RESEARCH PAPER

Impact of river dynamics on the genetic variation of Gypsophila repens (Caryophyllaceae): a comparison of heath forest and more dynamic gravel bank populations along an alpine river

C. Reisch [iD] & J. Sattler

Institute of Plant Sciences, University of Regensburg, Regensburg, Germany

ABSTRACT

- Alpine rivers are, despite anthropogenic water flow regulation, still often highly dynamic ecosystems. Plant species occurring along these rivers are subject to ecological disturbance, mainly caused by seasonal flooding. *Gypsophila repens* typically grows at higher altitudes in the Alps, but also occurs at lower altitudes on gravel banks directly along the river and in heath forests at larger distances from the river. Populations on gravel banks are considered non-permanent and it is assumed that new individuals originate from seed periodically washed down from higher altitudes. Populations in heath forests are, in contrast, permanent and not regularly provided with seeds from higher altitudes through flooding. If the genetic structure of this plant species is strongly affected by gene flow *via* seed dispersal, then higher levels of genetic diversity in populations but less differentiation among populations on gravel banks than in heath forests can be expected.
- In this study, we analysed genetic diversity within and differentiation among 15 populations of *G. repens* from gravel banks and heath forests along the alpine River Isar using amplified fragment length polymorphisms (AFLP).
- Genetic diversity was, as assumed, slightly higher in gravel bank than in heath forest populations, but genetic differentiation was, in contrast to our expectations, comparable among populations in both habitat types.
- Our study provides evidence for increased genetic diversity under conditions of higher ecological disturbance and increased seed dispersal on gravel banks. Similar levels of genetic differentiation among populations in both habitat types can be attributed to the species' long lifetime, a permanent soil seed bank and gene flow by pollinators among different habitats/locations.

INTRODUCTION

Plant species occurring along rivers face great challenges, mainly caused by the unidirectional movement of water (Honnay *et al.* 2010; Davis *et al.* 2018). Taking into account the seed dispersal of species growing along rivers with flowing water, it has been suggested that not only seeds but also alleles should drift downstream (Ritland 1981). Consequently, it is possible that without compensation by upstream dispersal, genetic diversity should accumulate in populations along the lower course of rivers, whereas upstream populations should become genetically depleted. This theory has been described previously as the 'unidirectional diversity hypothesis' (Ritland 1981; Markwith & Scanlon 2007).

Although some studies provide evidence for this assumption (Liu *et al.* 2006; Pollux *et al.* 2009), most investigations have failed to report increased levels of genetic diversity in downstream plant populations (Tero *et al.* 2003; Jacquemyn *et al.* 2006; Kropf *et al.* 2018). This may be because one-dimensional stepping-stone models assuming gene flow between adjacent populations or dominant long-distance dispersal are too simple to describe the complex process of gene flow among populations along rivers. Instead, it is suggested that the patterns of genetic variation are shaped by local extinction and recolonization events, and that gene flow along rivers follows rather a meta-population than a stepping-stone model (Honnay *et al.* 2010). Moreover, man-made corrections such as dams or other barriers may affect the dispersal processes along rivers and, consequently, also the pattern of genetic variation (Werth & Scheidegger 2014; Werth *et al.* 2014).

The processes of both local extinction and recolonization strongly depend on ecological disturbance, which destroys existing populations and creates gaps for founding new populations. In ecosystems along rivers ecological disturbance is mainly caused by flooding and the related erosion of the substrate (Jacquemyn *et al.* 2006; Džubàkova *et al.* 2015). The vegetation along alpine rivers arising from higher mountains is, under natural conditions, particularly dynamic (Kudrnovsky 2013). Although most rivers are now controlled and the dynamics of alpine rivers has strongly decreased in the last century due to flow regulation and human manipulation of sediment supply (Gurnell *et al.* 2009), the gravel banks directly along alpine rivers are still at least partly affected by spring flooding after snow melt at higher altitudes of the mountains

(Bill 2000). The intensity of ecological disturbance decreases, however, with increasing distance to the river. Plant communities located directly at the border of alpine rivers are, therefore, subjected to a higher level of disturbance than communities occurring at more distant sites from the river (Müller 1995).

Ecological disturbance has a strong impact on species and genetic diversity (Evanno *et al.* 2009; Frey *et al.* 2015) and is considered an important mechanism for the correlation of both of the above levels of diversity. It has already been demonstrated that man-made disturbance can have serious impacts on genetic diversity and differentiation (Reisch & Scheitler 2008; Rusterholz *et al.* 2009; Vogler & Reisch 2011).

The gradient of disturbance along alpine rivers may, therefore, have a strong effect on the genetic variation of plant populations, since key biological processes shaping genetic variation, such as mortality, reproduction or dispersal, depend on ecological disturbance (Banks *et al.* 2013). For both wet and dry grasslands, for example, it has been demonstrated that the establishment of new individuals from seed strongly depends on the existence of vegetation gaps, mainly caused by grazing (Jensen & Gutekunst 2003; Ruprecht *et al.* 2010). Similarly, many plant species growing along alpine rivers require a periodic rearrangement of the substrate and dispersal of seed through water for successful population establishment and survival (Bill *et al.* 1999; Bill 2000; Gurnell *et al.* 2012; Kudrnovsky 2013; Caponi *et al.* 2019). Differences in patterns of genetic variation can, therefore, be expected for populations growing in habitats subjected to different levels of disturbance caused by flooding.

