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Fine-scale population structure and riverscape genetics of brook trout (*Salvelinus fontinalis*) distributed continuously along headwater channel networks

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Abstract

Linear and heterogeneous habitat makes headwater stream networks an ideal ecosystem in which to test the influence of environmental factors on spatial genetic patterns of obligatory aquatic species. We investigated fine-scale population structure and influence of stream habitat on individual-level genetic differentiation in brook trout (*Salvelinus fontinalis*) by genotyping eight microsatellite loci in 740 individuals in two headwater channel networks (7.7 and 4.4 km) in Connecticut, USA. A weak but statistically significant isolation-by-distance pattern was common in both sites. In the field, many tagged individuals were recaptured in the same 50-m reaches within a single field season (summer to fall). One study site was characterized with a hierarchical population structure, where seasonal barriers (natural falls of 1.5–2.5 m in height during summer base-flow condition) greatly reduced gene flow and perceptible spatial patterns emerged because of the presence of tributaries, each with a group of genetically distinguishable individuals. Genetic differentiation increased when pairs of individuals were separated by high stream gradient (steep channel slope) or warm stream temperature in this site, although the evidence of their influence was equivocal. In a second site, evidence for genetic clusters was weak at best, but genetic differentiation between individuals was positively correlated with number of tributary confluences. We concluded that the population-level movement of brook trout was limited in the study headwater stream networks, resulting in the fine-scale population structure (genetic clusters and clines) even at distances of a few kilometres, and gene flow was mitigated by 'riverscape' variables, particularly by physical barriers, waterway distance (i.e. isolation-by-distance) and the presence of tributaries.

Keywords: dispersal, genetic clusters, isolation-by-distance, landscape genetics, *Salvelinus fontinalis*, streams

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Introduction

Understanding how population structure is influenced by landscape attributes is a major challenge in evolutionary ecology and conservation biology. Landscape genetics provides a set of tools allowing us to identify spatial genetic patterns and to relate them to landscape and environmental features (Manel *et al.* 2003; Storfer *et al.* 2007, 2010). Such analyses provide important insights into spatial patterns of population connectivity. The association between genetic patterns and landscape factors has been examined across many taxa (e.g. Funk *et al.* 2005; Spear *et al.* 2005; Crispo *et al.* 2006; Chaput-Bardy *et al.* 2008; Gomez-Uchida *et al.* 2009; Zalewski *et al.* 2009; Goldberg & Waits 2010).

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Landscape genetics approaches are most useful when applied at a fine spatial scale to individual-level genetic differentiation (Manel *et al.* 2003). Stream fish populations may be spatially structured just over a few kilometres (Carlsson *et al.* 1999; Crispo *et al.* 2006; Cook *et al.* 2007; Hudy *et al.* 2010) because of limited dispersal (Hansen *et al.* 1997; Hudy *et al.* 2010), kin recognition (Carlsson *et al.* 2004) or highly skewed reproductive success by a small number of breeders (Tatarenkov *et al.* 2010). Therefore, stream fish represent model populations for landscape genetics studies. Further, the linear habitat structure makes headwater stream networks an ideal ecosystem in which to apply landscape genetics approaches. The linear habitat makes identification of dispersal corridors more straightforward than in two-dimensional terrestrial or marine ecosystems in which organisms can potentially take many movement paths (Spear *et al.* 2005; Measey *et al.* 2007; Wang 2009). The linear spatial structure of running waters thus simplifies analyses characterizing movement corridor habitat and assessing its impact on the spatial genetic structure.

