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

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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 (Hon-nay 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 (Hon-nay 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; Dzubakova 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.
Genetic variation of Gypsophila repens

Reisch & Sattler

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...
DNA stock solutions were diluted with water to 7.8 ng was extracted using the CTAB method established by Rogers & From the sampled leaf material, DNA for molecular analyses

### 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  | 15 | 15 | 0.00    | 2313.8 | 67.31 | 0.27 | 0.40 |
| P02  | Gravel bank   | 15 | 15 | 21.90   | 14.6   | 79.81 | 0.34 | 0.49 |
| P03  | Heath forest  | 15 | 15 | 26.71   | 256.1  | 71.15 | 0.28 | 0.41 |
| P04  | Heath forest  | 15 | 15 | 33.11   | 97.9   | 61.54 | 0.24 | 0.35 |
| P05  | Gravel bank   | 15 | 15 | 56.82   | 2.4    | 76.92 | 0.31 | 0.45 |
| P06  | Gravel bank   | 15 | 15 | 58.04   | 672.0  | 61.54 | 0.22 | 0.33 |
| P07  | Gravel bank   | 15 | 15 | 65.25   | 169.9  | 74.04 | 0.29 | 0.42 |
| P08  | Heath forest  | 15 | 15 | 63.13   | 23.7   | 75.96 | 0.31 | 0.44 |
| P09  | Gravel bank   | 15 | 15 | 63.13   | 23.7   | 77.88 | 0.31 | 0.46 |
| P10  | Gravel bank   | 15 | 15 | 69.08   | 106.6  | 69.23 | 0.30 | 0.42 |
| P11  | Gravel bank   | 15 | 15 | 74.11   | 8.6    | 65.38 | 0.24 | 0.36 |
| P12  | Gravel bank   | 15 | 15 | 77.47   | 161.6  | 57.69 | 0.25 | 0.36 |
| P13  | Heath forest  | 15 | 15 | 87.59   | 125.4  | 62.50 | 0.26 | 0.37 |
| P14  | Heath forest  | 15 | 15 | 95.88   | 157.8  | 67.31 | 0.25 | 0.37 |
| P15  | Heath forest  | 15 | 15 | 104.50  | 12.0   | 57.77 | 0.22 | 0.33 |
| Mean | Gravel banks  | 15 | 15 | 144.92  | 70.31  | 70.31 | 0.28 | 0.41 |
| Mean | Heath forests | 15 | 15 | 426.68  | 65.93  | 65.93 | 0.26 | 0.38 |

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 \( H = 1 - \sum (p_i)^2 \) and Shannon’s information index \( SI = \sum (p_i) \ln (p_i) \), 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 using 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 (100,000 for *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 non-metric 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...
Genetic variation of *Gypsophila repens* 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<sub>PT</sub> 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 (Φ<sub>PT</sub> = 0.119 and 0.122, respectively). The genetic differentiation among populations upstream and downstream of the most conspicuous barrier, the man-made reservoir Sylvensteinsee, was nearly identical to the differentiation among all populations or among habitats (Φ<sub>PT</sub> = 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).

| Level of variation | df | SS  | MS   | %   | Φ<sub>PT</sub> | *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  |               |      |

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

![Fig. 2.](image-url) 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.](image-url) Results of Bayesian cluster analysis for *Gypsophila repens*. Populations were assigned to two groups (ΔK = 97.58), which did no, however, reflect the two habitat types.

![Fig. 4.](image-url) 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 (Pollex et al. 2007; Pollex 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 (Irvani 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 species studied is quite common on gravel banks in the study area, especially along the upper course of the 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 upstream 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.

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**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.

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**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 Harvester 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.
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