The vegetation along alpine rivers arising from the northern calcareous Alps and running through the pre-alpine landscape follows a characteristic gradient (Müller 1995). Gravel banks located directly beside the river are typically colonized by the shrub *Myricaria germanica* (Kudrnovsky 2013). These gravel banks are rich in species originally occurring at higher mountain altitudes (Leuschner & Ellenberg 2017). Their populations are supposed to be non-permanent and it is assumed that new individuals originate from seed or plant fragments periodically washed down from higher altitudes *via* inflows feeding the wild river (Bill 2000). At some distance behind the gravel banks heath forests dominated by *Pinus sylvestris* and *P. mugo* border the wild river (Hölzel 1996). Some alpine plant species growing on the gravel banks can also be found in the undergrowth of these heath forests. Here they form, in contrast to the gravel banks, more permanent populations which are not regularly provided with seed or plant material from higher altitudes.

For our study we selected the creeping baby's breath (*Gypsophila repens* L.), an alpine plant species typically growing on calcareous screes at higher altitudes in the northern calcareous Alps. However, this species also occurs both on gravel banks and in heath forests along alpine rivers (Hegi 1979). Considering the different intensity of disturbance by flooding between these two habitats, we expect higher levels of genetic diversity in populations but less differentiation among populations of *G. repens* on gravel banks than in heath forests. In our study, we attempt to answer the following questions: (i) is genetic diversity of *G. repens* distribution unidirectional along the river; (ii) are populations on gravel banks genetically more diverse than populations in heath forests; and (iii) are populations in heath forests genetically more differentiated than populations on gravel banks?

MATERIAL AND METHODS

Species description

Gypsophila repens (*Caryophyllaceae*) is a long-lived perennial species. Plants are glabrous, with branched rhizomes forming numerous vegetative and flowering stems up to 25-cm long. Leaves are 10- to 30-mm long and often falcate. Inflorescences comprise five to 30 flowers, with pedicels mostly two or more times as long as the calyx. Petals are white, lilac or pale purplish (Tutin *et al.* 1964). *Gypsophila repens* flowers from June to late October. The most common pollinators are syrphid flies and small solitary bees (López-Villavicencio *et al.* 2003). *Gypsophila repens* typically occurs in the mountains of western and central South and Central Europe (Hegi 1979). Here, the species is a pioneer plant growing on calcareous screes. Plant fragments and seeds are, however, frequently washed downwards by streams and rivers. *Gypsophila repens* occurs, therefore, also in heath forests and on gravel banks along alpine rivers flowing out of the surrounding mountains (Sebald *et al.* 1998).

Study design and sampled populations

For our study we selected 15 populations of *G. repens* along the upstream part of the River Isar in Germany and Austria (Fig. 1; Table 1; Table S1). Eight populations were located on gravel
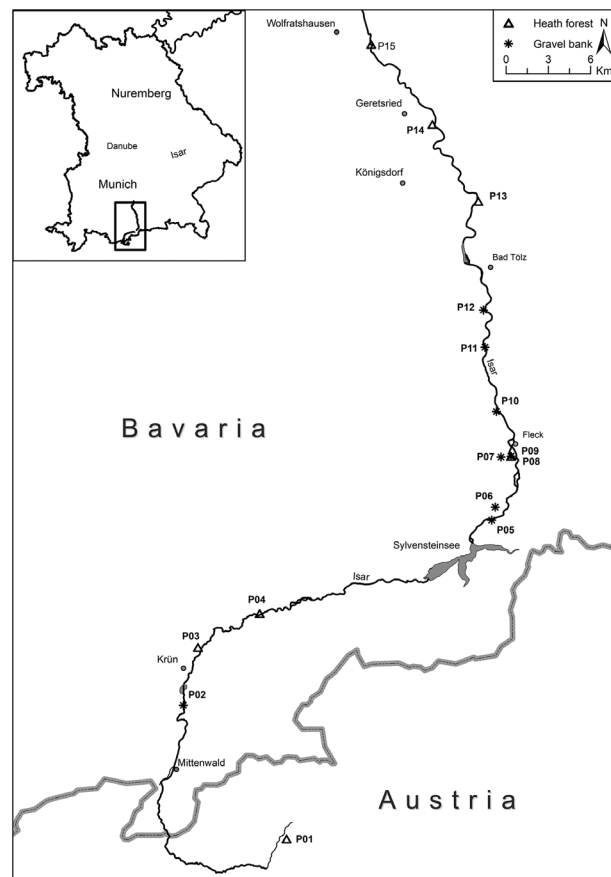

Fig. 1. Map of the sampling region and geographic position of the analysed populations of *Gypsophila repens* located in heath forests and on gravel banks along the River Isar in Germany and Austria.

Table 1. Studied populations of *Gypsophila repens* from heath forests and gravel banks along the River Isar, with population size (Ps), sample size (Ss), geographic distance to the source spring of the river (Ds), geographic distance to the river (Dr) and genetic variation within populations measured as a percentage of polymorphic bands (PB), Nei's gene diversity (H) and Shannon's information index (SI).

