

Dams and canyons disrupt gene flow among populations of a threatened riparian plant

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SUMMARY

1. Natural and man-made barriers have a significant impact on the population structure and genetic diversity of aquatic species. Little is known whether terrestrial, riparian species are affected in the same way by barriers.

2. Here, we analysed the genetic structure of a threatened riparian shrub based on 20 microsatellite markers and 1176 individuals collected from 34 localities in four Alpine rivers in Europe (Isar, Lech, Rhine and Tagliamento). The catchments comprised a variety of potential barriers to gene flow for terrestrial riparian plants: dams, channelised stretches of river and canyons. We compared the average level of genetic differentiation (pairwise F_{ST}) between connected localities and localities isolated by natural or man-made barriers.

3. Genetic differentiation was higher between localities isolated by a barrier than between connected localities, indicating genetic isolation caused by barriers. This increased degree of genetic differentiation across barriers was evident in geographic tree models.

4. Our results demonstrate genetic isolation effects due to large dams in a characteristic plant of Central European floodplains, highlighting that these types of barriers may negatively impact riparian plant populations. We also found genetic isolation effects in populations separated by natural barriers (canyons). These results have important implications for understanding the dynamics of riparian plant populations and for designing suitable management strategies for an effective conservation of riparian plants.

Keywords: barriers, gene flow, genetic structure, habitat fragmentation, *Myricaria germanica* (German Tamarisk), population genetics, population subdivision, riparian

Introduction

River corrections, including the straightening and channelisation of river beds, as well as the interruption of connectivity through dams and other barriers, have long-lasting negative impacts on the biodiversity of riparian species. Often, specialised species which require dynamic habitats are affected (Jansson, Nilsson & Dynesius, 2000a; Jansson, Nilsson & Renofalt, 2000b). Numerous examples have shown that the fragmentation of riparian habitats by the construction of dams, or by straightening and channelisation of rivers, may either

lead to local extinction of specialist species, or to a drastic decrease in their local population sizes (Andersson, Nilsson & Johansson, 2000; Jansson *et al.*, 2000a,b; Merritt & Wohl, 2006; Merritt, Nilsson & Jansson, 2010). The remaining populations in a fragmented riparian habitat may suffer the negative consequences of small population size, which include increased risk of extinction and negative effects of inbreeding (Barrett & Kohn, 1991; Ellstrand & Elam, 1993; Lande, 1993; Honnay *et al.*, 2005).

A number of plant species are confined to riverine habitats, that is the floodplains, islands and gravel bars along rivers. For many of these riparian plants, hydroch-

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ory is a key dispersal mode (Müller & Scharm, 2001; Nilsson *et al.*, 2010; Wang *et al.*, 2011), and as a consequence, unregulated rivers where longitudinal connectivity is retained often receive more propagules, which may lead to a marked increase in plant diversity, relative to that of regulated rivers where propagule flow is reduced (Jansson *et al.*, 2005). These effects are most visible in plants whose seeds are predominantly dispersed by hydrochory; dams may not disrupt the dispersal of seeds as severely in wind-dispersed species (Nilsson *et al.*, 2010). While the effect of river regulation on plant diversity has received some attention, the consequences that river regulations may have at other levels of biodiversity, such as the genetic diversity and the genetic structure of species, are less well understood (but see Kikuchi, Suzuki & Sashimura, 2011).

The fragmentation of riparian habitats by impoundments, dams and reservoirs can lead to increased isolation of populations upstream and downstream of barriers. For example, in aquatic species, numerous studies have documented increased isolation up- and downstream of man-made barriers (Neraas & Spruell, 2001; Meldgaard, Nielsen & Loeschke, 2003; Yamamoto *et al.*, 2004; Heggenes & Roed, 2006). Consequences of fragmentation of riparian habitats for terrestrial species are less studied. One might argue that especially in small populations, random genetic drift is an important mechanism that leads to an expected shift in allele frequencies if population size is small over several generations and hence leads to an increase in population structure. In terrestrial ecosystems such as forests, habitat fragmentation may ironically result in increased connectivity among fragments in insect-pollinated species. This may be the case, for example, if plant density is reduced by habitat fragmentation, leading to increased long-distance movement of insect pollinators, and thus, higher rates of gene flow among the isolated fragments than within continuous populations (Sork *et al.*, 2005). For riparian habitats, we would not expect this effect for two main reasons: (i) populations are most often not dense in the first place; and (ii) the barriers created by human activities are often large and represent man-made structures or artificial lakes, at the scale of hundreds to thousands of metres and are thus unattractive for pollinator movement. Such a distance may also rarely be crossed by long-distance seed dispersal events.

Some riparian plant species persist as metapopulations along rivers (Liu, Wang & Huang, 2006; Honnay *et al.*, 2009). The colonised patches within metapopulations are characterised by colonisation–extinction dynamics (Levins, 1966, 1968, 1969; Hanski, 1994, 1998) and a high

turnover of patches, with local extinctions (e.g. because of flooding and new colonisations of newly formed patches). A metapopulation can only persist in the long term if riparian habitats are functionally connected. Breaks in longitudinal connectivity – for instance through dams or other barriers – may hinder colonisation and, if severe, might eventually lead to the extinction of the entire metapopulation. On the other hand, a species can persist in a river segment as long as the disconnected parts of a riparian metapopulation are above a critical threshold of dispersal, enabling a sufficient number of patch colonisations to balance local extinctions (Hanski, 1994; Hanski & Gaggiotti, 2004). If local population sizes in a metapopulation are small over the course of several generations, the combined effects of founder events and genetic drift are expected to lead to genetic differentiation between the isolated parts of the metapopulation.