However, these advantages have not been fully exploited among studies of landscape genetics in stream fishes. Most commonly, researchers have sampled a collection of arbitrarily defined 'populations' at a spatial scale broader than the dispersal potential of individuals during a single lifespan and have examined the landscape influence on population-level measures of genetic differentiation such as F_{ST} (Wenburg & Bentzen 2001; Wofford *et al.* 2005; Lehtonen *et al.* 2009). Two common landscape factors that have been identified to contribute to genetic differentiation are physical barriers (i.e. dams and natural waterfalls: Yamamoto *et al.* 2004; Wofford *et al.* 2005; Pritchard *et al.* 2007) and waterway distance (i.e. isolation-by-distance: Castric *et al.* 2001; Wenburg & Bentzen 2001; Whiteley *et al.* 2006; Lehtonen *et al.* 2009). Stream position within a watershed also can influence genetic patterns; downstream-biased gene flow and dendritic patterns of watersheds typically lead to lower genetic diversity within and higher genetic differentiation among upstream sites (Fagan 2002; Hänfling & Weetman 2006; Neville *et al.* 2006a; Chaput-Bardy *et al.* 2008; Morrissey & de Kerckhove 2009). Clearly, many studies have attempted to answer ecological questions at a broader spatial scale (i.e. among watersheds or from distant sites within large watersheds), but the general lack of studies that have examined other landscape variables at a fine spatial scale is surprising because streams are recognized as spatially continuous and heterogeneous ecosystems along channel networks (Fausch *et al.* 2002; Benda *et al.* 2004; Meeuwig *et al.* 2010). Accordingly, distribution and abundance of stream fish is not homogenous along stream channels (Angermeier & Winston 1998; Peterson & Rabeni 2001), and fish dispersal differs with characteristics of the movement corridors such as stream depth and size (Gilliam & Fraser 2001; Albanese *et al.* 2004; Meeuwig *et al.* 2010). In this study, we use the phrase 'riverscape' (*sensu* Ward 1998; Fausch *et al.* 2002) genetics as an aquatic counterpart to landscape genetics in terrestrial ecosystems, and we consider how genetic differentiation of an aquatic organism is influenced by riverscape variables, namely seasonal barriers (natural falls of 1.5–2.5 m in height during summer base-flow condition), waterway distance, stream gradient (the ratio of elevation drop per unit distance of stream channel), stream temperature and number of tributary confluences.

Analysis of riverscape genetics is useful for species of conservation concern whose ecological requirements are relatively well known. Brook trout (*Salvelinus fontinalis*) is native to eastern North America, but populations have declined in much of the native range (Hudy *et al.* 2008). Lotic populations of brook trout are currently restricted to small, cold headwater streams in the central and southern parts of the native range (Hudy *et al.* 2008; Kanno & Vokoun 2008); brook trout become rare or absent when stream water temperatures exceed 20 °C for extended periods (Hartman & Cox 2008; Robinson *et al.* 2010). Stream temperature may also be important at localized spatial scales because brook trout preferentially spawn in areas with groundwater upwelling (Essington *et al.* 1998). Brook trout populations are also affected by stream gradient (Isaak & Hubert 2000), and it is an important determinant of stream channel geomorphology. These observations suggest that the fine-scale population structure, if present, might be associated with riverscape characteristics.

We investigated fine-scale population structure and riverscape influence on individual genetic differentiation in brook trout by quantifying riverscape characteristics in two headwater channel networks (channel network length 7.7 and 4.4 km) in Connecticut, USA. Brook trout were distributed continuously along the channel networks in both study sites and our field survey of fish and habitat characteristics covered the entire study stream networks, providing a truly continuous view of spatial genetic patterns at the headwater watershed scale. Our data were used to answer the following questions:

1 Does a spatial population structure exist in the absence of permanent physical barriers at the spatial scale of a few kilometres? If so, does such a pattern follow a gradient (i.e. isolation-by-distance) or clusters (i.e. genetically distinguishable groups of individuals)?

2 Given that a spatial population structure is present, what riverscape variables can describe the observed spatial population structure? Riverscape variables included seasonal barriers, waterway distance, stream gradient, stream temperature and number of tributary confluences.
3 Are the answers to the aforesaid questions different between the two study sites? Consistent results in the two study sites should indicate the potential importance of given riverscape factors on genetic patterns.

