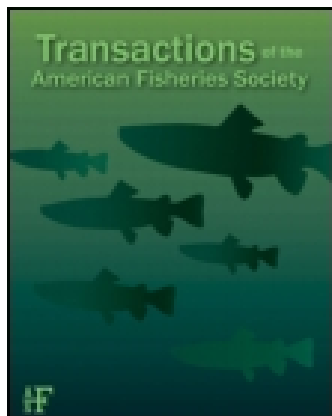


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Influence of Forest Road Culverts and Waterfalls on the Fine-Scale Distribution of Brook Trout Genetic Diversity in a Boreal Watershed

Jean-Baptiste Torterotot^{ab}, Charles Perrier^b, Normand E. Bergeron^{ac} & Louis Bernatchez^b

^a Institut National de la Recherche Scientifique, Centre Eau Terre Environnement, 490 Rue de la Couronne, Quebec, Quebec G1K 9A9, Canada

^b Institut de Biologie Intégrative et des Systèmes, Pavillon Charles-Eugène-Marchand, Université Laval, 1030 Avenue de la Médecine, Quebec, Quebec G1V 0A6, Canada

^c Groupe de Recherche Interuniversitaire en Limnologie et en Environnement Aquatique, Département de Sciences Biologiques, Université de Montréal, CP 6128, Succursale Centre-Ville, Montreal, Quebec H3C 3J7, Canada

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ARTICLE

Influence of Forest Road Culverts and Waterfalls on the Fine-Scale Distribution of Brook Trout Genetic Diversity in a Boreal Watershed

Jean-Baptiste Torterotot*

Institut National de la Recherche Scientifique, Centre Eau Terre Environnement, 490 Rue de la Couronne, Quebec, Quebec G1K 9A9, Canada; and Institut de Biologie Intégrative et des Systèmes, Pavillon Charles-Eugène-Marchand, Université Laval, 1030 Avenue de la Médecine, Quebec, Quebec G1V 0A6, Canada

Charles Perrier

Institut de Biologie Intégrative et des Systèmes, Pavillon Charles-Eugène-Marchand, Université Laval, 1030 Avenue de la Médecine, Quebec, Quebec G1V 0A6, Canada

Normand E. Bergeron

Institut National de la Recherche Scientifique, Centre Eau Terre Environnement, 490 Rue de la Couronne, Quebec, Quebec G1K 9A9, Canada; and Groupe de Recherche Interuniversitaire en Limnologie et en Environnement Aquatique, Département de Sciences Biologiques, Université de Montréal, CP 6128, Succursale Centre-Ville, Montreal, Quebec H3C 3J7, Canada

Louis Bernatchez

Institut de Biologie Intégrative et des Systèmes, Pavillon Charles-Eugène-Marchand, Université Laval, 1030 Avenue de la Médecine, Quebec, Quebec G1V 0A6, Canada

Abstract

Habitat fragmentation has become an increasing concern in conservation biology and is of prime importance with the expansion of forest road networks toward boreal and arctic regions. The aim of this study was to evaluate the effects of artificial and natural barriers to fish movement on the fine-scale distribution of genetic diversity in Brook Trout *Salvelinus fontinalis*. We genotyped 995 individuals sampled from 25 locations in a boreal watershed fragmented by culverts and natural waterfalls. Using a landscape genetics approach, we tested whether the presence of both types of barrier resulted in decreased genetic diversity and increased divergence within isolated locations. Results showed that genetic divergence was enhanced between sites separated by barriers and that genetic diversity was reduced within sites located upstream of barriers. Moreover, the observed changes in levels of divergence and diversity were correlated with the number of barriers. Overall, our results suggested that the effects of culverts and natural waterfalls were similar. To our knowledge, this is the first study illustrating the effects of forest road culverts and natural waterfalls on the distribution of genetic diversity in Brook Trout within a boreal watershed. We discuss our results in the context of boreal forest road network expansion and in light of the need to better understand the potential impacts of road infrastructure on the long-term persistence of fish populations.

With the expansion and intensification of land conversion for human activities, habitat fragmentation has become an increasing concern in conservation biology (Fagan 2002;

Vitousek et al. 1997). This landscape-level phenomenon (McGarigal et al. 2002) can be described as the disruption of continuous habitats into smaller habitat patches that are

*Corresponding author: jeanbaptiste.torterotot@gmail.com

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isolated from each other by a matrix of dissimilar habitats (Wilcove et al. 1986; Fahrig 2003). From a biological perspective, such spatial rearrangement results in the geographical separation of small local populations whose stability and persistence depend on the quality, extent, and connectivity of remaining habitats (Levins 1969; Groom et al. 2005). For most species, habitat fragmentation results in patch size reduction and in partial or complete isolation of populations (Fahrig 2003). Decreases in connectivity and reductions in patch size have been linked to a variety of negative ecological consequences (Collinge 1996), such as alteration of dispersal behavior (Mader 1984; Stow et al. 2001; Schtickzelle et al. 2006) and decreases in species richness and abundance (Andr en 1994; Hinsley et al. 1996; Coudrain et al. 2013). With pervasive ecological impacts on both terrestrial and aquatic ecosystems, habitat fragmentation is considered to be one of the most serious threats to biodiversity (Zwick 1992; Hanski 1999; Fagan 2002; Fahrig 2003).

From a population genetics perspective, numerous studies have demonstrated that breaks in connectivity resulting from habitat fragmentation can modify the genetic structure and diversity of a variety of organisms, including mammals (Epps et al. 2005), birds (Harrisson et al. 2012), invertebrates (Keller et al. 2004), and fishes (Morita et al. 2009). However, empirical studies have shown that genetic sensitivity to habitat fragmentation is variable among species and is mainly conditioned by their dispersal abilities (Debinski and Holt 2000; Blanchet et al. 2010). With respect to predictions from metapopulation theory (Levins 1969) and population genetics principles, habitat fragmentation may result in reduced gene flow between fragmented patches, hence increasing the level of genetic differentiation among remnant populations via accentuated genetic drift (Templeton et al. 1990; Young et al. 1996; Yamamoto et al. 2004). Moreover, patch size reduction may in turn lead to a decrease in effective population size, increased inbreeding, and a loss of genetic diversity, which may ultimately lead to a higher risk of extinction (Hanski 1999; Couvet 2002; Dixo et al. 2009).

Road construction ranks among the main sources of habitat fragmentation with potential negative ecological and genetic consequences for terrestrial and aquatic communities (Forman and Alexander 1998; Trombulak and Frissell 2000; Coffin 2007). Roads act as total or partial barriers to individual dispersal and gene flow in many taxa, including amphibians (Vos and Chardon 1998; Lesbarr eres et al. 2006), invertebrates (Keller et al. 2004), and both large and small mammals (Oxley et al. 1974; Epps et al. 2005). However, little attention has been given to fish communities and to the potential genetic impacts of stream-crossing structures, such as culverts, that are associated with the construction of roads in riverine landscapes. When poorly installed or maintained, culverts can become barriers to fish movement (Warren and Pardew 1998) due to outlet drops that exceed fish jumping abilities or due to excessive water velocities and/or accumulation of debris in the

structure. The lack of knowledge about the genetic impacts of culverts is surprising given that several studies have already reported negative impacts of culverts on fish distribution (Wheeler et al. 2005; P epino et al. 2012) and dispersal (Warren and Pardew 1998; Gibson et al. 2005; Benton et al. 2008; Park et al. 2008). Despite the relevance of these observations and the possible negative consequences for fish population persistence, to our knowledge no previous work has been conducted concerning the genetic consequences of habitat fragmentation by culverts.

In this study, we aimed to document the impact of forest road culverts on the fine-scale distribution of genetic diversity in a wild resident population of Brook Trout *Salvelinus fontinalis* inhabiting a small, North American boreal watershed. Pr evost et al. (2002) estimated that the expansion of forest road networks linked to forestry and mining activities involves the installation of more than 10,000 stream-crossing structures per year in the province of Quebec alone. Given this information and considering the lack of knowledge about the possible consequences of these infrastructures on fish population persistence, there is a need for a better understanding of the potential genetic impacts of habitat fragmentation induced by forest road culverts. This need is even more compelling in light of current economic and development programs that involve the construction of new transportation infrastructure in riverine landscapes, particularly in boreal regions.