Various structures in a catchment may represent more or less strong barriers to gene movement (Alp *et al.*, 2012; Hudman & Gido, 2013). We expect that canyons and channelised river segments function as semipermeable barriers, and neither one will completely impede the gene movement of riparian plants. Canyons are semipermeable for plants due to a lack of habitats and stepping stones for colonisation and the tendency of seeds to sink to the bottom as an effect of water turbulence. Yet, we would expect occasional seed movement across canyons. Hence, a less severe effect on the genetic structure of populations is expected than in the case of artificial lakes which may completely prevent migration via hydrochory because the standing water does not transport seeds. Channelised stretches of rivers may act as barriers to gene flow in riparian species if the channel width is narrow, leaving little or no habitat to be colonised. In cases where the distance between embankments is high enough to leave some riparian habitat, the remaining habitat is often prone to severe flooding and, as a consequence, may experience a high mortality of juvenile plants – areas within narrow channelised stretches are thus expected to behave as sinks for dispersed seeds and shoots of riparian species. In Central Europe, most dams and channelised river stretches have been constructed over the past two centuries. Genetic patterns will to some degree reflect the historical connectivity among localities, unless local population sizes are small. Natural barriers typically date back hundreds of generations and could thus leave visible traces in the genetic structure of riparian species even if they lead to only small decreases of migration rates. Genetic effects of isolation could be apparent if connectivity is hindered

subtly by old natural barriers, or if it is impeded strongly by man-made barriers.

Myricaria germanica is a typical riparian shrub growing on gravel banks along the upstream reaches of rivers. This character plant of riparian vegetation (Moor, 1958; Ellenberg, 1963) has declined severely in Central Europe due to habitat loss associated with river channelisation and gravel extraction during the past two centuries (Endress, 1975). The species requires habitats with an intermediate frequency of gravel-bank transforming flooding events (Alp *et al.*, 2011). Being a habitat specialist and a species forming metapopulations along rivers, it is expected that this species responds strongly to various properties of the riparian landscape, such as the extent of river dynamics (shaping the rates of turnover and age of patches), channel width and the presence of barriers.

Here, we studied the genetic structure of natural populations of *M. germanica* in four major Alpine rivers of Central Europe which are fragmented by natural and anthropogenic barriers. Our first aim was to compare genetic diversity among catchments to test the hypothesis that catchments showed similar levels of diversity. Second, we tested the hypothesis that physical barriers lead to reduced gene movement between populations. If habitat fragmentation by barriers had no impact on gene flow, genetic differentiation should be similar between populations separated by physical barriers and connected populations. Moreover, if gene movement across barriers was frequent, genetic groups detected by Bayesian cluster analysis should be found on either side of a physical barrier. The results will thus have important implications for the conservation of riparian plant populations.

Methods

Study species

Myricaria germanica (Tamaricaceae) is a riparian shrub which occurs along natural and near-natural rivers in Europe and Asia. Maximum age of individuals of 21 years (Schweingruber, Münch & Schwarz, 2007) and 70 years (Frisendahl, 1921) has been reported in the literature, but due to the natural dynamics of its habitat, most individuals will live shorter. *Myricaria germanica* is expected to have a short generation time, similar to other species belonging to the same genus. For instance, the riparian shrub *M. laxiflora* starts flowering early, after its first year (Chen & Xie, 2007). Details of the mating system in the insect-pollinated, hermaphroditic plant *M. germanica* are not known, but another species of the

genus is predominantly selfing (Liu *et al.*, 2006). Also *M. germanica* is capable of selfing (Werth & Scheidegger, 2014). The species' natural worldwide distribution is restricted to mountain regions of Europe and Asia, that is the Alps, Pyrenees, Scandes, Appenine, Carpathians, Caucasus and the Himalayas (Hultén & Fries, 1986). As the only species of its family naturally occurring in Central Europe, *M. germanica* grows on gravel banks along rivers from the colline to the subalpine altitudinal zone (500–2100 m a.s.l.). Being a pioneer species on gravel bars, *M. germanica* forms metapopulations, with frequent colonisations of new patches and extinction of existing populations after disturbance by flooding. When rivers are channelised in a way that suitable habitat is lacking, in particular localities with intermediate disturbance frequencies, the species goes locally extinct. The combined effects of river channelisation and habitat destruction have led to a severe decline of *M. germanica* in Central Europe in the past decades. For example, once a rather common species on the Swiss Plateau, *M. germanica* is now restricted to few local populations in this region. Nevertheless, the species is still locally abundantly along several Alpine rivers (Müller & Scharm, 2001; Werth & Scheidegger, 2014).

Study area

Our sampling included nine localities in the Isar catchment in southern Germany, six localities in the Lech catchment in north-western Austria, 10 localities situated in the Rhine catchment in eastern Switzerland and nine localities in the Tagliamento catchment located in north-eastern Italy (Table 1, Fig. 1).

In the Isar catchment, a channelised stretch of river spanning over 1 km and lacking suitable habitat for *M. germanica* separated localities ISA4 and ISA9 from locality ISA3. Next, the 'Stauwehr Krün' dam built in 1924 separated localities ISA4 and ISA9 from localities IS10, ISA5, ISA8 and RIS1. The dam was constructed in a place where the river was wide and braided (i.e. no prior natural barrier). A second dam, the large artificial lake 'Sylvensteinspeicher' finalised in 1959 separated the latter localities from ISA6 and IS12. In the place where the Sylvenstein dam was constructed, there was a small canyon. Between 1924 and 1989, most of the Isar water was pumped into the Walchensee lake from Stauwehr Krün for the production of hydroelectric power, having left a large stretch of river falling dry periodically. Water ran on average for only 60 days a year due to diversion. Since 1990, a continuous minimum discharge has been enforced.

Table 1 Summary statistics of the collecting localities included in the analysis of *Myricaria germanica*, showing population name, biogeographic region (Region), affiliation of localities to catchments and rivers, number of samples analysed, GPS coordinates (map datum WGS84), altitude [m a.s.l.], mid-point of the estimated interval of population size (Pop. size), gene diversity H_E and allelic richness A_R