Pop.	habitat	Ps	Ss	Ds (km)	Dr (m)	PB	H	SI
P01	Heath forest	150	15	0.00	2313.8	67.31	0.27	0.40
P02	Gravel bank	19	15	21.90	14.6	79.81	0.34	0.49
P03	Heath forest	50	15	26.71	256.1	71.15	0.28	0.41
P04	Heath forest	100	15	33.11	97.9	61.54	0.24	0.35
P05	Gravel bank	200	14	56.82	2.4	76.92	0.31	0.45
P06	Gravel bank	>400	15	58.04	672.0	61.54	0.22	0.33
P07	Gravel bank	20	15	65.25	169.9	74.04	0.29	0.42
P08	Heath forest	>400	15	63.13	23.7	75.96	0.31	0.44
P09	Gravel bank	70	15	63.13	23.7	77.88	0.31	0.46
P10	Gravel bank	70	13	69.08	106.6	69.23	0.30	0.42
P11	Gravel bank	>400	15	74.11	8.6	65.38	0.24	0.36
P12	Gravel bank	100	15	77.47	161.6	57.69	0.25	0.36
P13	Heath forest	100	15	87.59	125.4	62.50	0.26	0.38
P14	Heath forest	NA	15	95.88	157.8	67.31	0.25	0.37
P15	Heath forest	>400	15	104.50	12.0	55.77	0.22	0.33
Mean	Gravel banks				144.92	70.31	0.28	0.41
Mean	Heath forests				426.68	65.93	0.26	0.38
P (*t*-test)					0.42	0.34	0.33	0.32

banks and seven other populations were located in heath forests along the river (Table 1).

Using GIS (Arc Info 10.0; Esri) based on corrected aerial photos, we identified the geographic distance of each population to the river and to the initial spring of the river. In the field we determined the size of each population by estimating the number of occurring individuals and sampled fresh leaf material from 15 individuals per population (in total 225 samples) for molecular analyses. We placed the leaf material in plastic bags and stored it in a freezer at −20 °C in the lab until the analyses.

Molecular analyses

From the sampled leaf material, DNA for molecular analyses was extracted using the CTAB method established by Rogers & Bendich (1994) with slight modifications by Reisch (2007). The DNA stock solutions were diluted with water to 7.8 ng·µl^{-1} and then subjected to molecular analysis. We applied amplified fragment length polymorphism (AFLP) analysis to determine genetic variation within and among populations, in accordance with the protocol from Beckmann Coulter (Brea, CA, USA) as described previously (Bylebyl *et al.* 2008; Reisch 2008).

For AFLP analysis, two primer combinations, after a wider-scale screening of 30 different combinations (D2 (DY-751)-AGC-CTA and D4 (Cyanine 5)-ACA-CTA) were chosen. PCR products were separated by capillary gel electrophoresis on an automated sequencer (GeXP; Beckmann Coulter). Results were examined using the GeXP software (Beckman Coulter) and analysed using the software Bionumerics 4.6 (Applied Maths, Kortrijk, Belgium). From the computed gels, only those fragments that showed intense and distinct bands were taken into account for further analyses. Due to weak banding patterns, three individuals were finally excluded from the analyses. Reproducibility of molecular analyses was investigated by

means of estimating the genotyping error rate (Bonin *et al.* 2004). We replicated 10% of all analysed samples (24 individuals), scored the fragments and calculated the percentage of fragments where differences between original and replicate occurred. Following this procedure, we determined a genotyping error rate of 1.3%.

Statistical analysis

Based upon 104 AFLP fragments, a binary matrix was created. Genetic variation within populations was determined by applying the program PopGene 1.32 (Yeh *et al.* 1997) as a percentage of polymorphic bands (PB), Nei's gene diversity H = $1 - \Sigma(p_i)^2$ and Shannon's information index SI = $\Sigma(p_i)\ln(p_i)$, where p_i represents the allele frequency. After testing residuals for normal distribution with Shapiro–Wilk test, differences between heath forest and gravel bank populations of *G. repens* in terms of population size, distance to the river and genetic variation were analysed using Student's *t*-tests. Correlations of population size, distance to the river and distance to the source spring of the River Isar with genetic variation were tested using Spearman rank correlations. All tests were done in R 3.5.1, package stats (R-Core-Team 2018).

Using the AFLP matrix, a hierarchical AMOVA based on pairwise Euclidean distances between samples was performed uising GenAlEx 6.5 (Peakall & Smouse 2006) to analyse the genetic relationships within and between populations and study regions. A Mantel test was used to analyse whether genetic and geographic distances between populations were correlated (Mantel 1967).

Furthermore, we calculated among-population distances with the program AFLP-SURV (Vekemans 2002) as Nei's standard (Ds) with non-uniform prior distribution of allele frequencies, and constructed a consensus Neighbour-Net graph with Splitstree4 (Huson & Bryant 2006) based on these Ds distances.

We finally applied Bayesian statistics in order to determine the number of clusters most appropriate to represent our AFLP dataset. For this, we ran a Structure analysis with the program STRUCTURE 2.3.4 (Pritchard *et al.* 2000) a simulation run of 10,000 and 100,000 Markov Chain Monte Carlo (MCMC) repeats after burn-in. As ancestry model we chose admixture and applied the Structure analysis for a *K* of 1 to 16 with 20 iterations each. The output data from STRUCTURE were then visualized using STRUCTURE Harvester 0.6.34 (Earl & von Holdt 2012).