The Brook Trout is a salmonid that is native to eastern North America (MacCrimmon et al. 1971) and is sensitive to habitat fragmentation (Letcher et al. 2007; Whiteley et al. 2013). We addressed the hypothesis that culverts, acting as partial or complete barriers to fish dispersal, can affect the distribution of genetic diversity within Brook Trout populations, as has been reported for dams (Yamamoto et al. 2004), weirs (Meldgaard et al. 2003; Blanchet et al. 2010), and mills (Raeymaekers et al. 2009) in relation to other fish species. We predicted lower genetic diversity in locations upstream of culverts and increased genetic differentiation among sites separated by culverts. Although culverts may affect dispersal and gene flow among fish populations, waterfalls may also affect fish movement (Carlsson et al. 1999; Castric et al. 2001). Therefore, when estimating the extent to which culverts affect Brook Trout, it is important to take into account the presence of natural potential barriers to dispersal (e.g., waterfalls) as well as other key riverscape variables (Ward 1998), such as elevation, channel width, and waterway distance, which have been shown to impact the distribution of genetic diversity in salmonids and other fishes (Hebert et al. 2000; Castric et al. 2001; Neville et al. 2006; Raeymaekers et al. 2008; Gomez-Uchida et al. 2009; Kanno et al. 2011).

Using a landscape genetics approach (Manel et al. 2003) accounting for the effects of anthropogenic and natural factors on the distribution of genetic diversity in Brook Trout populations, we estimated genetic diversity within and genetic differentiation among Brook Trout samples obtained at 25 locations

in a boreal watershed. We sought to address the following questions: (1) “Does fine-scale population structure exist in the study watershed?”; (2) “What are the main riverscape variables associated with the genetic diversity and differentiation observed in the watershed?”; (3) “What is the effect of the number of culverts and waterfalls on the observed distribution of genetic diversity?”; and (4) “Do the physical characteristics of culverts have an effect on the distribution of genetic diversity?” We discuss the implications of these results for management strategies and restoration programs in dendritic riverine networks that are fragmented by culverts.

METHODS

Study site.—The study was conducted in the Saint-Louis River, a small, boreal watershed in central Quebec, Canada (Figure 1; study site encompasses 48°48'N, 70°53'W and above). The Brook Trout is the principal species inhabiting the Saint-Louis River and its tributaries. The Saint-Louis River has a drainage area of 239 km² and an average wetted width of 25 m; its tributaries have an average wetted width of 3 m.

The area was dominated by logging activities during the last four decades or so, resulting in a notable expansion of the forest road network and in the installation of numerous culverts throughout the watershed. During summer 2011, we surveyed the watershed to identify all potential barriers (natural and artificial) to fish movement and gene flow. The height of natural waterfalls and the physical characteristics of each culvert (slope, length, and outlet drop height) were measured. The outlet drop height of the culvert was the distance between the outlet's lowest edge and the water surface of the downstream pool.

Fish sampling.—Fish sampling was designed to statistically evaluate the potential effects of culverts and natural waterfalls on the spatial distribution of genetic diversity (Figure 1; Supplementary Table S.1 in the online version of this article). An attempt was made to select widely distributed culverts comprising a wide variety of physical characteristics to evaluate the relative influence of these characteristics on the observed spatial genetic patterns. Brook Trout were electrofished at 25 locations throughout the entire watershed during July 2012 (Figure 1; Table 1). Seven sites were chosen within the Saint-Louis River main stem (MS1–MS7), 15 sites were established on tributaries upstream of barriers (culverts and/or waterfalls; T1–T9, T11, T13–T15, T17, and T18), and three sites were located on tributaries downstream of barriers (T10, T12, and T16). For each sampling location, elevation (m above sea level) and channel width were recorded. Following recommendations for preventing the sampling of closely related individuals (Hansen et al. 1997), an effort was made to avoid collecting age-0 fish and to spread the sampling effort over long river sections. In total, 995 Brook Trout were electrofished, weighed, and measured for FL. The number of fish sampled per site varied from 38 to 40 (Table 1). A small piece of tissue (either adipose fin or anal fin) was clipped from each individual and stored in 95% ethanol.

Genotyping.—The salt extraction method described by Aljanabi and Martinez (1997) was used to extract DNA from fin tissue. Amplification by PCR was realized at 16 microsatellite loci: *SfoB52*, *SfoC24*, *SfoC28*, *SfoC86*, *SfoC88*, *SfoC113*, *SfoC129*, *SfoD75*, *SfoD91*, *SfoD100*, and *SfoD105* (T. L. King, U.S. Geological Survey, unpublished data); *Sfo23* (Angers et al. 1995); and *Sfo226*, *Sfo266*, *Sfo269*, and *Sfo308* (Perry et al. 2005). The PCR products were electrophoresed on an Applied Biosystems (ABI), 3100 automated capillary sequencer. Alleles were scored with ABI GeneMapper. Genotypes were checked for scoring errors arising from stutter products and large-allele dropout by using Micro-Checker version 2.2.3 (Van Oosterhout et al. 2004).

Analysis of the distribution of genetic diversity.—The genetic diversity within sampling sites was quantified in terms of allele number and allelic richness (A_R) using FSTAT version 2.9.3.2 (Goudet 1995). Expected heterozygosity (H_E), observed heterozygosity (H_O), and the presence of private alleles were calculated with GENETIX version 4.05.2 (Belkhir

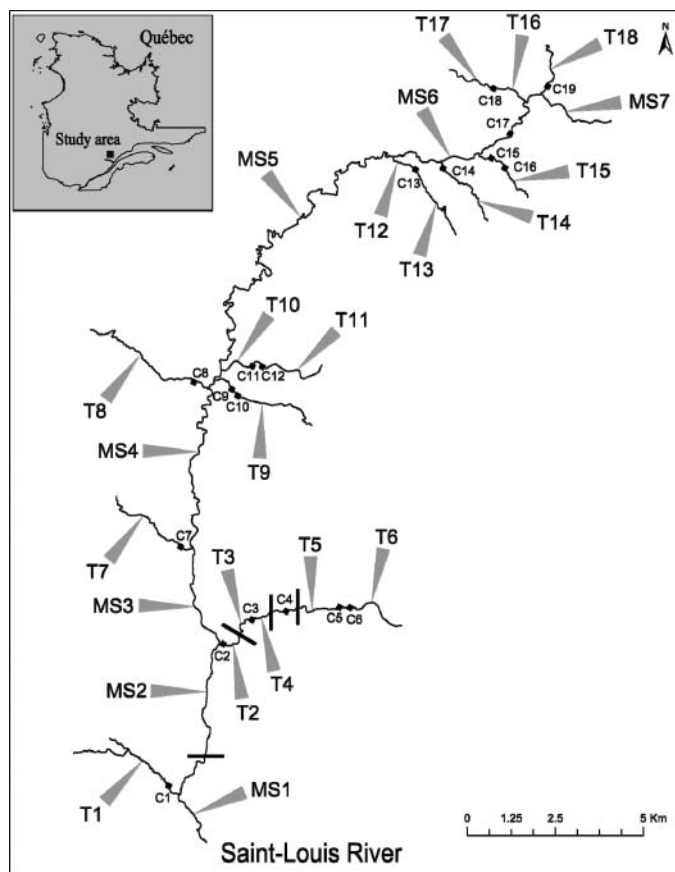


FIGURE 1. Locations of the 25 sites where Brook Trout were sampled in the Saint-Louis River watershed, Quebec, Canada. Black squares represent culverts ($n = 19$); solid bars represent impassable waterfalls ($n = 4$). Location codes correspond to those in Table 1 (MS = main-stem site; T = tributary site; C = culvert).

TABLE 1. Sample size (n), allelic richness (A_R ; standardized for 38 individuals), unbiased expected heterozygosity (H_E), and number of private alleles (P_A) for Brook Trout sampled at 25 locations in the Saint-Louis River watershed, Quebec, Canada (MS = main-stem site; T = tributary site).