Materials and methods

Study area

This study was conducted in two headwater watersheds located in north-western Connecticut, USA (Fig. 1). Both study streams contained self-reproducing brook trout populations in a branching stream channel network predominantly characterized by boulder (>256 mm), cobble (64–256 mm) and pebble (16–63 mm) substrates (Bain 1999). The Jefferson Hill-Spruce Brook watershed (drainage area: 14.56 km^2) spanned approximately 7.7 km in stream channel length (mean wetted width = 4.3 m, mean stream depth = 18.4 cm under baseflow condition). It drained into a large stream (mean wetted width 20 m) at the downstream end of the study area (Fig. 1). Brook trout were stocked every year by the state fisheries agency in this large stream and few individuals were observed in our study area. However, genetic assignment analysis indicated that stocked trout had made no detectable reproductive contribution at least in recent times in the study area (Kanno *et al.* 2011). Stocked trout were reliably identified in the field from a combination of body size and external characteristics, and our analysis considered only wild trout. Common fish species observed in Jefferson Hill-Spruce Brook included blacknose dace (*Rhinichthys atratulus*), longnose dace (*Rhinichthys cataractae*), and white sucker (*Catostomus commersoni*).

The Kent Falls Brook watershed had a drainage area of 14.06 km^2 and included approximately 4.4 km of stream channel network (mean wetted width = 4.8 m, mean stream depth = 19.8 cm under baseflow condition) (Fig. 1). The headwater channel network was isolated because of large natural waterfalls located approximately 750 m downstream of the lower end of the study area. There was no known record of brook trout stocking above the waterfalls including the study area, although the presence of naturalized non-native brown trout (*Salmo trutta*) in our study area suggested that trout stocking had occurred in the past. Blacknose dace was common in Kent Falls Brook. A second permanent barrier (a series of natural waterfalls >5 m in height) existed in a tributary to Kent Falls Brook ('KT3' in Fig. 1). No brook trout were found above this permanent barrier.

Field sampling

Brook trout were collected in a spatially continuous manner throughout the entire stream channel networks in both study sites (Fig. 1). Prior to collection, the study

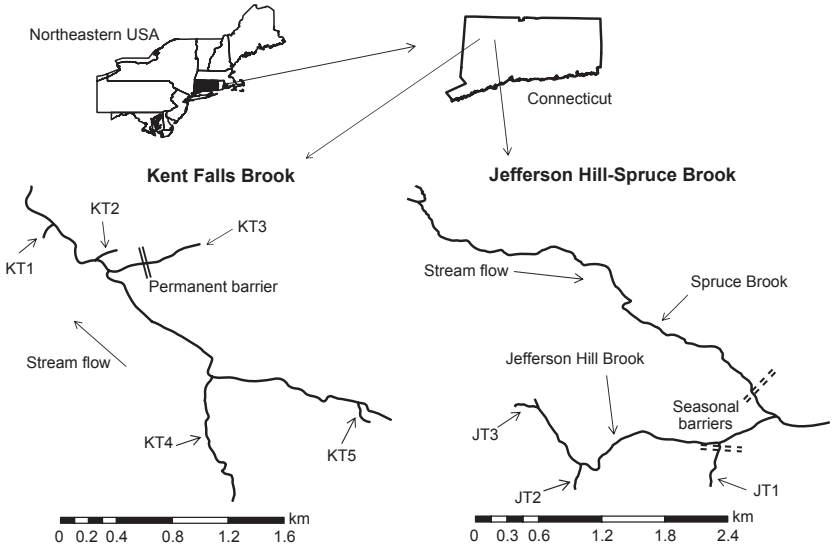

Fig. 1 Locations of Kent Falls Brook and Jefferson Hill-Spruce Brook in the State of Connecticut, Northeastern USA. Tributaries are labelled in each study site (e.g. 'KT1' for 'Kent Falls Tributary #1, 'JT2' for Jefferson Hill Brook Tributary #2). Brook trout were sampled throughout the entire stream channel networks shown in a spatially continuous manner.

streams were travelled by foot, and riparian trees were marked at an interval of 50 m (each 50 m-zone is called a 'reach' hereafter). Single-pass backpack electrofishing surveys (a pulsed DC waveform, 250–350 V: Smith-Root model LR-24, Vancouver, WA, USA) were conducted without blocknets in June, August and October of 2008. Trout count was recorded by each reach, and each fish was measured for total length and weight. During the June and August surveys, an anal fin clip was removed from all trout captured, except age 0+ individuals that had hatched in early spring of 2008. Age 0+ trout were easily distinguished from older fish owing to their body size. Adipose fins were also removed and used as a permanent mark, so that tissue samples were not collected twice from the same individuals. In June and August, trout >150 mm were tagged with Visible Implant Alpha tags (Northwest Marine Technology Inc., Shaw Island, WA, USA) in the adipose eyelid tissue; these tags had unique alphanumeric numbers identifying fish as individuals upon recapture.