In order to visualize our data in space, we conducted nonmetric multidimensional scaling (NMDS) with three dimensions in the R package 'vegan' 2.5-3 (Oksanen *et al.* 2017; R-Core-Team 2018). We applied the function 'metaMDS', which attempts to find a stable solution for the ordination of residuals in space using several random pitches, here 100 at maximum. The distance matrix was calculated using Bray-Curtis dissimilarity, and ordination was performed with a stress level of 0.19, which means that the ordination adequately summarizes the observed distances among the individual samples.

RESULTS

Genetic diversity measured as a percentage of polymorphic bands (PB) ranged from 55.77 to 76.92, Nei's gene diversity

Table 2. Molecular variance within and among populations of *Gypsophila repens* from heath forests and gravel banks along the River Isar calculated with different analyses of molecular variance (AMOVA).

Level of variation	df	SS	MS	%	Φ_{PT}	P
All populations						
Among habitat types	1	20.0	19.954	0	0.110	0.001
Among populations	13	528.8	40.680	12		
Within populations	207	2778.9	13.424	88		
Heath forests						
Among populations	6	233.295	38.883	12	0.119	0.001
Within populations	98	1256.933	12.826	88		
Gravel banks						
Among populations	7	295.544	42.221	12	0.122	0.001
Within populations	109	1521.926	13.963	88		
Upstream and downstream of sylvensteinsee						
Above and below reservoir	1	40,666	40,666	0	0.115	0.001
Among populations	13	508,128	39,087	11		
Within populations	207	2778,859	13,424	88		

SS, sum of squares; MS, mean squares; %, proportion of genetic variability. Levels of significance are based on 999 iteration steps.

(H) varied between 0.22 and 0.31 and Shannon's information index (SI) ranged from 0.33 to 0.46. Generally, genetic variation was slightly lower in heath forest populations than in gravel bank populations (Table 1), but the differences between the two habitat types were not significant (*t*-test $P > 0.05$). Genetic variation was not significantly correlated with population size, geographic distance to the river or to geographic distance from the source spring of the River Isar (Spearman rank correlation $P > 0.05$; Table S2).

With a Φ_{PT} value of 0.110, analyses of molecular variance (AMOVA) revealed moderate differentiation between populations (Table 2). However, in a three level AMOVA arranging the populations into two groups from heath forests or gravel banks, we found no variance among the two habitat types. The degree of genetic variance among populations within the two habitat types was nearly identical ($\Phi_{PT} = 0.119$ and 0.122, respectively). The genetic differentiation among populations upstream and downstream of the the most conspicuous barrier, the man-made reservoir Sylvensteinsee, was nearly identical to the differentiation among all populations or among habitats ($\Phi_{PT} = 0.115$).

Genetic distance among populations was not significantly correlated with geographic distance among populations (Mantel test $r = 0.08$, $P = 0.18$), and in the consensus Neighbour-Net graph, populations from the two habitat types were not separated from each other (Fig. 2); the Bayesian cluster analysis supported this observation (Table S3; Figure S1). Although the dataset most likely consisted of two groups (Table S3), these did not reflect the two habitat types (Fig. 3). Similarly, the populations from heath forests and gravel banks were not separated from each other in the NMDS analysis. Individuals from heath forests were, however, more similar to each other than individuals from the gravel banks, supporting the slight differences in genetic diversity of the populations found in the two habitat types (Fig. 4).
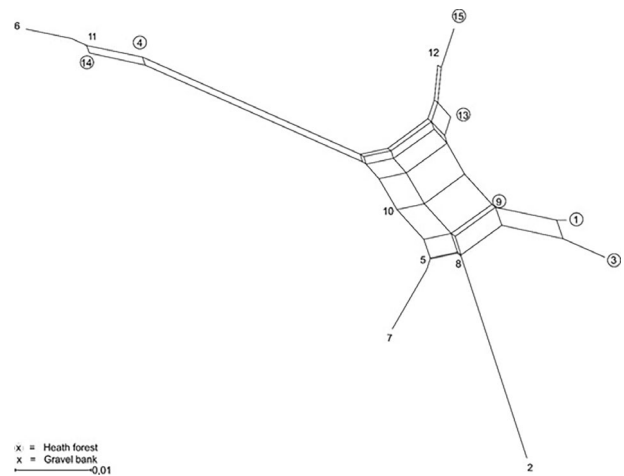

Fig. 2. Neighbour-Net graph visualizing the relationship between the study populations of *Gypsophila repens* based on Nei's genetic distance, determined using the Bayesian method with 999 permutations in the program AFLP-surv. The graph is modified from output derived from Splitstree4 and shows population ID, encircled for heath forest populations and without a circle for gravel bank populations.
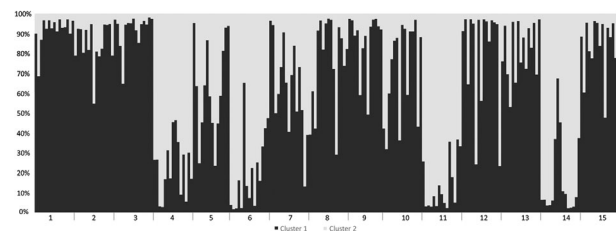