Location	Latitude (°N)	Longitude (°W)	n	A_R	H_E	P_A
MS1	48°36'27.4'	70°56'12.6'	40	9.78	0.69	13
MS2	48°37'21.5'	70°55'43.2''	40	8.20	0.62	0
MS3	48°40'18.7'	70°56'06.0'	40	9.15	0.66	0
MS4	48°43'03.3'	70°55'37.4'	40	8.47	0.63	0
MS5	48°45'40.1'	70°53'29.4'	40	8.12	0.63	1
MS6	48°46'32.5'	70°50'28.4'	39	6.71	0.61	0
MS7	48°47'36.4'	70°48'07.6'	40	7.26	0.61	0
T1	48°38'58.5'	70°55'21.1'	39	9.99	0.70	9
T2	48°38'58.0'	70°55'18.9'	40	8.68	0.67	0
T3	48°39'11.9'	70°54'58.1'	38	6.43	0.59	1
T4	48°39'24.2'	70°54'28.7'	40	6.83	0.61	0
T5	48°39'32.2'	70°53'36.5'	40	7.05	0.60	0
T6	48°39'29.4'	70°53'19.8'	40	6.53	0.59	2
T7	48°40'28.2'	70°56'17.2'	40	7.36	0.59	2
T8	48°42'59.2'	70°55'50.5'	40	7.70	0.64	1
T9	48°42'55.4'	70°54'55.8'	40	6.65	0.64	0
T10	48°43'15.8'	70°55'20.8'	40	7.51	0.63	0
T11	48°43'24.0'	70°55'11.5'	40	7.72	0.63	0
T12	48°46'31.3'	70°51'18.8'	40	7.08	0.64	0
T13	48°46'24.1'	70°50'56.5'	40	7.63	0.65	0
T14	48°46'27.8'	70°50'19.1'	40	5.52	0.55	0
T15	48°46'35.5'	70°49'17.5'	40	6.28	0.58	2
T16	48°47'33.4'	70°48'30.9'	40	6.07	0.60	0
T17	48°47'38.1'	70°47'59.8'	40	4.82	0.56	0
T18	48°47'30.5'	70°48'26.3'	40	6.17	0.61	0

et al. 2000). The inbreeding coefficient (F_{IS}) was used to measure deviation from Hardy–Weinberg equilibrium and was estimated for each sampling site and each locus by using FSTAT.

The differentiation among sampling sites was quantified by pairwise genetic differentiation index (F_{ST}) values calculated in FSTAT. Statistical significance of pairwise F_{ST} values was tested using 1,000 permutations, and the significance level α was adjusted using the sequential Bonferroni technique (Rice 1989) implemented in FSTAT. The program BAPS (Bayesian Analysis of Population Structure; Corander et al. 2003) was used to identify clusters of genetically related individuals and to detect migrants and admixed individuals among the genetic clusters identified in the Saint-Louis River watershed. The BAPS program is a Bayesian genotypic clustering model that allows for the simultaneous (1) identification of genetically differentiated populations (clusters) and (2) assignment of individuals to populations based on individual multilocus genotypes. The program also allows the quantification of individual admixture proportions (q -values) in a two-tiered approach: once the clustering solutions are determined, the admixture of individual genotypes can be then estimated by establishing the clusters from which an individual's alleles originate. We first

used BAPS to cluster groups of individuals, testing for a number of clusters (k) from 1 to 20. An admixture model was then used to identify migrants and admixed individuals. Admixture model settings were as follows: 150 iterations to estimate the admixture coefficients for individuals, 50 reference individuals from each cluster, and 15 iterations to estimate the admixture among individuals. With respect to previous publications (Lecis et al. 2006; Vähä and Primmer 2006; Bergl and Vigilant 2007), individuals were classified as local, migrant, and admixed when their individual admixture proportions of the local cluster were greater than 0.80, less than 0.20, and between 0.20 and 0.80, respectively.

GENETIX was used to compute a multidimensional correspondence factorial analysis (CFA). The program ARLEQUIN version 3.5 (Excoffier and Lischer 2010) was used for analysis of molecular variance (AMOVA) among the genetic units identified by BAPS and to evaluate the amount of genetic variation within clusters and corresponding to lower hierarchical levels of genetic structure.

Riverscape genetics.—The potential influence of several riverscape variables on genetic differentiation of Brook Trout between sampling sites and on genetic diversity within sites was tested by using the following statistical methods. First,

linear regressions were conducted to independently test the correlations between genetic diversity measures (namely A_R and H_E) and watershed position, site elevation, channel width, and degree of habitat fragmentation. Watershed position was measured using ArcGIS version 10.0 (ESRI 2011) as the site's river distance from the downstream-most sampling site; the degree of habitat fragmentation was based on the number and types of barriers located downstream of each sampling location. The strength of the linear correlation between each explanatory variable and the response variable was measured using Pearson's correlation coefficient. All regressions were conducted and tested in R software (R Development Core Team 2010).

Multiple linear regression was then performed to accurately disentangle the effects of the above-mentioned riverscape variables on genetic diversity in terms of mean A_R per site. Model selection was conducted by exhaustive search based on the Schwarz Bayesian information criterion (BIC; Schwarz 1978; Excoffier and Lischer 2010) using the R package LEAPS (Lumley and Miller 2009). Multicollinearity among variables was tested using the variance inflation factor (VIF) that was implemented in the R package CAR (Fox and Weisberg 2011). Variables with a VIF less than 10 were retained in the final model (Neter et al. 1996). Residuals were tested for normality and heteroscedasticity. Statistical significance of final predictors was accepted at P -values less than 0.05.

Mantel tests (Mantel 1967) and multiple regression on distance matrices (MRDM; Lichstein 2007) were used to measure the effect of riverscape variables on genetic differentiation between sampling sites ($n = 300$ pairwise combinations). Essentially, the Mantel test examines the correlation between a dependent matrix and a predictor matrix, whereas MRDM simultaneously examines the effect of a group of predictor matrices on the dependent matrix. Statistical significance was evaluated by permutations. To test for isolation by distance (Wright 1943), the Mantel test was performed between pairwise F_{ST} values and streamwise distances between site pairs; streamwise distances were calculated using the Network Analyst tool implemented in ArcGIS version 10.0. To separate the effects of river distance and fragmentation, the data set was split into two different groups to test for isolation by distance at barrier-separated sites and at sampling sites located on continuous reaches. Mantel tests were also used to examine for correlations between the number (and types) of barriers between sites, pairwise differences in channel width, pairwise differences in sampling site elevation, and the response matrix of pairwise F_{ST} values. Multiple regressions on distance matrices were conducted to simultaneously test the relative correlation of all aforementioned variables with the pairwise F_{ST} matrix. Model selection was based on the minimization of the Schwarz BIC, and statistical significance of explanatory variables was accepted at P -values less than 0.05. Mantel tests and MRDM were performed using the ECODIST package (Goslee and Urban 2007) in R,

and statistical significance for both analyses was obtained after 10,000 permutations.

Finally, following the method of Raeymaekers et al. (2008), the models obtained from the two multiple regressions described above were extended to more accurately assess the relative effect of culvert physical characteristics on genetic diversity within sites and genetic differentiation between sites. Detailed culvert characteristics were added to the data set and were tested as explanatory variables: namely the cumulative and mean height of the outlet drops, cumulative and mean slope of the culverts, and cumulative and mean length of the culverts.

RESULTS

Characteristics of Barriers

Nineteen culverts and four major natural waterfalls (>2 m high) were identified and characterized. One waterfall was a long bedrock cascade located on the downstream part of the Saint-Louis River, isolating the upper portion of the drainage network (Figure 1). The other waterfalls were a series of three bedrock falls (2–7 m high) located on a small tributary in the lower part of the watershed. Apart from a few exceptions, all culverts were situated at the confluence of tributaries and the main-stem Saint-Louis River (Figure 1). All but two of the selected culverts (C8 and C16; Table S.1) were circular metallic pipes, with lengths ranging from 7.2 to 30.0 m. The slope of the selected culverts ranged from 0.10% to a maximum of 7.00% (for C6). Six of the culverts, referred to as “hanging culverts” below, presented outlet drops ranging from 0.15 m to a maximum of 1.17 m (for C14). Furthermore, C18 was almost completely obstructed by woody debris and sediments.