Stream habitat data were also collected in a spatially continuous manner across the study areas. Stream temperature was recorded at an interval of every three reaches (i.e. 150 m) throughout the study watersheds, using HOBO temperature data loggers that recorded every hour (Model U22-001; Onset Computer Inc., Bourne, MA, USA). Each stream reach was assigned temperature values that were recorded at the closest logger. For each reach, stream gradient was calculated as an elevation difference divided by a waterway distance (i.e. 50 m). Upstream and downstream boundaries of each reach were identified with a Juno ST Handheld GPS receiver (2-5 m accuracy; Trimble Inc., Sunnyvale, CA, USA) in early spring of 2009. Elevation values were assigned to the reach boundaries from the 3-m (10-ft) Digital Elevation Model GIS layer based on Light Detection and Ranging (LiDaR) remote-sensed data (available from the Center for Land Use Education and Research, University of Connecticut). Values of stream gradient calculated by this process were checked against detailed field notes for quality assurance.

Genotyping

A sub-sample of collected anal fins was genotyped for eight microsatellite loci: *Sfo*C-113 (trinucleotide), *Sfo*D-75 (tetranucleotide), *Sfo*C-88 (trinucleotide), *Sfo*D-100 (tetranucleotide), *Sfo*C-115 (dinucleotide), *Sfo*C-129 (trinucleotide), *Sfo*C-24 (trinucleotide) and *Ssa*D-237 (tetranucleotide). The same set of loci had been used in previous brook trout studies (Hudy *et al.* 2010; Kanno *et al.* 2011). We used genotype data on trout ranging 81–140 mm in both study sites, in order to target a single cohort as best as possible for statistical analysis. Larger trout were more unevenly distributed and were less abundant in tributaries (Kanno 2010), precluding an assessment at the watershed scale. Body size is a reasonable surrogate for age; however, it is not possible to determine age clearly for brook trout individuals after the age 0+ stage based on length–frequency distributions (see also Kennedy *et al.* 2003; Hudy *et al.* 2010). Presumably, fish in the size range genotyped were age 1+ individuals. Anal fin clips taken in June and August surveys were combined for analysis, because trout movement inferred from tagging was limited within summer (see Results). An attempt was made to genotype a similar number of trout (3–4 individuals) randomly selected from each 50-m reach; all individuals were genotyped in reaches where less than three trout of 81–140 mm were captured. Laboratory protocols for genomic DNA extraction and PCR amplification of microsatellite loci can be found in Kanno *et al.* (2011).

Descriptive statistics

Genotypes were checked for scoring errors arising from stutter products and large allele dropout, using MICRO-CHECKER, version 2.2 (Van Oosterhout *et al.* 2004). The frequency of null alleles per each locus was estimated using FREENA (Chapuis & Estoup 2007). Observed (H_O) and expected (H_E) heterozygosity was calculated using GENEPOP, version 4.0.10 (Raymond & Rousset 1995). Exact tests for gametic disequilibrium between loci, and calculation of the inbreeding coefficient, F_{IS}, for each locus were performed in FSTAT, version 2.9.3.2 (Goudet 1995). Departures from Hardy–Weinberg equilibrium were tested for each locus using the heterozygote deficiency option in GENEPOP.

Genetic clusters and local dispersal

The spatial population structure was investigated to identify clusters of genetically related individuals within the study watersheds, using the Bayesian clustering method (Pritchard *et al.* 2000) implemented in STRUCTURE, version 2.3.1, and the discriminant analysis of principal components (DAPC: Jombart *et al.* 2010) available in the adegenet package (Jombart 2008) for R, version 2.11 (R Development Core Team 2010). Both methods do not require a priori delineation of genetic clusters and are suitable for analysing spatially continuous data (Pritchard *et al.* 2000; Jombart *et al.* 2010). However, some important differences exist in the analytical approaches between the two methods. STRUCTURE attempts to cluster individuals by minimizing Hardy–Weinberg and gametic disequilibrium (Pritchard *et al.* 2000), and typically fails to identify some complex types of spatial structure such as isolation-by-distance

(Jombart *et al.* 2010) and hierarchical population structure (Evanno *et al.* 2005). The multivariate analysis used in DAPC does not make any assumption on the population genetic models and may be more efficient at identifying genetic clines and hierarchical structure (Jombart *et al.* 2010). The two dissimilar approaches were used in this study, because various clustering approaches may lead to different conclusions (e.g. Latch *et al.* 2005; Waples & Gaggiotti 2006; Frantz *et al.* 2009).