Pop	Region	Catchment	River	N	Longitude	Latitude	Altitude	Pop. size	H_E	A_R
MRH1	Eastern Central Alps	Rhine	Mittelrhein	40	8.85450	46.67430	1250	75	0.280	1.808
RHE1	North side of Alps	Rhine	Alpenrhein	33	9.54676	46.93969	540	350	0.384	2.545
RHE2	Eastern Central Alps	Rhine	Hinterrhein	40	9.41057	46.78643	610	350	0.338	2.440
RHE3	North side of Alps	Rhine	Alpenrhein	44	9.49150	47.07397	470	175	0.390	2.579
RHE4	North side of Alps	Rhine	Alpenrhein	40	9.54792	46.96048	570	50	0.358	2.397
RHE5	North side of Alps	Rhine	Alpenrhein	18	9.54192	47.27791	410	19	0.075	1.504
RHE6	North side of Alps	Rhine	Alpenrhein	17	9.50798	47.01930	500	17	0.395	2.473
VRH1	Eastern Central Alps	Rhine	Vorderrhein	41	9.24086	46.78521	900	350	0.259	1.743
VRH2	Eastern Central Alps	Rhine	Vorderrhein	21	9.17500	46.77272	710	20	0.302	2.059
VRH4	Eastern Central Alps	Rhine	Vorderrhein	41	8.95735	46.72708	900	75	0.422	2.500
IS10	North side of Alps	Isar	Isar	40	11.32675	47.53039	880	50–100	0.365	2.248
IS12	North side of Alps	Isar	Isar	39	11.58139	47.60847	710	≥39	0.365	2.324
ISA3	North side of Alps	Isar	Isar	20	11.25932	47.42278	1000	20	0.279	1.829
ISA4	North side of Alps	Isar	Isar	42	11.28016	47.47886	350	200–500	0.339	2.038
ISA5	North side of Alps	Isar	Isar	40	11.40353	47.55225	800	≥40	0.341	2.061
ISA6	North side of Alps	Isar	Isar	38	11.55689	47.59720	740	50–100	0.385	2.564
ISA8	North side of Alps	Isar	Isar	32	11.41287	47.55205	800	≥32	0.351	2.310
ISA9	North side of Alps	Isar	Isar	38	11.28092	47.47181	350	100–200	0.347	2.119
RIS1	North side of Alps	Isar	Rissbach	8	11.44014	47.53408	810	8	0.355	2.035
LEC1	North side of Alps	Lech	Lech	40	10.60328	47.43003	950	200–300	0.389	2.517
LEC2	North side of Alps	Lech	Lech	39	10.57707	47.42203	920	100–150	0.365	2.440
LEC3	North side of Alps	Lech	Lech	35	10.62825	47.43238	887	1000–1500	0.395	2.381
LEC4	North side of Alps	Lech	Lech	40	10.52215	47.32860	1028	500–600	0.352	2.373
LEC5	North side of Alps	Lech	Lech	7	10.67406	47.55460	800	7	0.391	2.350
HAL1	North side of Alps	Lech	Halblech	40	10.79632	47.64967	780	≥40	0.281	1.817
TAG1	South side of Alps	Tagliamento	Tagliamento	46	12.67208	46.38588	690	200–300	0.035	1.171
TAG2	South side of Alps	Tagliamento	Torrente But	19	13.00841	46.41395	330	19	0.130	1.418
TAG3	South side of Alps	Tagliamento	Torrente But	39	13.00950	46.41818	340	100–200	0.186	1.502
TAG4	South side of Alps	Tagliamento	Tagliamento	38	13.11175	46.36391	230	2000–3000	0.094	1.345
TAG6	South side of Alps	Tagliamento	Fella	40	13.20573	46.39952	308	200–300	0.089	1.311
TAG7	South side of Alps	Tagliamento	Tagliamento	42	12.97113	46.20287	140	200–300	0.165	1.531
TAG8	South side of Alps	Tagliamento	Tagliamento	38	12.99471	46.20678	140	50–100	0.211	1.902
TAG9	South side of Alps	Tagliamento	Tagliamento	42	13.00997	46.21103	150	50–100	0.184	1.774
TA10	South side of Alps	Tagliamento	Tagliamento	39	13.01790	46.21230	155	39	0.200	1.728

One single anthropogenic barrier is found at the Lech River, where the Forggensee dam built in 1952 separates HAL1 situated at Halblech from the remaining five localities in the Lech catchment. Halblech is a tributary of the river Lech.

The Rhine catchment is structured by multiple barriers: a canyon separating locality MRH1 from populations downstream, a channelised stretch of river separating locality VRH4 from downstream sites and a long, narrow canyon separating VRH1 and VRH2 from all other localities downstream. Additionally, the dam near Tamins built in 1962 separates RHE1 from all localities situated upstream.

Lech and Tagliamento included extensive stretches of river without any natural or anthropogenic barriers. There was a single natural barrier in the upstream area of Tagliamento, a canyon separating population TAG1

from the remaining populations. Moreover, large stretches of the river bed and alluvial plain at Tagliamento are dry during the summer months and are hence not suitable for the survival of juveniles. At Tagliamento, *M. germanica* is found only in certain areas, for example where ground water rises to the surface, creating suitable habitat, or where the river does not become entirely dry.

Sampling design

In each catchment, we intended to analyse 10 populations (referred to as 'localities') with a minimum distance of 500 m between them. Fewer localities were analysed in catchments where the species was less frequent. Tissue samples were collected from apical tips of branches of *M. germanica* lacking flowers. We carefully avoided