Analysis of the Distribution of Genetic Diversity

On the basis of Hardy–Weinberg equilibrium proportions, we rejected 2 of the 16 microsatellite markers that were tested. Indeed, the loci *SfoC24* and *SfoC129* exhibited significant F_{IS} for Brook Trout at 10 sampling locations. However, Micro-Checker analyses revealed no evidence of null alleles or large-allele drop-out at any marker. Genotypes were obtained for 995 Brook Trout (38–40 individuals/site; Table 1) based on the 14 retained loci (amplification success = 99.98%).

Allelic richness and H_E were variable among sampling locations (Figure 2; Tables 1, S.2). Over all locations, we observed 6–38 alleles/locus, with an average of 15.14 alleles/locus. Mean A_R per sampling location was 7.35 on average and ranged from 4.82 (T17) to 9.99 (T1). Notably, the lowest levels of A_R were observed at T14 (5.52) and T17 (4.82); T14 is located upstream of the highest hanging culvert identified in the watershed (C14; Table S.1), and T17 is upstream of an obstructed hanging culvert (C18). The highest diversity was observed for T1, the downstream-most sampling site in the

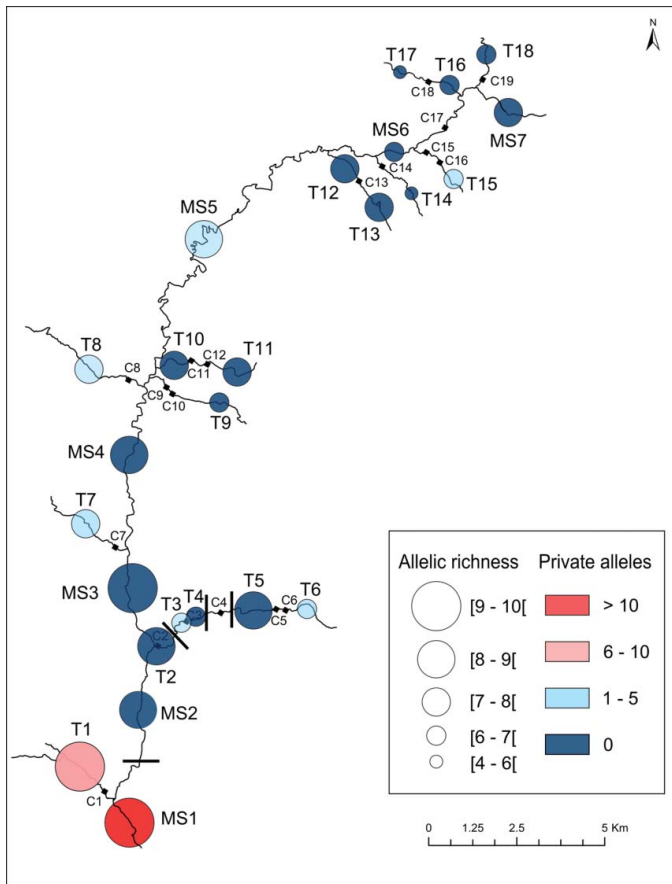


FIGURE 2. Geographical distribution of Brook Trout allelic richness and private alleles among the 25 sampling locations in the Saint-Louis River watershed. See Figure 1 for an explanation of site codes. [Figure available in color online.]

watershed. Mean H_E per location ranged from 0.55 (T15) to 0.70 (T1; Tables 1, S.2). Thirty-one private alleles were found for 11 of the 14 loci analyzed; 22 of these private alleles were observed in samples collected at MS1 and T1, which are located downstream of the impassable waterfall situated on the lower part of the Saint-Louis River (Figure 2; Table 1).

Pairwise F_{ST} values ranged from 0.001 (between T4 and T3; MS2 and MS3; MS2 and MS4; MS2 and MS5; and MS3 and MS5) to 0.144 (between T6 and T14; Table S.3), and overall genetic differentiation was 0.052. After Bonferroni correction, significant genetic differentiation was detected in 263 of the 300 pairwise comparisons. The mean pairwise F_{ST} value for sites separated by barriers (culverts or waterfalls) was significantly higher ($F_{ST} = 0.055$) than the mean pairwise F_{ST} for sites located on continuous river sections ($F_{ST} = 0.017$; Mann-Whitney test: $P < 0.01$), providing a first indication of the influence of barriers on the level of genetic differentiation between sampling locations. A comparable result was obtained when removing the possible influence of waterfalls (i.e., considering sites separated by culverts only). Particularly high F_{ST} values were found between T3, T4, T5, or T6 (located

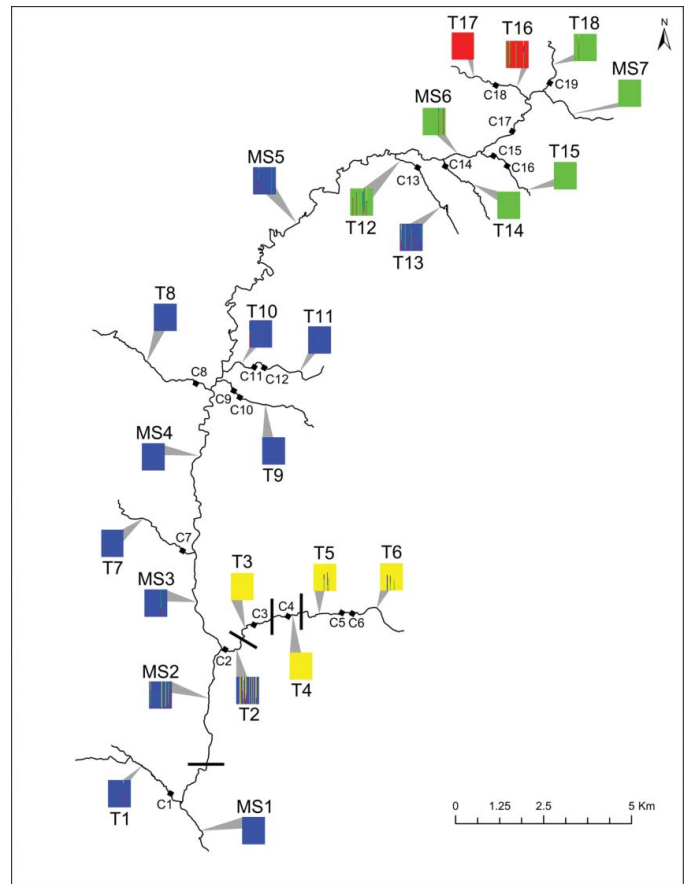


FIGURE 3. Results of Bayesian individual clustering with the program BAPS for $k = 4$ genetic clusters of Brook Trout in the Saint-Louis River watershed. See Figure 1 for an explanation of site codes. [Figure available in color online.]

above a series of major waterfalls) and all other sampling locations in the watershed (mean overall $F_{ST} = 0.097$). We also observed particularly high F_{ST} values between T17 (upstream of the obstructed culvert C18; Figure 1; Table S.1) and the remaining sampling locations (mean overall $F_{ST} = 0.092$). In contrast, F_{ST} was low and often nonsignificant between sites sampled on the main-stem Saint-Louis River (mean $F_{ST} = 0.013$), suggesting more pronounced gene flow and/or lower genetic drift for Brook Trout within the main stem.