Fig. 3. Results of Bayesian cluster analysis for *Gypsophila repens*. Populations were assigned to two groups ($\Delta K = 97.58$), which did no, however, reflect the two habitat types.
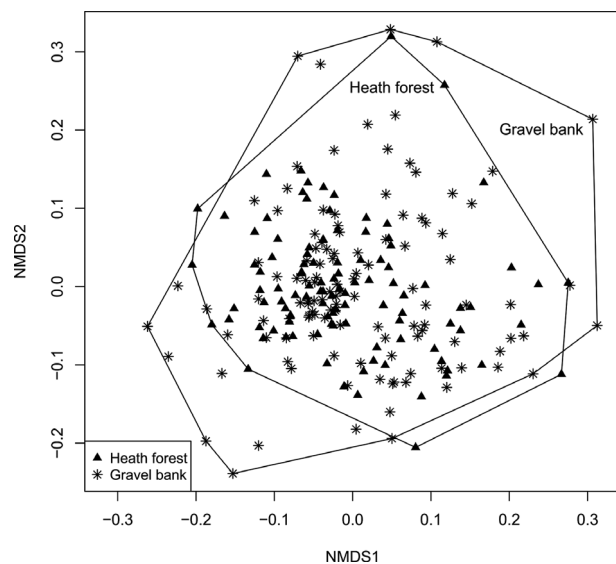

Fig. 4. Nonmetric multidimensional scaling (NMDS) of the binary AFLP dataset displaying the 222 individuals of *Gypsophila repens* from the two analysed habitats (heath forest and gravel bank). The distance matrix was calculated using Bray-Curtis-distances.

DISCUSSION

Genetic diversity and differentiation along the alpine river

In our study, genetic diversity of *G. repens* populations along the River Isar did not increase downstream. Consequently, the results presented here provide no evidence for the 'unidirectional diversity hypothesis' (Ritland 1981), postulating the accumulation of genetic diversity in populations along the lower course of the river. Our study corresponds, however, to many previous investigations, which also failed to demonstrate increased levels of genetic diversity in downstream plant populations (Tero *et al.* 2003; Jacquemyn *et al.* 2006; Kropf *et al.* 2018).

Several reasons can be identified for our observations. Although being more unlikely than the downstream movement of plant material, upstream dispersal of seeds and plant fragments may possibly be related to waterfowl (Pollux *et al.* 2007; Pollux *et al.* 2009). Previously, it was demonstrated that numerous plant species are dispersed by dabbling ducks (Soons *et al.* 2016). Furthermore, roe deer and red deer are quite common in the forests of the Alps and may also contribute to zoochoric upstream seed dispersal along the Isar (Iravani *et al.* 2011; Lepková *et al.* 2018). Moreover, the heath forests along the River Isar were formerly and are partly still grazed by sheep and cattle (Müller 1995; Helbing *et al.* 2014), which might also contribute to dispersal, at least among the populations of *G. repens* in the heath forests. Finally, gene flow among populations of *G. repens* may also be created by pollen transport *via* pollinating insects. The study species is quite common on gravel banks in the study area, especially along the upper course of this river. Pollinators may, therefore, also contribute to upstream gene flow, although pollen dispersal is limited to few kilometres (Kwak *et al.* 1998). Hence, there are numerous mechanisms that can potentially generating upstream gene flow which prevents populations of *G. repens* along the upper course of the river from becoming genetically depleted.

Genetic differentiation among populations along the river was lower than that reported for other perennial and moderately common species (Reisch & Bernhardt-Römermann 2014), reflecting the generally dynamic character of the riparian habitat. Moreover, we detected no impact of the Sylvensteinsee reservoir on genetic differentiation. Anthropogenic constructions, such as dams and reservoirs, seem therefore, in contrast to the results of previous studies (Werth & Scheidegger 2014; Werth *et al.* 2014), not to be a significant barrier for both up- and downstream gene flow among populations of *G. repens*.

Genetic diversity and differentiation within and among heath forests and gravel banks

Considering the postulated effects of ecological disturbance by flooding, we expected lower levels of genetic diversity in populations of *G. repens* in heath forests than on gravel banks. In our analyses we indeed observed slightly lower levels of genetic diversity in heath forests than on gravel banks, although the differences were not statistically significant. However, the results of the NMDS analysis support our observations since individuals located in the heath forests were more similar to each other than individuals located on gravel banks. Considering the comparatively low number of fragments used in our study, we conclude that there is at least weak evidence for increased genetic diversity under conditions of higher

ecological disturbance on gravel banks – a pattern of genetic diversity that has not yet been reported in previous studies for plant species along rivers. Generally, the continuous supply of plant fragments and seeds by hydrochoric dispersal from higher altitudes during flooding (Bill *et al.* 1999; Bill 2000), therefore, seems to promote immigration of new alleles and increases the level of genetic diversity. In contrast to our expectation, we observed similar levels of genetic differentiation among populations from the two habitat types. There are several reasons why the observed difference in genetic diversity was weak and why we found similar levels of genetic differentiation on gravel banks to those in heath forests.

First, water flow along the River Isar has been regulated since the beginning of the last century (Bill 2000). Since then, gravel banks directly along the river are still affected by flooding in spring after snow melt, but the magnitude of the disturbance is clearly smaller than under natural conditions. Both the hydrochoric dispersal of seeds through the river and the preparation of the habitat for successful germination due to flooding may thus be reduced due to water flow regulation. It can, therefore, be assumed that genetic diversity in populations from gravel banks is lower than it would be under natural conditions.