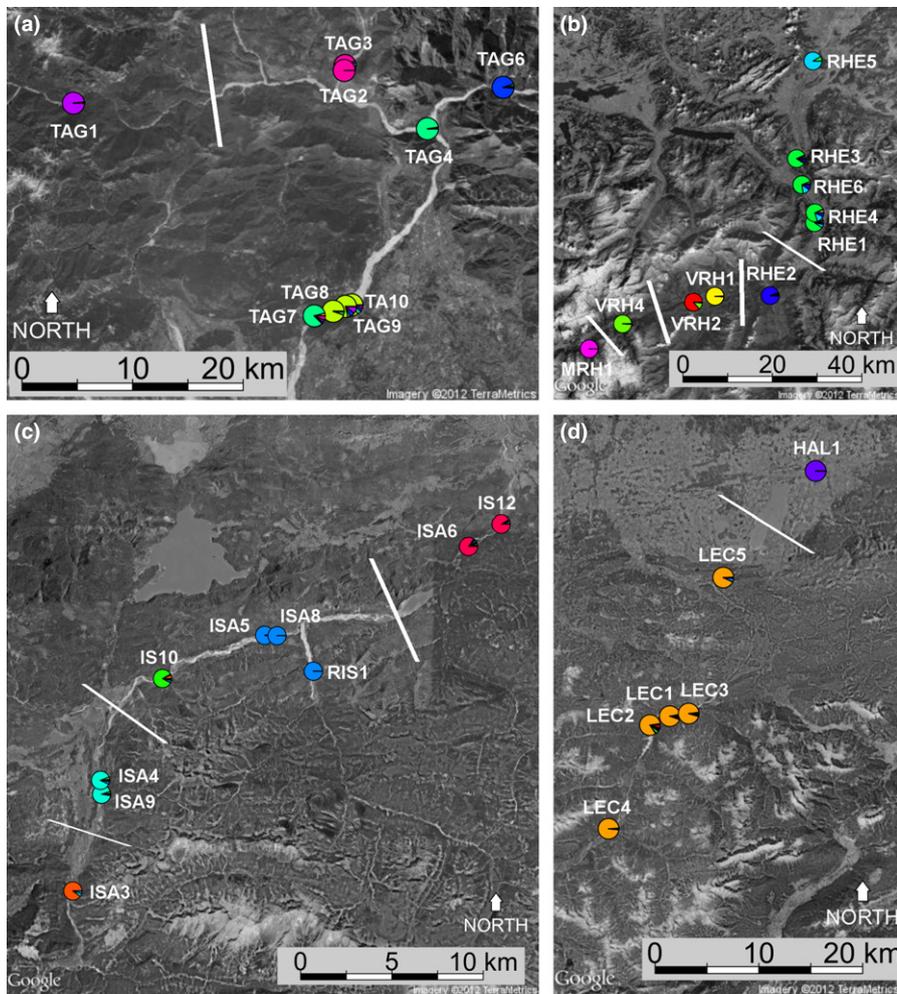


Fig. 1 Spatial location of sampling localities and affiliation to genetic clusters as determined by admixture analysis in BAPS. Natural and man-made barriers in a catchment are indicated by white lines. (a) Rhine. (b) Lech. (c) Isar. (d) Tagliamento. (a) Flow direction downwards. (b–d) Direction of water flow to the upper right.

including seeds deposited on the plant. The tissue was stored on silica gel at room temperature until DNA extraction. In large populations, we sampled 40 adult plants along a transect through the population following the flow direction of the river, leaving a minimum distance of 2 m between adjacent sampled plants. In small populations, all individuals were collected. In each locality, we recorded GPS coordinates and estimated population size either by counting all individuals (small populations), or by counting a subset of individuals and extrapolating to the estimated total area harbouring plants; the latter estimates are given as intervals to account for uncertainties in estimation of the total area of the population. In subsequent analyses, interval mid-points were used.

Molecular analysis

DNA extraction was performed using the Qiagen DNeasy 96 Plant Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. PCR, fragment

analyses and genotyping of 22 nuclear microsatellites were performed as described in Werth & Scheidegger (2011). We excluded the loci Mg461 and Mg482 because they did not amplify consistently across all populations. Thus, analyses are based on data from 20 loci.

Data analysis

Genetic variability of localities and inbreeding. Quantifying the genetic variability of populations of *M. germanica* at the level of collecting localities is a first step to understand the species' overall genetic structure and provides important background information for any interpretations of genetic data. Hence, we calculated Nei's gene diversity (H_E) and allelic richness (A_R) using FSTAT version 2.93 (Goudet 1995) for each population, and used these values for comparisons between catchments.

Mantel tests and F_{ST} analysis. Genetic distance between pairs of populations was quantified using pairwise F_{ST} values, calculated with Arlequin version 3.5 (Excoffier,

Laval & Schneider, 2005). We used Mantel tests (Mantel, 1967) to assess whether genetic distance and geographic distance were correlated, as expected under isolation by distance. Mantel tests were performed using the library 'vegan' (Dixon, 2003; Oksanen *et al.*, 2010) in R (R Development Core Team, 2013). Another set of Mantel tests quantified whether there was a correlation between genetic distance and the presence of putative barriers to gene flow (channelised river segments, canyons, embankments). Partial Mantel tests quantified: (i) whether there was an effect of barriers when geography had been accounted for; and (ii) whether there was an effect of geography when barriers had been accounted for. We also calculated the average differentiation (F_{ST}) between connected localities vs. localities isolated by barriers and used mean separation tests to infer whether the average F_{ST} differed between groups. Student's *t*-tests assuming unequal variance of groups were used for this purpose. We accounted for multiple testing using Holm's correction (Holm, 1979) using the function 'p.adjust' in R.

Population structure. To quantify the amount of variability in populations of *M. germanica* associated with various hierarchical levels of population structure, we performed analysis of molecular variance in Arlequin. The *F*-statistics in the analysis of molecular variance were based on the number of different alleles, and significance of variance components was tested with 1000 permutations (Excoffier, Smouse & Quattro, 1992). Populations were grouped: (i) by catchment, that is Rhine, Danube, Po and Rhone; or (ii) by grouping by catchments as well as man-made and natural barriers (impoundments, channelised river stretches, canyons). The aim of this analysis was to evaluate whether adding putative barriers to gene flow increased population structure, as expected if these barriers indeed hindered the movement of genes.

Secondly, within each catchment, we attempted to define groups of individuals with a similar genetic composition by means of Bayesian assignment to test whether natural or anthropogenic barriers had an isolating effect on populations, leading to different panmictic groups upstream and downstream of a barrier. Bayesian analysis of population structure was performed using BAPS version 5.4 (Corander, Waldmann & Sillanpää, 2003; Corander *et al.*, 2004). First, we used mixture analysis (Corander, Marttinen & Mäntyniemi, 2006; Corander *et al.*, 2008) clustering groups of individuals to find the best partitioning of the data set, using the number of localities ($N = 34$) as an upper limit of popula-

tions. For the best partition ($N = 19$), we repeated the analysis with 10 runs to get the best partitioning. Then, we used admixture analysis (Corander & Marttinen, 2006; Corander *et al.*, 2008) to identify admixed individuals. The results were plotted in R, using the R libraries 'RgoogleMaps' (Loecher, 2012), 'plotrix' (Lemon *et al.*, 2012), 'GISTools' (Brunsdon & Chen, 2012), 'SDMTools' (Vanderwal *et al.*, 2012), 'shape' (Soetaert, 2012) and 'mapplots' (Gerritsen, 2012).

Geographic tree models. We built geographic tree models using the software GenGIS version 2.0 (Parks *et al.*, 2009). First, neighbour-joining trees were generated with 1000 bootstraps using the models 'seqboot', 'gendist.exe' and 'neighbor' in phylip version 3.695 (Felsenstein, 1989) based on chord distance (Cavalli-Sforza & Edwards, 1967). Then, a majority-rule consensus tree was built using the 'consense' module of phylip. The consensus tree was mid-point-rooted with FigTree version 1.2.1 (Rambaut, 2008) and plotted onto a raster map of the Alps region with GenGIS version 2.0 (Parks *et al.*, 2009, 2013).