The Bayesian clustering performed with BAPS identified four distinct genetic clusters (Figure 3). The first genetic cluster was associated with T3, T4, T5, and T6, which are located above a series of impassable waterfalls. The second genetic cluster was observed in a headwater tributary and comprised T16 and T17, representing samples taken below and above C18. As mentioned above, the highest pairwise F_{ST} values were associated with sampling sites located between the first and second clusters, whereas pairwise F_{ST} was low among sampling sites within the two clusters (mean pairwise $F_{ST} = 0.024$ and 0.009, respectively). The third cluster (Figure 3) encompassed most of the sampling sites located on the main

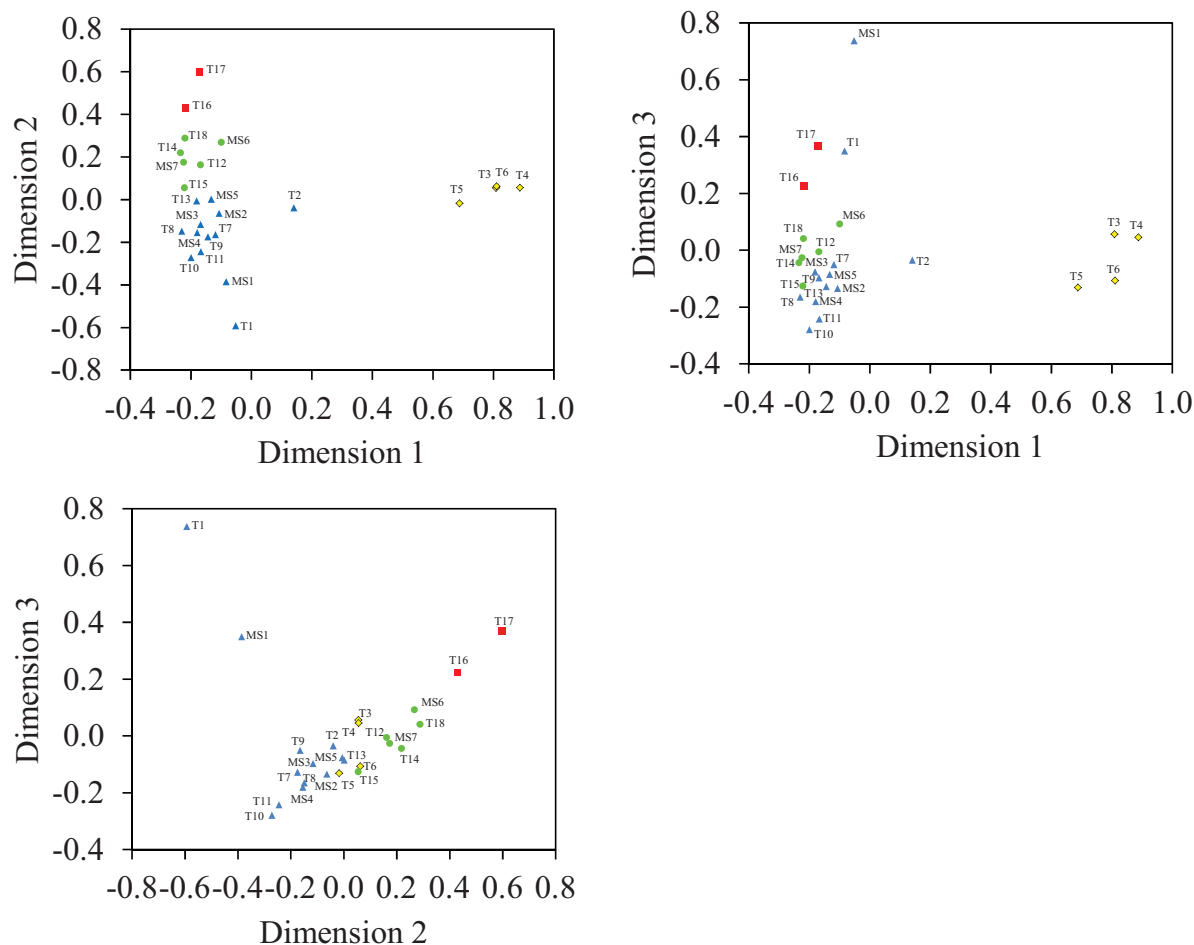


FIGURE 4. Correspondence factorial analysis illustrating Brook Trout genetic distances among locations in the Saint-Louis River watershed based on the three most significant axes. Symbol shading or color corresponds to that used in Figure 3 to illustrate the four BAPS clusters. See Figure 1 for an explanation of site codes. [Figure available in color online.]

stem (MS1–MS5) as well as T1, T2, T7–T11, and T13. The fourth cluster consisted of T12, T14, T15, T18, MS6, and MS7. Genetic differentiation within the third and fourth clusters was relatively low (mean pairwise $F_{ST} = 0.021$ and 0.024 , respectively). At the population level, a low level of admixture was detected, as only a few putative migrants from neighboring clusters were identified in samples from T2, T12, T13, T16, MS2, MS5, and MS6, further illustrating reduced gene flow between clusters. The low proportion of putative migrants or admixed individuals detected from sampling sites upstream of barriers suggested little evidence of upstream migration above barriers. The CFA (GENETIX) confirmed the genetic structure found by BAPS (Figure 4), with four distinct genetic groups being defined. According to the first axis, the first cluster obtained in BAPS was clearly discriminated from other clusters. The second axis discriminated among the second, third, and fourth clusters identified by BAPS. The CFA further revealed modest genetic differentiation among locations within the clusters delineated by BAPS. In particular,

according to the third axis, MS1 and T1 appeared to be differentiated from other locations contained within the same cluster (i.e., the third cluster identified from BAPS). Overall, AMOVA revealed that 4.79% of the total genetic variance was found among clusters identified by BAPS, whereas 2.00% of the variance was found among samples within clusters (Table 2).

Riverscape Genetics

We found that A_R was negatively correlated with a site's river distance from the downstream-most sampling site (Pearson's correlation: $r_{Pearson} = -0.65$, $P = 0.0003$); thus, higher A_R values were observed in downstream locations (Table 3; Figure 5). A similar pattern was found for H_E ($r_{Pearson} = -0.49$, $P = 0.0123$), which decreased with a site's river distance from the downstream-most location. A negative correlation was also observed between A_R and the number of barriers located downstream of the sampling site ($r_{Pearson} =$

TABLE 2. Analysis of molecular variance results showing the partitioning of genetic variance among Brook Trout genetic clusters identified by BAPS (F_{CT}), among samples within clusters (F_{SC}), and within samples (F_{ST}). Values in parentheses are 95% confidence intervals.

Source of variation	Percentage of variance	F-statistic	P
Among clusters	4.79	$F_{CT} = 0.048$ (0.035–0.060)	<0.0001
Among samples within clusters	2.00	$F_{SC} = 0.021$ (0.018–0.023)	<0.0001
Within samples	93.21	$F_{ST} = 0.068$ (0.055–0.080)	<0.0001

–0.45, $P = 0.0238$). This relationship was stronger and more significant for culverts ($r_{Pearson} = -0.44$, $P = 0.0262$) than for waterfalls ($r_{Pearson} = -0.37$, $P = 0.0819$; Figure S.1). As revealed by our analyses, sites that were located at higher elevations in the watershed were characterized by lower A_R ($r_{Pearson} = -0.83$, $P < 0.0001$) and lower levels of H_E ($r_{Pearson} = -0.70$, $P < 0.0001$). Finally, A_R ($r_{Pearson} = 0.59$, $P = 0.0019$) and H_E ($r_{Pearson} = 0.41$, $P = 0.0610$) tended to increase with channel width.

The multiple linear regression using the number of culverts and waterfalls, site elevation, channel width, and watershed position explained 70% of the variance in A_R among sampling sites (adjusted $R^2 = 0.70$, $F_{2, 22} = 29.44$, $P < 0.0001$; maximum VIF = 1.09; Table 4). Based on the BIC, the mean A_R at a given site was best explained by the number of culverts located downstream of the site ($\beta = -0.2298$, $P = 0.0765$) and by the site's elevation ($\beta = -0.0096$, $P < 0.0001$). At $\alpha = 0.05$, site elevation was the only significant predictor in the model, but the number of culverts was marginally nonsignificant (Table 4). When testing for detailed physical characteristics of culverts as additional predictors, the best model explained 75% of the variance in observed genetic diversity (adjusted $R^2 = 0.75$, $F_{4, 20} = 25.16$, $P < 0.0001$; maximum VIF = 1.19); this model integrated the number of waterfalls ($\beta = -0.2621$, $P = 0.0766$), the cumulative outlet drop height of culverts ($\beta = -0.0117$, $P = 0.0233$), and the site elevation ($\beta = -0.0087$, $P < 0.0001$) as predictors. Some riverscape variables (river distance and channel width) were no longer

significant in the multiple regressions, although they were statistically significant when tested with simple linear regressions. Among all predictors, site elevation was the most consistent riverscape variable, as it was significant in all tests performed on A_R .

