6	1.38
HD43	P	4391020	5686400	64	12	0.17	48.7	1.40
HD44	P	4393508	5658884	32	8	0.17	40.7	1.39
HD45	P	4395499	5657100	32	8	0.15	38.1	1.36
HD46	P	4412998	5675399	32	8	0.16	41.3	1.39
HD48	MP	4387100	5684800	64	12	0.17	47.1	1.40
HD50	MP	4389910	5683590	64	12	0.16	46.0	1.38
SA3	M	3539490	5363590	64	12	0.15	43.9	1.37
SA5	MP	3532600	5362100	64	10	0.15	41.3	1.36

SA6	MP	3532780	5362700	64	12	0.14	39.7	1.34
SA8	P	3536500	5365100	64	12	0.15	41.8	1.35
SA9	P	3537310	5361995	64	12	0.13	38.6	1.32
SA10	M	3519900	5361800	64	10	0.17	47.1	1.41
SA11	M	3525780	5372020	64	12	0.14	41.3	1.35
SA13	M	3526900	5361600	52	12	0.16	41.3	1.36
SA18	M	3538700	5360700	64	12	0.15	42.9	1.36
SA19	MP	3533200	5362300	28	7	0.14	34.9	1.35
SA20	P	3526420	5372480	64	12	0.15	40.2	1.35
SA22	M	3538090	5363080	56	11	0.14	39.2	1.34
SA24	MP	3536620	5362190	56	12	0.14	36.5	1.32
SA25	P	3519300	5361995	64	11	0.14	41.8	1.35
SA28	P	3536485	5369485	64	12	0.16	47.6	1.39
SA30	MP	3533900	5368820	64	12	0.15	39.7	1.35
SA32	P	3536000	5369900	56	10	0.17	42.3	1.39
SA33	P	3536400	5368300	64	11	0.15	43.4	1.37
SA34	P	3537100	5368800	64	12	0.17	47.1	1.41
SA36	M	3522200	5371200	64	12	0.15	42.9	1.36
SA38	M	3531540	5366950	64	12	0.16	46.0	1.39
SA40	M	3542020	5363710	36	9	0.15	38.6	1.36
SA41	M	3529600	5359200	44	11	0.15	40.7	1.35
SA42	MP	3527920	5362310	64	12	0.16	45.0	1.38
SA43	MP	3540200	5364100	64	12	0.17	47.6	1.40
SA44	P	3532300	5360110	64	12	0.16	42.3	1.37
SA49	P	3537400	5368900	64	9	0.15	41.3	1.37
SA50	MP	3534700	5363200	64	12	0.17	48.1	1.41

AFLP analysis

Genomic DNA was extracted from dried leaf tissue using the Qiagen DNeasy 96 Plant Kit. DNA (0.15 µg) was digested and the PCR adapters were ligated at 21 °C over night with 5 units *Eco*RI (Fermentas Inc.), 1 unit of *Mse*I (New England Biolabs Inc.), 67 units T4 DNA Ligase (BioLabs Inc.), in 11 µL of buffer comprising 10 mM Tris chloride, 0.5 mM EDTA (pH 9.0) (AE/Elution buffer, Qiagen), 0.5 M sodium chloride, 50 ng/µL BSA, 1.1 µL 10X T4 DNA Ligase buffer (BioLabs Inc.), 5 pmol *Eco*RI adapters and 50 pmol *Mse*I adapters. After the restriction-ligation reaction the mix was then diluted with sterile H_2O (1:3 v/v). Four microlitres of the diluted restriction-ligation mix was used as template for the pre-amplification. PCR was performed with 30 ng *Eco*-A and 30 ng of *Mse*-C primers with 0.8 units DreamTaq DNA polymerase (Fermentas Inc.) and 0.2 mM each dATP, dCTP, dGTP, dTTP, in a total of 20 µL of 10X DreamTaq buffer with 20 mM $MgCl_2$ (Fermentas Inc.). The PCR program consisted of 72 °C for 2 min then 20 cycles of (94 °C for 20 s, 56 °C for 30 s, 72 °C for 2 min) and 60 °C for 30 min. PCR products were diluted with sterile H_2O (1:5 v/v). One microlitre of the diluted pre-amplification product was used as template for selective amplification and was added to 2.2 µL of Multiplex PCR Master Mix (Qiagen), 0.6 µL fluorescently labelled *Eco*+ANN primer (1 pmol/µL) and 0.6 µL *Mse*+CNN primer (5 pmol/µL). Four selective PCR amplifications were performed with different primer combinations: FAM+*Eco*+ACT/ *Mse*+CTC, VIC+*Eco*+ACA/ *Mse*+CAC, NED+*Eco*+ACC/ *Mse*+CAC and PET+*Eco*+AGC/ *Mse*+CTA. The PCR program was: 95 °C for 15 min, 10 cycles of (94 °C for 20 s, 66 °C for 30 s, decrease 1 °C per cycle, 72 °C for 2 min), 20 cycles of (94 °C for 20 s, 56 °C for 30 s, 72 °C for 2 min) and 60 °C for 30 min. Two microlitres of each selective PCR product were used for fragment analysis with LIZ(500)-labelled size standard on a capillary sequencer (AB 3130 Genetic Analyzer).