Results

Locality TAG5 from the Tagliamento river was omitted as it contributed little information on diversity or genetic structure – it only contained two individuals. After pruning these individuals from the data, our total data set comprised microsatellite genotypes from 1176 individuals. Our final data set contained genotypes at 20 nuclear microsatellite loci for all but two individuals (ISA8-29, MRH1-15), which had missing data for one locus each (Mg442 and Mg452, respectively).

Genetic variability of localities and catchments. Genetic variability was low to moderate in the studied localities (A_R ranging from 1.2 to 2.6, H_E ranging from 0.04 to 0.42, Table 1). The Isar, Lech and Rhine catchments exhibited similar levels of genetic diversity, but genetic diversity was low at Tagliamento (Fig. 2).

Mantel tests and F_{ST} analysis. In three of four catchments, there was significant isolation by distance when the effect of barriers had been removed (Table 2). In two of four catchments, significant effects of barriers were found after accounting for the effect of geography. The catchments which lacked a significant association of genetic distance with barriers (Lech and Tagliamento) each had only a single collecting locality beyond the barrier and hence, fewer comparisons among populations

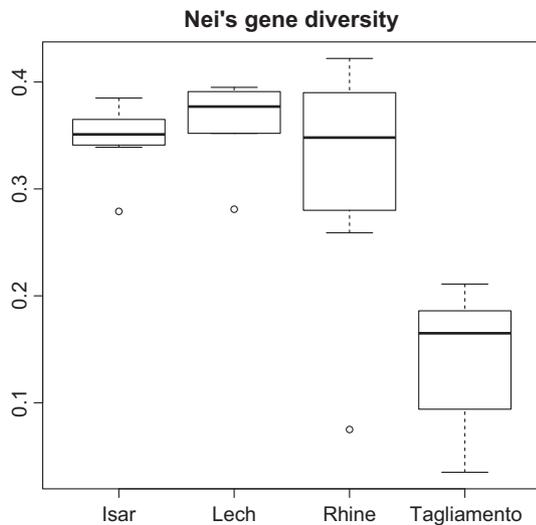


Fig. 2 Boxplots of Nei's gene diversity (H_E), grouping collecting localities by catchment.

were made, leading to lower power of the analysis. Nevertheless, mean separation tests indicated that the average F_{ST} of localities separated by a putative barrier differed significantly from that of connected localities, also in these two catchments (Table 2, Fig. 3). Our F_{ST} analysis revealed that average F_{ST} values differed between isolated and connected localities when canyons and impoundments were the putative barriers. F_{ST} values were lower for connected localities than for isolated ones, as expected if connected localities had higher rates of gene flow. Average F_{ST} values did not differ between connected and isolated localities when the putative barrier was a channelised river segment. In the Isar catchment, the average F_{ST} was significantly larger between populations separated by Sylvenspeicher than between those separated by Stauwehr Krün (one-sided Student's t -test, $t = -2.36$, d.f. = 7.7, $P = 0.023$; difference in means 0.046).

Population structure. Results from analysis of molecular variance showed that populations of *M. germanica* were significantly subdivided; when barriers and catchments were used to define groups of populations, the between-group differentiation was 21% higher than when the grouping was based on catchment alone (Table 3). In accordance with analysis of molecular variance which showed substantial population structure, admixture analysis performed with BAPS resulted in well-defined genetic clusters (Fig. 1). The spatial distribution of genetic clusters was related to the presence of physical barriers in the landscape. For instance, in all catchments, different genetic clusters were found on opposite sides

Table 2 Mantel tests, partial Mantel tests and F_{ST} values quantifying the genetic isolation effects of channelised river stretches, natural barriers (canyons) and of impoundments for the riparian shrub *Myricaria germanica**

Model	Mantel test		Mean F_{ST} value	
	r_M	P -value	Connected	Isolated
Isar catchment				
$F_{ST} \sim \text{geo.dist}$	0.6871	0.001		
$F_{ST} \sim \text{barrier}$	0.6542	0.002		
$F_{ST} \sim \text{geo.dist (barrier)}$	0.4239	0.010		
$F_{ST} \sim \text{barrier (geo.dist)}$	0.3333	0.022		
Channelised segment			0.1123 ^a	0.1453 ^b
$F_{ST} \sim \text{barrier}$	0.2946	0.231		
$F_{ST} \sim \text{barrier (geo.dist)}$	0.1539	0.238		
Impoundment			0.0706 ^a	0.1385 ^b
$F_{ST} \sim \text{barrier}$	0.6542	0.003		
$F_{ST} \sim \text{barrier (geo.dist)}$	0.3333	0.028		
Lech catchment				
$F_{ST} \sim \text{geo.dist}$	0.6561	0.084		
$F_{ST} \sim \text{barrier}$	0.9623	0.175		
$F_{ST} \sim \text{geo.dist (barrier)}$	-0.0946	0.440		
$F_{ST} \sim \text{barrier (geo.dist)}$	0.9334	0.082		
Channelised segment			0.099 ^a	0.112 ^a
$F_{ST} \sim \text{barrier}$	0.0739	0.3866		
$F_{ST} \sim \text{barrier (geo.dist)}$	-0.2293	0.7501		
Impoundment			0.055 ^a	0.216 ^b
$F_{ST} \sim \text{barrier}$	0.9623	0.1674		
$F_{ST} \sim \text{barrier (geo.dist)}$	0.9334	0.0798		
Rhine catchment				
$F_{ST} \sim \text{geo.dist}$	0.7018	0.001		
$F_{ST} \sim \text{barrier}$	0.6312	0.013		
$F_{ST} \sim \text{geo.dist (barrier)}$	0.5681	0.004		
$F_{ST} \sim \text{barrier (geo.dist)}$	0.4441	0.047		
Canyon			0.222 ^a	0.411 ^b
$F_{ST} \sim \text{barrier}$	0.6097	0.0047		
$F_{ST} \sim \text{barrier (geo.dist)}$	0.3593	0.0565		
Channelised segment			0.218 ^a	0.341 ^a
$F_{ST} \sim \text{barrier}$	0.1660	0.1675		
$F_{ST} \sim \text{barrier (geo.dist)}$	-0.0781	0.6806		
Impoundment			0.278 ^a	0.382 ^b
$F_{ST} \sim \text{barrier}$	0.3368	0.0073		
$F_{ST} \sim \text{barrier (geo.dist)}$	-0.2473	0.8880		
Tagliamento catchment				
$F_{ST} \sim \text{geo.dist}$	0.5705	0.003		
$F_{ST} \sim \text{barrier}$	0.6100	0.134		
$F_{ST} \sim \text{geo.dist (barrier)}$	0.3102	0.010		
$F_{ST} \sim \text{barrier (geo.dist)}$	0.3982	0.165		
Canyon			0.228 ^a	0.499 ^b
$F_{ST} \sim \text{barrier}$	0.6100	0.1122		
$F_{ST} \sim \text{barrier (geo.dist)}$	0.3982	0.1599		