In each study site, ten independent STRUCTURE runs were executed for each of $K = 1$–10 (K = number of genetic clusters), based on the admixture model with correlated allele frequencies. Each run had a 100 000 burn-in followed by 500 000 replicates. The number of clusters that best fitted the observed genotype data was determined by examining the estimated log probability of the data and the second order rate of change in log probability between successive K values (Evanno *et al.* 2005). Because STRUCTURE is known to identify the uppermost hierarchical level of population structure when individuals are structured in a hierarchical manner (Evanno *et al.* 2005), subsequent runs (100 000 burn-in and 500 000 replicates) were executed for detecting the presence of sub-structure within each cluster when $K > 1$ was identified at the watershed scale.

Evidence of genetic clusters was also examined in DAPC by running successive K-means clustering in the *find.clusters* function and using Bayesian Information Criterion (BIC) (i.e. K with the lowest BIC value is ideally the optimal number of clusters). However, BIC values may keep decreasing after the true K value in case of genetic clines and hierarchical structure (Jombart *et al.* 2010). Therefore, the rate of decrease in BIC values was visually examined to identify values of K, after which BIC values decreased only subtly (Jombart *et al.* 2010). We tested values of $K = 1$–20, with ten runs at each value of K. Once the number of genetic clusters was selected, each study site was sub-divided into groups of spatially continuous stream reaches (the number of groups equal to the number of genetic clusters indentified) by taking into account the locations of seasonal barriers and tributary confluences, and STRUCTURE results. The *dapc* function was then executed using this grouping, retaining axes of Principal Components Analysis sufficient to explain ≥90% of total variance of data. The result was presented in an ordination plot with the first two axes. Finally, pair-wise F_{ST} values among genetic clusters were calculated using FSTAT.

The presence of evident genetic clusters identified in Jefferson Hill-Spruce Brook (see Results) prompted us to estimate contemporary dispersal rates between the clusters. Bi-directional dispersal rates were estimated using BAYESASS, version 1.3 (Wilson & Rannala 2003). This analysis was not used for Kent Falls Brook because BAYESASS assumes a low immigration rate that cannot exceed one-third of a population (Wilson & Rannala 2003) and this assumption was likely violated in Kent Falls Brook (see Results). We followed the approach of Faubet *et al.* (2007) to obtain accurate dispersal rates. Specifically, ten independent runs of 21×10^6 iterations (the first 2×10^6 burn-in) were carried out with different random seed values. The Bayesian deviance information criterion (DIC) value was calculated for each run and was used to identify runs that did not converge. Estimated dispersal rates among runs that appeared to have converged varied only subtly; we present estimated dispersal rates from the run with the lowest DIC value.

Isolation-by-distance

Spatial autocorrelation analysis was performed to examine the effect of waterway distance on genetic differentiation (i.e. isolation-by-distance). Because seasonal barriers played an important role in mitigating gene flow in Jefferson Hill-Spruce Brook (see Results), this study area was split into two different subsets of continuous reaches that were not influenced by seasonal barriers; (i) reaches upstream of the seasonal barrier in Spruce Brook, and (ii) the rest of the reaches, minus those above the seasonal barrier in a tributary ('JT1') in Jefferson Hill Brook (Fig. 1). Genetic differentiation between all pairs of individuals was calculated using the method of Smouse & Peakall (1999) available in GENALEX, version 6.3 (Peakall & Smouse 2006). This method quantifies the squared genetic distance between a pair of individuals by the extent to which the two individuals share the same alleles across loci. Geographic distance followed stream channel distance between individuals, and a value of zero was assigned for pairs of individuals collected from the same reaches. The autocorrelation coefficient (r) was calculated as a measure of genetic relatedness between all pairs of individuals within the specified distance classes in GENALEX. Statistical significance of r at each distance class was declared when the 95% error bar of r estimated by bootstrap trials is greater than zero and the estimated mean value of r does not overlap with the 95% confidence interval about the null hypothesis of no spatial genetic structure by permutation (Peakall *et al.* 2003; Neville *et al.* 2006b).