The Mantel test revealed an isolation-by-distance effect in the Saint-Louis watershed ($r_{Mantel} = 0.33$, $P < 0.0001$). This relationship was particularly pronounced among sites that were separated by barriers ($r_{Mantel} = 0.33$, $P < 0.0001$) but was nonexistent among sites that were located on continuous reaches ($r_{Mantel} = -0.12$, $P = 0.0106$; Figure 5; Table 3). Consequently, Mantel tests revealed a strong positive correlation between pairwise F_{ST} values and the total number of barriers ($r_{Mantel} = 0.66$, $P < 0.0001$). The correlation coefficient r_{Mantel} was slightly higher when considering only the number of culvert barriers ($r_{Mantel} = 0.63$, $P < 0.0001$) than when considering the number of natural waterfalls ($r_{Mantel} = 0.55$, $P < 0.0001$) between sites. Other variables (channel width and elevation differences) had no significant effect ($r_{Mantel} = -0.26$, $P = 0.9964$; $r = 0.01$, $P = 0.4811$).

In the MRDM analysis, 49% of the genetic differentiation between sample locations was explained by the best model (adjusted $R^2 = 0.49$, $F_{4, 295} = 72.31$, $P < 0.0001$; maximum VIF = 1.86). The final model integrated the pairwise geographic distance ($\beta = 0.0001$, $P = 0.0010$), the number of culverts between sites ($\beta = 0.0091$, $P = 0.0150$), the pairwise difference in site elevation ($\beta = -0.0001$, $P = 0.1300$), and the number of natural waterfalls between sites ($\beta = 0.009$, $P = 0.0900$; Table 4) as predictors of pairwise F_{ST} values. Thus, pairwise geographic distances and the number of culverts between sites were the two significant predictors in the model at $\alpha = 0.05$; the effect of waterfalls was marginally nonsignificant. Based on the adjusted R^2 , the extended model for pairwise F_{ST} values was not more predictive than the general model on the level of genetic differentiation between sampling sites.

TABLE 3. Results of Pearson's correlation (coefficient $r_{Pearson}$) between landscape variables and allelic richness; and results of Mantel matrix correlation (coefficient r_{Mantel}) between landscape variables and pairwise values of the genetic differentiation index F_{ST} for Brook Trout sampled in the Saint-Louis River watershed (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). "Culverts," "waterfalls," and "barriers" refer to the number of barriers of the specified type located downstream of ($r_{Pearson}$) or between (r_{Mantel}) sampling sites.

Geographical feature	$r_{Pearson}$	r_{Mantel}
River distance (km)	–0.65***	0.32***
Culverts	–0.44*	0.63***
Waterfalls	–0.37	0.55***
Barriers (culverts + waterfalls)	–0.45*	0.66***
Elevation (m above sea level)	–0.82***	0.01
Channel width (m)	0.59**	–0.26

DISCUSSION

The aim of this study was to evaluate the effect of forest road culverts and natural waterfalls on the fine-scale distribution of genetic diversity in Brook Trout within a boreal watershed. We tested whether the presence of both types of barrier resulted in a decrease in genetic diversity and an increase in divergence among isolated sampling sites. Results

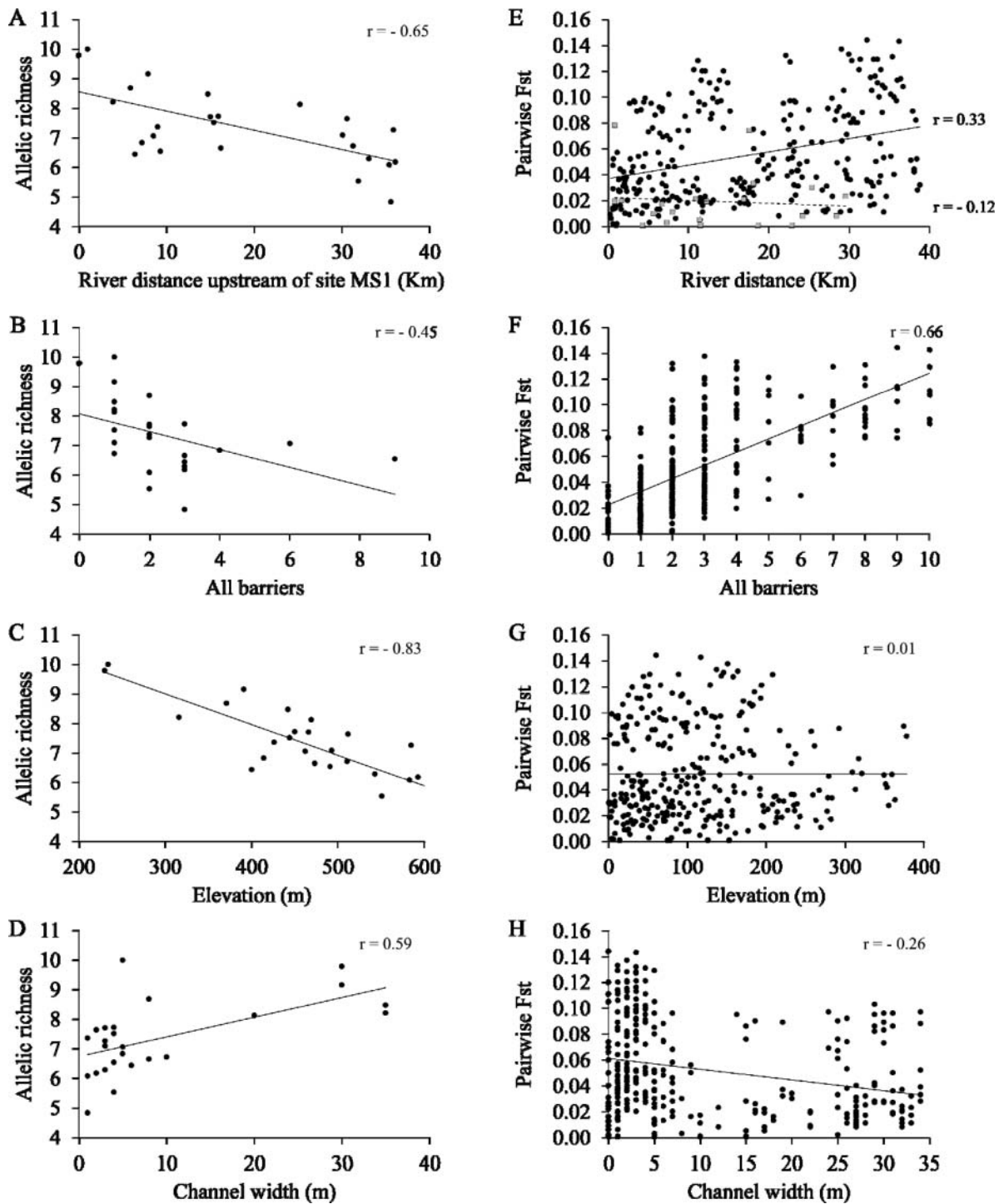


FIGURE 5. Relationships between geographic features and mean allelic richness (left panels) or pairwise F_{ST} estimates (right panels) based on 14 microsatellite loci in Brook Trout from the 25 sampling locations. In panel E, results are presented for pairs of sampling sites located on continuous reaches (gray squares and dashed line) or separated by barriers (black dots and solid line).

demonstrate that the distribution of genetic diversity was shaped in part by forest road culverts and natural waterfalls. Genetic divergence was enhanced between sites separated by barriers, and genetic diversity tended to be reduced within sites

located upstream from barriers. Moreover, the effect of barriers appeared to be cumulative since the observed changes in divergence and diversity were correlated with the number of barriers.