*In partial Mantel tests, the relationship between the first and second matrix is investigated while keeping the third matrix (in parentheses) constant. The table gives average genetic differentiation (pairwise F_{ST}) of connected vs. isolated localities in the fourth and fifth column, given the respective type of barrier. Letters in superscripts indicate whether average values of F_{ST} differed significantly among connected and isolated localities, as determined by paired Student's t -tests assuming unequal variance of groups.

Table 3 Results from analysis of molecular variance (AMOVA) in the threatened riparian shrub *Myricaria germanica*, nesting populations within four catchments (above), or defining groups by the location of physical barriers (canyons, reservoirs) within catchments and by catchment, resulting in a total of 12 groups[†]

Model	Source	d.f.	SSq	Var	Perc
Four catchments (Rhine, Lech, Isar, Tagliamento)					
	Between catchments	3	2423.9	1.272	25.5
	Between populations within catchment	30	1891.0	0.880	17.6
	Within populations	2318	6589.7	2.843	56.9
$F_{CT} = 0.255^*$ $F_{SC} = 0.236^*$ $F_{ST} = 0.431^*$					
Barriers and catchments					
	Between groups	11	3577.3	1.560	32.1
	Between populations within groups	22	737.6	0.463	9.5
	Within populations	2318	6589.7	2.843	58.4
$F_{CT} = 0.321^*$ $F_{SC} = 0.140^*$ $F_{ST} = 0.416^*$					

* $P < 0.001$.

[†]The table gives the AMOVA model, source of variance, degrees of freedom (d.f.), sum of squares (SSq), variance (Var), the percentage of variation (Perc), as well as F -statistics.

of physical barriers (canyons, reservoirs). However, at the Tagliamento, two tributaries were occupied by different genetic clusters. Along the main channel of Tagliamento, two genetic clusters occurred repeatedly and in one case far apart. In Rhine and Tagliamento, downstream populations showed a higher degree of admixture than upstream populations, indicating a downstream accumulation of genetic diversity originating from historically stable upstream populations.

Geographic tree models. The geographic plotting of neighbour-joining trees in GenGIS (Fig. 4) indicated that there were genetic discontinuities corresponding to natural or anthropogenic barriers in all catchments as evidenced by longer branches and deeper bifurcations. In some cases, spatially proximate populations were genetically dissimilar.

Discussion

Overall, genetic diversity of populations of *M. germanica* was comparable with the diversity of populations of its Asian congener *M. laxiflora* (Liu *et al.*, 2006, 2010). Genetic variability was rather homogenous across catchments and moderately high, with the exception of the low diversity Tagliamento catchment. In a prior study on *M. germanica*, the Inn catchment showed a similarly low diversity as the Tagliamento catchment (Werth & Scheidegger, 2014). To our knowledge, populations at

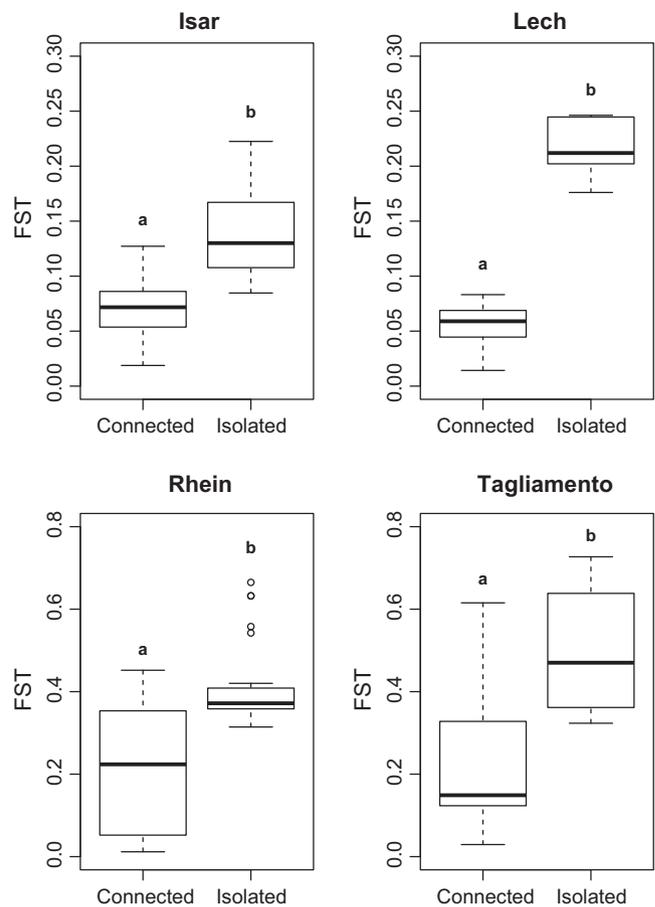


Fig. 3 Boxplots of genetic differentiation (pairwise F_{ST}) contrasting connected localities with localities isolated by natural or anthropogenic barriers. The letters state whether groups differed significantly from one another in one-sided t -tests assuming unequal variance of groups, after Holm's correction of P -values for multiple tests had been applied.

Tagliamento have been little affected by human activities; the area we studied can be characterised as largely natural. It seems likely that the low diversity of the Tagliamento catchment might be a consequence of different processes: the populations are located at the southern periphery of the range of the species in the Alps and are geographically isolated from other populations by large mountain ranges. As vast stretches of river are dry during summer, habitats allowing the survival of seedlings are rare at Tagliamento, leading to a lack of stepping stone habitats for dispersal, as the river stretches containing habitat are geographically isolated from one another. The small, isolated populations are likely to be influenced substantially by genetic drift, resulting in a loss of genetic diversity.