Second, *G. repens* is a long-lived perennial species, which can last for up to 20 years (Schweingruber & Poschlod 2005). Such high plant longevity may decelerate the progressive loss of genetic variation under conditions of lower immigration, as expected for heath forest populations. Moreover, gravel bank and heath forest populations are located geographically close to each other, and gene flow *via* the transport of pollen by pollinators is likely. This may also allow the immigration of new alleles into heath forest populations of *G. repens*. In the AMOVA, we observed no genetic differentiation among the two habitat types and similar levels of genetic differentiation among populations from both heath forests and gravel banks. This result was supported also by the neighbour net, Bayesian cluster and NMDS analysis. A previous study reported genetic differentiation between riparian species growing in different vegetation types along a river system in Australia, which was mainly attributed to the effect of different pollinator communities (Hopley & Byrne 2018). In our study, populations from heath forests and gravel banks seem, however, to be connected by pollen transport between the two habitat types, which are located close to each other. Previous studies also revealed a slight trend for lower differentiation among populations of species growing closer to the river (Honnay *et al.* 2010), which might indicate higher gene flow among populations through water flow (Kudoh & Whigham 1997). The species analysed in these investigations were, however, short-lived, in contrast to the long-lived *G. repens* studied, here. High longevity and close spatial proximity might, therefore, contribute to the pattern of genetic diversity and differentiation observed in the present study.

Finally, it is thought that *G. repens* forms a long-term persistent seed bank (Bill 2000), which supports the survival of species in dynamic habitats. Generally, seed banks contain a considerable amount of genetic variation (Mandák *et al.* 2006; Mandák *et al.* 2012). In a previous meta-study, populations of species building a persistent seed bank were genetically less differentiated than populations of species having no such seed bank (Honnay *et al.* 2008). Furthermore, the emergence of seedlings from the seed bank may represent a form of genetic rescue (Ottewell *et al.* 2011), increasing genetic diversity in

populations suffering from isolation and lack of gene flow. A potential long-term persistent seed bank of *G. repens* may, therefore, contribute significantly to the low differentiation among heath forest and gravel bank populations and will preserve comparatively high levels of genetic diversity in the heath forest populations.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets supporting the conclusions of this article are included within the article. AFLP raw data are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Geographic location and altitude of the study sites.

Table S2. Correlation of genetic diversity in terms of percentage of polymorphic bands (PB), Nei's gene diversity (H), and Shannon's information Index (SI) with geographic distance to the spring of the river, geographic distance to the river and population size.

Table S3. Results of calculations for K values of 1–16 groups with 20 repeats each, derived from the STRUCTURE Harverster output. The maximum of Delta K value at 97.58 for a $K = 2$ indicates that the dataset can be represented appropriately by two groups.

Figure S1. Detection of the number of clusters K that best fits the AFLP-dataset as derived from Structure analysis with 20 replications and $K = 16$. Delta K as well as the estimated mean of Ln (K) indicate that $K = 2$ is adequate to describe the structure in the dataset. The graph is modified from the output derived from Structure Harvester.

REFERENCES

Banks S.S., Cary G.J., Smith A.L., Davies I.D., Driscoll D.A., Gill A.M., Lindenmayer D.B., Peakall R. (2013) How does ecological disturbance influence genetic diversity? *Trends in Ecology & Evolution*, **28**, 671–679.

Bill H.-C. (2000) *Besiedlungsdynamik und Populationsbiologie charakteristischer Pionierpflanzenarten nordalpiner Wildflüsse*. Görich & Weiershauser, Marburg, Germany, 202 pp.

Bill H.-C., Poschlod P., Reich M., Plachter H. (1999) Experiments and observations on seed dispersal by running water in an Alpine floodplain. *Bulletin of the Geobotanical Institute ETH*, **65**, 13–28.

Bonin A., Belleman E., Eidesen P.B., Pompanon F., Brochmann C., Taberlet P. (2004) How to track and assess genotyping errors in population genetic studies. *Molecular Ecology*, **13**, 3261–3273.

Bylebyl K., Poschlod P., Reisch C. (2008) Genetic variation of *Eryngium campestre* L. (Apiaceae) in Central Europe. *Molecular Ecology*, **17**, 3379–3388.

Caponi F., Koch A., Bertoldi W., Vetsch D.F., Siviglia A. (2019) When does vegetation establish on gravel bars? Observations and modeling in the alpine Rhine river. *Frontiers in Environmental Science*, **7**, 124.

Davis C.D., Epps C.W., Flitcroft R.L., Banks M.A. (2018) Refining and defining riverscape genetics: how rivers influence population genetic structure. *Wiley Interdisciplinary Reviews: Water*, **5**, e1269.

Džubàkova K., Molnar P., Schindler K., Trizna M. (2015) Monitoring of riparian vegetation response to flood disturbances using terrestrial photography. *Hydrology and Earth System Sciences*, **19**, 195–208.

Earl D.A., von Holdt B.M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.

Evanno G., Castella E., Antoine C., Paillat G., Goudet J. (2009) Parallel changes in genetic diversity and species diversity following a natural disturbance. *Molecular Ecology*, **18**, 1137–1144.

Frey D., Arrigo N., Granereau G., Sarr A., Felber F., Kozlowski G. (2015) Parallel declines in species and genetic diversity driven by anthropogenic disturbance: a multispecies approach in a French Atlantic dune system. *Evolutionary Applications*, **9**, 479–488.