TABLE 4. Multiple regression analysis results for allelic richness (A_R ; adjusted $R^2 = 0.70$, $F_{2, 22} = 29.44$, $P < 0.001$) and for the distance matrix of pairwise F_{ST} values (adjusted $R^2 = 0.49$, $F_{4, 295} = 72.31$, $P < 0.001$) of Brook Trout sampled in the Saint-Louis River watershed. "Culverts" and "waterfalls" refer to the number of barriers of the specified type located downstream of (A_R) or between (F_{ST}) sampling sites.

Geographical feature	A_R		Pairwise F_{ST}	
	β	P	β	P
Intercept	12.0121	<0.0001	0.0174	0.0998
Elevation (m above sea level)	-0.0096	<0.0001	-0.0001	0.1300
Culverts	-0.2298	0.0765	0.0090	0.0150
River distance (km)			0.0010	0.0010
Waterfalls			0.0091	0.0900

Fine-Scale Distribution of Genetic Diversity

Brook Trout in the Saint-Louis River watershed were characterized by notable fine-scale genetic variability. Position in the watershed was a good predictor of the level of observed genetic diversity, as A_R and H_E decreased with locations further upstream. These results are consistent with the results of previous studies on salmonids and other fishes (Hänfling and Weetman 2006; Primmer et al. 2006; Morrissey and de Kerkhove 2009; Raeymaekers et al. 2009; Blanchet et al. 2010), which have shown a decrease in genetic diversity from downstream to upstream locations in linear or dendritic networks. However, we found that A_R within sampling sites was predominantly influenced by site elevation rather than by river distance, and thus we excluded river distance from our final models of A_R . Elevation was a consistent predictor in our models: Brook Trout samples collected at higher elevations tended to be less diverse. Similar observations were reported in earlier empirical studies of Brook Trout and other fish species, suggesting limited gene flow and increased genetic drift with increasing elevation (Hernandez-Martich and Smith 1990; Shaw et al. 1991; Castric et al. 2001). As was noted by Castric et al. (2001), populations located at higher elevations are more geographically isolated and thus may be more subject to unidirectional (i.e., downstream) gene flow because of the increased probability of steep river sections and physical barriers (e.g., woody debris and small waterfalls) that can prevent upstream migration. These results therefore highlight the importance of considering site elevation in future riverscape genetics studies.

Our results revealed the existence of a significant within-watershed genetic structure, identifying four distinct genetic clusters that exhibited a clear geographical pattern in their distribution. The AMOVAs corroborated these clustering results, demonstrating significant genetic differentiation among the four groups of sampling locations. These observations are consistent with earlier studies (Carlsson et al. 1999; Hebert et al. 2000; Castric et al. 2001; Adams and Hutchings 2003; Meldgaard et al. 2003; Wilson et al. 2004; Hudy et al. 2010; Junker et al. 2012), which have reported the existence of genetic structure in resident Brook Trout and other fish species at a fine geographic scale within small watersheds. Significant

genetic differences were also found between almost all pairs of sampling locations, regardless of the presence or absence of barriers, thus revealing further (albeit less-pronounced) genetic structure within each of the four clusters as highlighted by AMOVA. Considering that only a few migrants are required to maintain homogeneous allele frequencies among locations (Slatkin and Barton 1989), these observations indicate that effective migration within this watershed is limited, particularly among the four main clusters we identified. Limited dispersal was also evident from our Bayesian clustering analysis, which detected very few putative admixed individuals or migrants among the genetic clusters. The present findings support earlier empirical studies indicating limited dispersal of individual Brook Trout between relatively close areas (Hebert et al. 2000; Hudy et al. 2010; Kanno et al. 2011).

Impact of Culverts on the Distribution of Genetic Diversity

Results revealed a significant isolation effect attributable to culverts in the study drainage. As mentioned above, significant genetic differentiation was found between almost all sampling locations, regardless of the presence or absence of barriers. However, we found that sampling sites separated by culverts were characterized by higher levels of genetic differentiation than locations separated by open dispersal pathways. These results suggest that gene flow among locations was lower when the sites were separated by culverts. Our findings complement those of previous empirical studies using mark-recapture techniques, which demonstrated that movements of Brook Trout and other fish species were an order of magnitude lower through culverts than through natural reaches (Thompson and Rahel 1998; Warren and Pardew 1998; Benton et al. 2008). Our observations complement these earlier findings in that even if upstream migration is hampered by the presence of culverts, downstream movements remain physically possible and could have been sufficient to limit genetic differentiation with Brook Trout at locations upstream of culverts. The present findings also corroborate observations from previous genetic

studies on fish habitat fragmentation by artificial barriers, such as weirs and mills (Wofford et al. 2005; Raeymaekers et al. 2008; Junker et al. 2012).

Moreover, the multivariate analysis revealed that the level of divergence in allele frequencies increased as the number of culverts present between sampling sites increased. This can be attributed to the fact that the number of culverts between two locations is often correlated with the streamwise distance between the sites. However, we observed a weak correlation between the number of culverts and streamwise distance. Such observations have been previously reported in empirical studies of other fish species and other types of artificial barrier, including dams, weirs, and mills (Meldgaard et al. 2003; Leclerc et al. 2008; Raeymaekers et al. 2008; Horreo et al. 2011), but to our knowledge, our study is the first to demonstrate this genetic pattern for culverts.

We were surprised that the sample from T13 appeared to belong to the third cluster identified by BAPS, while the sample downstream of this was classified into the fourth cluster. One explanatory hypothesis could be that T12 and T13 previously belonged to the same cluster (i.e., the third cluster); fish then migrated from the headwater areas (fourth cluster) toward T12 but have not yet reached T13, potentially due to the presence of C13. Furthermore, the second cluster encompassed T16 and T17, which are situated below and above C18, indicating that this obstructed culvert has had a significant impact on genetic connectivity with the rest of the watershed. It is likely that C18 impedes migration from downstream areas, leading to the genetic isolation of individuals located at T17. However, the fact that T16 was also classified into the second cluster indicates that upstream migration from the main stem is limited. Gene flow instead likely originates from individuals located upstream of the culvert and presumably occurs at high-discharge events. This supports the findings of Whiteley et al. (2013), who demonstrated that genetic variation can also be lost from locations downstream of barriers.

We found a negative correlation between the level of genetic diversity and the number of culverts separating sampling sites from the outlet of the watershed, independent of their streamwise separation distance. Reduction in genetic diversity due to genetic drift can occur rapidly when migration is reduced and when population size is small (Lacy 1987; Young et al. 1996; Dixo et al. 2009). Our findings thus indicate that spatial fragmentation due to successive culverts limited upstream migration and led to the isolation of reduced-size populations subject to increased genetic drift. Our results also suggest that in addition to the cumulative effect of culverts revealed by the general model of A_R , culvert physical characteristics also affected the degree of genetic connectivity. Indeed, we found that the level of A_R among samples was better predicted by the cumulative outlet drop height of culverts than by the number of culverts, thus implying that hanging culverts had a stronger impact on genetic diversity within sampling locations than culverts without outlet drops. Few

culverts in the study drainage present an outlet drop height that exceeds the jumping capabilities of Brook Trout (Kondratieff and Myrick 2006). We therefore suggest that the occurrence of outlet drops, even if small, enhanced the negative effects of culverts on upstream dispersal, resulting in a stronger asymmetric gene flow toward locations downstream of hanging culverts. These results support previous observations (Meldgaard et al. 2003; McKay et al. 2013) indicating that cumulative barriers along a watercourse can have substantial cumulative effects on population connectivity, even when individual barriers have a negligible impact. Even if our analysis had not revealed a role of other culvert physical characteristics, it would be of interest in further research to assess the impacts of culvert slope or roughness, given that these physical characteristics are known to alter flow dynamics and thus fish movement and gene flow (Coffman 2005).