Our results showed that populations of *M. germanica* were substantially differentiated, as expected under

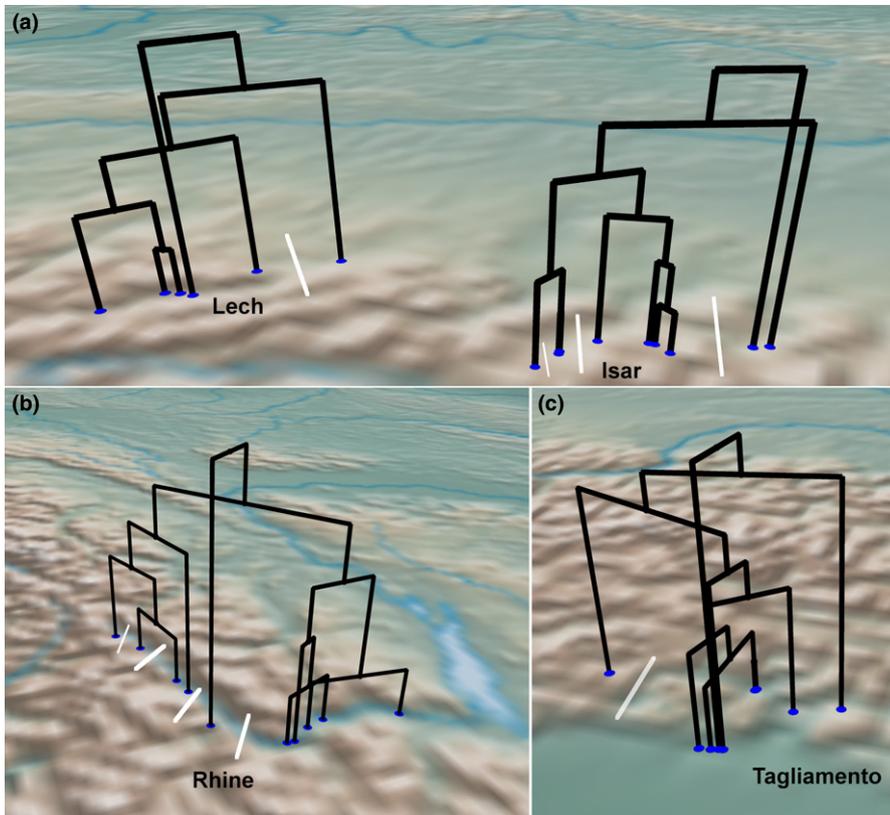


Fig. 4 Geographic tree models for *Myricaria germanica*, based on mid-point-rooted neighbour-joining trees of micro-satellite data. Trees were projected with GenGIS. (a) Isar and Lech catchments. (b) Rhine catchment. (c) Tagliamento catchment. Natural and man-made barriers are indicated by white lines.

genetic isolation. This is different from what has been found for the riparian tree species black poplar (Smulders *et al.*, 2008), a wind-pollinated and wind-dispersed species. We found substantial genetic differentiation between catchments (Electronic Supplement, Table S1) and between populations within catchments. These results are similar to those of Mitsui, Isagi & Setoguchi (2010), Kondo, Nakagoshi & Isagi (2009), Hudman & Gido (2013) and Van Looy *et al.* (2009). For other species, far less genetic differentiation between populations situated in different catchments and less of an ordered population structure along rivers has been reported, with frequent migration within and between catchments obscuring geographic genetic patterns (e.g. Smulders *et al.*, 2008). Also, no isolation by distance was found in some other species (Honnay *et al.*, 2010; Brzyski & Culley, 2011).

We found a significant effect of barriers on the genetic composition of localities. Owing to the natural dynamics of their floodplain habitat, populations of *M. germanica* are frequently disturbed, leading to high mortality of individuals and to a high turnover. For example, only one of the many populations of *M. germanica* at Hinterrhein that Endress (1975) had mapped in the 1970s was re-found by Kolly (2007) in the exact same site – an area protected from river dynamics by the presence of a

bridge pillar. The species was still found at Hinterrhein in 2007, but with fewer populations and lower local population sizes. When revisiting one of our populations at Rhine a year after sampling, the gravel bank had been eroded, and only a few individuals were found of the forty that we had sampled the year before. Therefore, only few individuals may survive over several decades in a site, even though a rare individual in a sheltered site may live long. Due to high population turnover, few individuals will have survived since the construction of dams. Thus, it seems likely that the increased genetic differentiation observed between sites fragmented by anthropogenic barriers is indeed a result of those barriers, or a combination of the isolating effects of the new barriers and of pre-existing natural barriers (e.g. Sylvenstein dam).

Pairwise F_{ST} values were significantly higher between localities fragmented by natural and man-made barriers than between connected localities, pointing towards reduced gene flow among localities separated by barriers; geographic tree models also showed this effect. Moreover, Bayesian clustering revealed different populations on either side of physical barriers in all catchments. For aquatic species, several studies have found similar genetic isolation effects among populations separated by physical barriers (Horreo *et al.*, 2011; Alp *et al.*,

2012; Franssen, 2012; Hudman & Gido, 2013), but this issue has received less attention in riparian species. In riparian plants, reduced gene flow would be expected if barriers reduced migration rates, for example by lowering the rate of seed-mediated gene flow. It is easy to picture how dams could disrupt seed movement via hydrochory. Effects of fragmentation of rivers by dams have previously been reported, leading to lower seed dispersal via hydrochory, which has repercussions on species diversity of riparian habitats (Jansson *et al.*, 2000a,b). However, not only water dispersal of seeds could be impacted in catchments fragmented by dams, but also wind dispersal. This is because an artificial lake provides a large area with no suitable habitat, and seeds could only move over this unfavourable area via long-distance dispersal, which is rare relative to local dispersal of seeds (Nathan, 2006).