Gurnell A., Bertoldi W., Corenblit D. (2012) Changing river channels: the roles of hydrological processes, plants, and pioneer fluvial landforms in humid temperate, mixed load, gravel bed rivers. *Earth Science Reviews*, **111**, 129–141.

Gurnell A., Surinan N., Zanoni L. (2009) Multi-thread river channels: a perspective on changing European alpine river systems. *Aquatic Sciences*, **71**, 253–365.

Hegi G. (1979) *Illustrierte Flora von Mitteleuropa. Pteridophyta – Spermatophyta*. Blackwell, Berlin, Germany.

Helbing F., Blaeser T.P., Löffler F., Fartmann T. (2014) Response of Orthoptera communities to succession in alluvial pine woodlands. *Journal of Insect Conservation*, **18**, 215–224.

Hölzel N. (1996) Schneeheide-Kiefernwälder in den mittleren nördlichen Kalkalpen. *Laufener Forschungsberichte*, **3**, 1–192.

Honnay O., Bossuyt B., Jacquemyn H., Shimono A., Uchiyama K. (2008) Can a seed bank maintain the genetic variation in the aboveground plant population? *Oikos*, **117**, 1–5.

Honnay O., Jacquemyn H., Nackaerts K., Breyne P., Van Looy K. (2010) Patterns of population genetic diversity in riparian and aquatic plant species along rivers. *Journal of Biogeography*, **37**, 1730–1739.

Hopley T., Byrne M. (2018) Connectivity in riparian plants: influence of vegetation type and habitat fragmentation overrides water flow. *Oecologia*, **188**, 465–478.

Huson D.H., Bryant D. (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, **23**, 254–267.

Iravani M., Schütz M., Edwards P.J., Risch A.C., Scheidegger C., Wagner H.H. (2011) Seed dispersal in red deer (*Cervus elaphus* L.) dung and its potential

importance for vegetation dynamics in subalpine grasslands. *Basic and Applied Ecology*, **12**, 505–515.

Jacquemyn H., Honnay O., Van Looy K., Breyne P. (2006) Spatiotemporal structure of genetic variation of a spreading plant metapopulation on dynamic riverbanks along the Meuse river. *Heredity*, **96**, 471–478.

Jensen K., Gutekunst K. (2003) Effects of litter on establishment of grassland plant species: the role of seed size and successional status. *Basic and Applied Ecology*, **4**, 579–587.

Kropf M., Huppenberger A.S., Karrer G. (2018) Genetic structuring and diversity patterns along rivers – local invasion history of *Ambrosia artemisiifolia* (Asteraceae) along the Danube River in Vienna (Austria) shows non-linear pattern. *Weed Research*, **58**, 131–140.

Kudoh H., Whigham D.F. (1997) Microgeographic genetic structure and gene flow in *Hibiscus moscheutos* (Malvaceae) populations. *American Journal of Botany*, **84**, 1285–1293.

Kudrnovsky H. (2013) Alpine rivers and their ligneous vegetation with *Myricaria germanica* and riverine landscape diversity in the Eastern Alps: proposing the Isel river system for the Natura 2000 network. *eco.mont*, **5**, 5–18.

Kwak M.M., Velterop O., van Andel J. (1998) Pollen and gene flow in fragmented habitats. *Applied Vegetation Science*, **1**, 37–54.

Lepková B., Horčičková E., Vojta J. (2018) Endozoochorous seed dispersal by free-ranging herbivores in an abandoned landscape. *Plant Ecology*, **219**, 1127–1138.

Leuschner C., Ellenberg H. (Eds) (2017) *Ecology of Central European Non-Forest Vegetation: Coastal to Alpine, Natural to Man-Made Habitats. Vegetation Ecology of Central Europe*, Volume II. Springer, Basel, Switzerland.

Liu Y., Wang Y.-H., Huang H. (2006) High interpopulation genetic differentiation and unidirectional linear migration patterns in *Myricaria laxiflora* (Tamaricaceae), an endemic riparian plant in the

Three Gorges valley of the Yangtze river. *American Journal of Botany*, **93**, 206–215.

López-Villavicencio M., Collin C.L., Shykoff J.A. (2003) No evidence of sex-differential pollen limitation at the flower level in the gynodioecious *Gypsophila repens* infected by *Microbotryum violaceum*. *International Journal of Plant Science*, **164**, 899–905.

Mandák B., Bímová K., Mahelka V., Plačkova I. (2006) How much genetic variation is stored in the seed bank? A study of *Atriplex tatarica* (Chenopodiaceae). *Molecular Ecology*, **15**, 2653–2663.

Mandák B., Zákravský P., Mahelka V., Plačkova I. (2012) Can soil seed banks serve as a genetic memory? A study of three species with contrasting life history strategies? *PLoS ONE*, **7**, e49471.

Mantel N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

Markwith S.H., Scanlon M.J. (2007) Multiscale analysis of *Hemerocallis coronaria* (Amaryllidaceae) genetic diversity, genetic structure, and gene movement under the influence of unidirectional stream flow. *American Journal of Botany*, **94**, 151–160.

Müller N. (1995) Wandel von Flora und Vegetation nordalpiner Wildflußlandschaften unter dem Einfluß des Menschen. *Berichte der Akademie für Naturschutz und Landschaftspflege*, **19**, 125–187.