Overall, our observations suggest that similar to dams (Yamamoto et al. 2004) or impassable waterfalls (Carlsson and Nilsson 2001; Gomez-Uchida et al. 2009), culverts can act as barriers to gene flow, with a cumulative effect enhanced by the presence of outlet drops. Such impacts may furthermore increase the process of genetic drift through reduced population size and inbreeding, thereby enhancing the risk of local extinction of isolated populations (Carlsson and Nilsson 2001; Letcher et al. 2007).

Limitations Related to the Geographic Distribution of Culverts

Although we found significant effects of culverts on the distribution of genetic diversity in Brook Trout, we acknowledge that the strength of our analyses was slightly limited by the distribution of culverts in the study watershed. Some studies (Rodríguez 2002; Neville et al. 2006; Kanno et al. 2011) have demonstrated that salmonids can display variable movement life history strategies, with some individuals adopting a “resident” strategy and others exhibiting a “migratory” strategy. These contrasting dispersal patterns still remain poorly understood, but it was suggested that they may be influenced by riverscape structure and complexity (Neville et al. 2006). Small headwater habitats may therefore promote residency behavior, while large main stems may favor migratory life histories. It logically follows that these movement strategies may influence gene flow and the distribution of genetic diversity in stream-living salmonids. It was thus documented that natural genetic differentiation can sometimes occur between populations from main stems and tributaries even in the absence of barriers. To detect such potential natural genetic variability, we attempted to sample systematically throughout the watershed, including reaches that lacked culverts. However, as illustrated in Figure 1, every tributary in the watershed is separated from the main stem by at least one culvert installed very near to the tributary mouth. Consequently, most of our control sites were located

on the main stem. Nevertheless, three control sites were located downstream of culverts on tributaries. As revealed by our analysis, the level of genetic differentiation between sampling sites and the level of genetic diversity within sites were influenced in part by the number of culverts. Without any information on life history strategies in the study watershed, it is difficult from the confounded sampling design here to disentangle the potential natural genetic variability from the impact of the presence of culverts. We can therefore only postulate that the presence of culverts exacerbated the natural spatial genetic patterns. Future studies should attempt to account for this by either (1) comparing with the genetic structure found in another watershed that is not impacted by culverts or (2) finding tributaries without culverts.

Impacts of Waterfalls on the Distribution of Genetic Diversity

We found evidence that major waterfalls affected the distribution of genetic diversity. In accordance with our expectations and with observations from previous studies (Skaala and Nævdal 1989; Hindar et al. 1991; Carlsson and Nilsson 2001; Gomez-Uchida et al. 2009), we determined that Brook Trout at sampling sites isolated above major waterfalls were differentiated from those at all other sampling sites in the drainage. The influence of waterfalls on the genetic structure of Brook Trout was also particularly evident from our Bayesian clustering analysis, as one genetic cluster corresponded to the four sampling sites that were isolated above a group of impassable waterfalls. This observation is not surprising given that the heights of these waterfalls greatly exceed the jumping abilities of Brook Trout (Hindar et al. 1991), most likely preventing all upstream movement of individuals and acting as barriers to upstream gene flow from the rest of the watershed. As was observed for culverts, results of the multivariate analysis revealed that the level of divergence in allele frequencies was positively correlated with the number of intervening waterfalls between locations. This result once again demonstrates the cumulative effect of barriers on the distribution of genetic diversity within a dendritic network.

However, the effect of the number of waterfalls on genetic diversity was not evident from our models of A_R . It is plausible that the mitigated evidence of the waterfalls' impact may reflect a statistical bias attributable to the configuration of the study watershed. Three of the four impassable waterfalls were located on a single tributary, and the remaining one was located on the very lower part of the drainage; thus, most of the sampling sites were isolated from the outlet of the watershed by only one waterfall. As a consequence, the resulting lack of heterogeneity in our data set may reduce the ability of our models of A_R to detect significant effects of the number of waterfalls on genetic diversity within sampling sites. There was nevertheless some evidence that a loss of genetic diversity was induced by the presence of waterfalls. We found that 71%

of the private alleles detected among our sampling sites were observed at one of the two sites below the impassable waterfall located on the Saint-Louis River, which clearly demonstrates that this geomorphic structure impedes gene flow to upstream locations, as reported previously (Wofford et al. 2005; Pritchard et al. 2007; Gomez-Uchida et al. 2009).

Management Implications of Culverts' Impacts on the Distribution of Genetic Diversity

To our knowledge, the present study is the first to provide a watershed-wide picture of the genetic impacts of forest road culverts in a stream network, taking into consideration other environmental factors that potentially affect patterns of genetic diversity. With strong evidence that breaks in genetic connectivity are induced by the presence of culverts, our data complement earlier empirical studies of habitat fragmentation, which have reported that fish populations receive significant negative genetic effects from anthropogenic barriers, including weirs (Meldgaard et al. 2003; Blanchet et al. 2010), dams (Neraas and Spruell 2001; Yamamoto et al. 2004; Alò and Turner 2005), and mills (Raeymaekers et al. 2009). Our study not only provides information for the prioritization of restoration programs in this watershed but also highlights useful guidelines for the general management of culvert-fragmented stream networks. First, based on the low levels of A_R and H_E detected at T14 and T17, there is clear evidence that improper installation or a lack of maintenance of C14 and C18 led to the isolation of their upstream Brook Trout populations. With no potential immigration from the main stem, these populations are subject to increased effects of inbreeding and genetic drift and may thus be exposed to an increased risk of extinction (Fagan 2002). We suggest that priority in the survey watershed should be given to the restoration of linkages among these two tributaries and the Saint-Louis River. We also found that culvert outlet drops, even if small, may have increased the loss of genetic diversity within isolated upstream populations. A reduction in genetic diversity may reduce the adaptive potential of populations and increase the risk of extirpation by demographic and environmental stochastic events (Yamamoto et al. 2004). Considering that similar results may be observable in comparable systems and following the recommendations made in earlier studies (Raeymaekers et al. 2009; Blanchet et al. 2010), we recommend that restoration priority be given to the tallest barriers and thus to hanging culverts in river systems that are fragmented by culverts. However, we must additionally consider the fact that natural barriers, such as waterfalls, also shape the distribution of genetic diversity within drainages (Carlsson et al. 1999; Blanchet et al. 2010). Therefore, in planning their prioritizations for restoration, managers should use a watershed-scale approach when considering the natural breaks in genetic connectivity induced by the presence of such geomorphic features. The position of culverts within the

system should also be taken into account when prioritizing restoration programs. In a recent study, Kanno et al. (2013) found that half of the surviving offspring of adult Brook Trout from a given stream were spawned in the stream's tributaries. These results support previous studies (Petty et al. 2005, 2012) that have highlighted the importance of small tributaries for the persistence of Brook Trout populations and thus the necessity of maintaining genetic connectivity among main stems and tributaries for the proper conservation of Brook Trout and other salmonids with similar life cycles. As was illustrated in our study system, forest roads are generally built along main stems, leading to the installation of culverts near tributary mouths. When acting as barriers to fish movement, culverts can lead to the physical and genetic isolation of entire tributaries, depriving salmonids of the demographic and genetic benefits of population connectivity. Letcher et al. (2007) demonstrated that, against predictions, Brook Trout can sometimes persist as small, isolated populations. However, those authors noted that whether rescue will occur in small, fragmented populations depends on the race between local adaptation and reduced survival—hence, there is no justification for failing to apply the precautionary principle in management strategies. From a conservation perspective, barriers to gene flow located near tributary junctions should be given high restoration priority in order to (1) preserve linkages among main stems and tributaries and (2) avoid the loss of genetic diversity in small, isolated patches that may already support limited genetic diversity due to enhanced genetic drift (Whiteley et al. 2013). As was underscored by Roni et al. (2002), the quality and quantity of upstream habitat isolated by problematic culverts should be evaluated when prioritizing restoration actions. Our recommendations support those made in previous studies (Roni et al. 2002; Poplar-Jeffers et al. 2009) emphasizing the necessity of adopting watershed-scale management strategies for proper conservation of fish populations and making a prioritized list to account for the costs and benefits of restoration actions.

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