The average differentiation was significantly higher between populations separated by Sylvenspeicher than between those separated by Stauwehr Krün. A large artificial lake such as Sylvensteinspeicher may have an entirely different effect on the riparian ecosystem than a rather small dam (Stauwehr Krün) where sediment is occasionally released. At Sylvensteinspeicher, the river bedload gets completely trapped by a specially constructed dam before entering the reservoir, and therefore, localities downstream from the reservoir are depleted of sediment. The lack of sediment supply causes an incision of the river into the sediment and a decrease of the water table, leading to a loss of habitat and making large parts of the flood plain unsuitable for germination of *M. germanica*. Moreover, the Sylvensteinspeicher is likely to act as a seed trap for *M. germanica* as the water is collected in the reservoir and the water flowing out first passes through two hydroelectric power plants. Finally, the Sylvenstein dam was constructed in a narrow river stretch, which may have operated as a natural barrier to gene flow prior to dam construction. This was not the case in Stauwehr Krün.

Stauwehr Krün occasionally releases bedload during high water levels to remove sediment accumulation. Even though Stauwehr Krün is also likely to trap seeds of *M. germanica*, the flooding events to release sediment might in principle help the species to occasionally spread across the dam. At a distance of 3–5 km from Stauwehr Krün, the conditions are not suitable for growth and survival of *M. germanica*, mainly due to severe flooding and deposition of excessive amounts of sediment. Entire plants are washed away or covered with sediment, but the redistribution of plants of *M. germanica* with successful subsequent growth in a locality

downstream has been observed twice in the past 15 years after severe flooding (M. Schödl pers. obs.). The bedload release at Stauwehr Krün is associated with a separation of sediment by size, with coarse material being deposited close to the dam and fine material reaching further downstream. This process eliminates many (but not all) potential germination habitats of *M. germanica* downstream from the dam as the species requires moist sand for germination (Benkler & Bregy, 2010).

Our data show a significant isolating effect of one type of 'soft' barrier: canyons. As these natural barriers have persisted in riverscapes for hundreds of generations, even if the number of migrants per generation was only slightly decreased, isolation effects would be expected, and this was indeed found. In contrast, for the other type of 'soft' barrier (regulated river stretches), as found in our data, we expected to find no genetic isolation due to the small expected effect and short time that had passed since these barriers were erected. Small reductions of the migration rate may not have had any discernible effect on population structure after a few generations. We are not aware of prior studies that have focused on different types of 'soft' barriers, nor on the temporal scale of barriers. However, a number of other studies have compared localities along regulated stretches of river with unregulated stretches and have found reduced seed dispersal via hydrochory (Nilsson & Jansson, 1995; Jansson *et al.*, 2000a,b, 2005) and reduced genetic diversity of localities along regulated river stretches (Pollux *et al.*, 2007; Mosner *et al.*, 2012). For example, Mosner *et al.* (2012) found higher genetic diversity of willows in natural floodplains as compared to localities along rivers regulated with dikes. The oldest natural floodplains exhibited the highest genetic diversity. The arguably most drastic effect of river regulation on a plant species has been found by Pollux *et al.* (2007). In the aquatic plant *Sparganium emersum*, populations were exclusively clonal in channelised stretches of river and predominantly sexual in natural river segments, leading to drastic differences in genetic diversity. In contrast, our data showed no such effect of channelised stretches of rivers on gene flow in *M. germanica*, possibly because the regulated river stretches were rather short (~1 km).

In riparian plant populations that are frequently disturbed and influenced by colonisation-extinction dynamics, we would not expect a close correspondence between genetic and geographic distance. For example, episodes of long-distance dispersal and extreme flooding events are likely to introduce stochasticity. However,

geographic tree models showed a correspondence between the genetic relationships among populations and geography. These results are in accordance with the findings of another study that reported significant isolation by distance in *M. germanica*, with gene flow following a one-dimensional stepping stone model (Werth & Scheidegger, 2014). Nevertheless, in the geographic tree models, spatially proximate populations were genetically dissimilar in some cases even though there were no natural or anthropogenic barriers between them, indicating stochasticity. This effect was especially pronounced in the Tagliamento catchment, where the river dynamics has not been altered by humans, and where large-scale disturbances of the floodplain are extremely common. The pattern revealed by geographic tree models at Tagliamento would be expected for populations which are not in migration-drift equilibrium, such as metapopulations. Moreover, the large genetic distances among populations in the upper part of Tagliamento might be the result of spatial isolation among sites, as large parts of the upper Tagliamento fall periodically dry, implying a lack of stepping-stone habitats.

In plants dispersing their seeds via hydrochory, downstream populations should show an accumulation of genetic diversity, a concept coined the 'unidirectional dispersal hypothesis'. Some empirical evidence for this hypothesis has been found (e.g. Pollux *et al.*, 2009). We also found empirical support for this hypothesis. In the Inn and Rhine catchments, downstream populations were mixed with respect to their affiliations to genetic clusters (Fig. 1). However, a number of other studies have found no downstream accumulation of diversity in riparian plants (Imbert & Lefèvre, 2003; Tero *et al.*, 2003; Liu *et al.*, 2006; Honnay *et al.*, 2010; Hu *et al.*, 2010). Populations of riparian plants growing in dynamic floodplains are characterised by complex population histories, as colonisation–extinction dynamics are important in this habitat type (Tero *et al.*, 2003; Jäkäläniemi *et al.*, 2005; Honnay *et al.*, 2009); resulting in complex patterns in genetic diversity along streams (Honnay *et al.*, 2010).

We found that dams and canyons disrupt gene flow among populations of *M. germanica*, as evidenced by strong effects of genetic isolation. This result has important implications for the conservation of populations of riparian plants. Connectivity is an important property to consider during restorations of riparian habitats. For example, in species forming metapopulations along rivers, disruption of gene flow increases the extinction risk of the entire metapopulation, because long-term persistence can only be guaranteed if dispersal rates are high enough that the rate of new colonisations exceeds the rate

of extinctions of local populations (Hanski & Gilpin, 1991; Hanski, 1994, 1998; Hanski & Gaggiotti, 2004). But also for populations of riparian plants that do not form metapopulations, connectivity among sites in a catchment is crucial for long-term persistence (Nilsson & Jansson, 1995; Jansson *et al.*, 2000a,b, 2005). For the long-term survival of species such as the riparian character plant *M. germanica*, care has to be taken to not further disrupt gene flow between populations. Restoration of channelised rivers may create additional stepping stones between existing populations and thus restore metapopulation dynamics in this rare and threatened riparian plant.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Pairwise F_{ST} values (below diagonal) and significance ± 1 standard error (above diagonal). Non-significant numbers printed in bold.

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