Oksanen J., Blanchet F.G., Friendly M., Kindt R., Legendre P., McGlinn D., Minchin P.R., O'Hara R.B., Simpson G.L., Solymos P., Stevens M.H.H., Szoecs E., Wagner H. (2017) *vegan: Community Ecology Package. R package version 2.4-3*. R Foundation for Statistical Computing, Vienna, Austria.

Ottewell K.M., Bickerton D.C., Lowe A.J. (2011) Can a seed bank provide demographic and genetic rescue in a declining population of the endangered shrub *Acacia pinguifolia*? *Conservation Genetics*, **12**, 669–678.

Peakall R., Smouse P.E. (2006) GENALEX 6: Genetic analyses in Excel. Population genetic software for teaching and reseach. *Molecular Ecology Notes*, **6**, 288–295.

Pollux B.J.A., Jong M.D.E., Steegh A., Verbruggen E., van Groenendael J.M., Ouborg N.J. (2007) Reproductive strategy, clonal structure and genetic diversity in populations of the aquatic macrophyte

Sparganium emersum in river systems. *Molecular Ecology*, **16**, 313–325.

Pollux B.J.A., Luijten S.H., Van Groenendael J.M., Ouborg N.J. (2009) Gene flow and genetic structure of the aquatic macrophyte *Sparganium emersum* in a linear unidirectional river. *Freshwater Biology*, **54**, 64–76.

Pritchard J.K., Stephens M., Donelly P. (2000) Inferring of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

R-Core-Team (2018) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-project.org/

Reisch C. (2007) Genetic structure of *Saxifraga tridactylites* (Saxifragaceae) from natural and man-made habitats. *Conservation Genetics*, **8**, 893–902.

Reisch C. (2008) Glacial history of *Saxifraga paniculata* (Saxifragaceae) – molecular biogeography of a disjunct arctic-alpine species in Europe and North America. *Biological Journal of the Linnean Society*, **93**, 385–398.

Reisch C., Bernhardt-Römermann M. (2014) The impact of study design and life history traits on genetic variation of plants determined with AFLPs. *Plant Ecology*, **215**, 1493–1511.

Reisch C., Scheitler S. (2008) Disturbance by mowing affects clonal diversity: the genetic structure of *Ranunculus ficaria* (Ranunculaceae) in meadows and forests. *Plant Ecology*, **201**, 699–707.

Ritland K. (1981) Genetic differentiation, diversity and inbreeding in the mountain monkeyflower (*Mimulus caespitosus*) of the Washington Cascades. *Canadian Journal of Botany*, **67**, 2017–2024.

Rogers S.O., Bendich A.J. (1994) Extraction of total cellular DNA from plants, algae and fungi. In: Gelvin S.B., Schilperoort R.A. (Eds), *Plant molecular biology manual*. Kluwer Academic, Dordrecht, the Netherlands, pp 1–8.

Ruprecht E., Enyedi M., Eckstein R.L., Donath T.W. (2010) Restorative removal of plant litter and vegetation 40 years after abandonment enhances re-emergence of steppe grassland vegetation. *Biological Conservation*, **143**, 449–456.

Rusterholz H.P., Kissling M., Baur B. (2009) Disturbance by human trampling alters the performance, sexual reproduction and genetic diversity in a clonal woodland herb. *Perspectives in Plant Ecology, Evolution and Systematics*, **11**, 17–29.

Schweingruber F.H., Poschlod P. (2005) Growth rings in herbs and shrubs: life span, age determination and stem anatomy. *Forest, Snow and Landscape Research*, **79**, 195–415.

Sebald O., Seybold S., Philippi G., Wörz A. (1998) *Farn- und Blütenpflanzen Baden-Württembergs*. Ulmer, Stuttgart, Germany.

Soons M.B., Brochet A.-L., Kleyheeg E., Green A.J. (2016) Seed dispersal by dabbling ducks: an overlooked dispersal pathway for a broad spectrum of plant species. *Journal of Ecology*, **104**, 443–455.

Tero N., Aspi J., Siikamäki P., Jäkäläniemi A., Tuomi J. (2003) Genetic structure and gene flow in a metapopulation of an endangered plant species, *Silene tatarica*. *Molecular Ecology*, **12**, 2073–2085.

Tutin T.G., Heywood V.H., Burgess N.A., Moore D.M., Valentine D.H., Walters S.M., Webb D.A. (Eds) (1964) *Flora Europaea*. Cambridge University Press, Cambridge UK.

Vekemans X. (2002) AFLP-surv version 1.0. Distributed by the author. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.

Vogler F., Reisch C. (2011) Genetic variation on the rocks – the impact of climbing on the population ecology of a typical cliff plant. *Journal of Applied Ecology*, **48**, 899–905.

Werth S., Scheidegger C. (2014) Gene flow within and between catchments in the threatened riparian plant *Myricaria germanica*. *PLoS ONE*, **9**, e99400.

Werth S., Schödl M., Scheidegger C. (2014) Dams and canyons disrupt gene flow among populations of a threatened riparian plant. *Freshwater Biology*, **59**, 2502–2515.

Yeh F.C., Yang R.C., Boyles T.B.J., Ye Z.H., Mao J.X. (1997) *POPGENE, the user-friendly shareware for population genetic analysis*. Molecular Biology and Biotechnology Centre, Alberta, Canada.