Riverscape genetics analysis

A suite of riverscape variables was tested for their influence on genetic differentiation between individuals in each study site (see Table 1 for details). Identical to

Table 1 Riverscape variables tested for their effect on genetic differentiation between individuals in the current study

Riverscape variable	Description	Hypothesis
Waterway distance	Geographic distance (m) along stream channels between individuals. Calculated at the resolution of 50 m to correspond to the scale at which stream reaches were delineated. Log10 transformed prior to data analysis	Genetic differentiation increases with waterway distance (i.e. isolation-by-distance)
Seasonal barrier	Presence of natural falls with a (semi-)vertical drop of 1.5–2.5 m during summer base-flow condition. Binary data ('1' when a seasonal barrier exists between individuals, and '0' otherwise). Tested in Jefferson Hill-Spruce Brook only	Seasonal barriers prevent fish movement temporarily and increase genetic differentiation
Mean stream gradient	Mean stream gradient across stream reaches located between individuals. Stream gradient was calculated as the ratio of elevation drop per unit distance of stream channel	High gradient stream reaches (i.e. steep slope) impede fish movement and increase genetic differentiation
Mean stream temperature	Mean stream temperature across stream reaches located between individuals. Late July stream temperatures were used because they covered the hottest periods in 2008 (July 16–31)	Stream reaches with warmer temperature are avoided by brook trout in summer when temperature exceeds or approaches their upper thermal limit. Warmer reaches therefore fragment brook trout populations
Number of confluences	Number of tributary confluences between individuals	Brook trout typically spawn in uppermost headwaters and tributaries, and disperses downstream (Witzel & MacCrimmon 1983; Petty *et al.* 2005). If so, given the dendritic arrangement of stream networks, tributaries support genetically related individuals and the presence of tributaries increase genetic differentiation at the watershed scale

spatial autocorrelation analysis, genetic differentiation between all pairs of individuals was calculated in the matrix format using the method of Smouse & Peakall (1999) in GENALEX. Riverscape variables characterized stream channel habitat between pairs of individuals in the matrix format, and a positive correlation was hypothesized between the genetic distance matrix and each riverscape variable matrix (Table 1). Waterway distance between individuals tested evidence for isolation-by-distance. Seasonal barriers, mean stream gradient and mean stream temperature in late July were examined for 'isolation-by-resistance', because its presence (seasonal barriers) or high values (gradient and temperature) were hypothesized to impede gene flow for brook trout (Table 1). We originally considered several stream temperature variables, but the mean temperature at loggers (range 16.6–21.1 °C; SD 0.64 °C) was significantly correlated with the mean daily maximum temperature [Pearson's correlation $r = 0.95$, $P < 0.0001$; range 17.6–22.9 °C; standard deviation (SD) 0.87 °C)], the single maximum temperature recorded ($r = 0.88$, $P < 0.0001$; range 18.6–24.4 °C; SD 0.98 °C) and cumulative number of days exceeding 20 °C ($r = 0.86$, $P < 0.0001$; range 0–15 days; SD 4.5 days) during the period of July 16–31, 2008. Thus, the mean stream temperature was used in our statistical analysis because this is a commonly used variable in previous thermal studies on stream fishes (e.g. Sloat *et al.* 2005; Xu *et al.* 2010). Finally, number of tributary confluences was included because tributaries were known to be important spawning habitat for brook trout (Witzel & MacCrimmon 1983; Petty *et al.* 2005) and the mere presence of tributaries may increase genetic differentiation if dispersal is downstream-biased in the dendritic habitat (Morrissey & de Kerckhove 2009) (see Table 1).

Each riverscape variable was quantified for all possible pairs of individuals in each study site, based on values across all stream reaches between and including the two reaches in which the pair of individuals was collected. When a pair of individuals was collected from the same reach, a value of zero was assigned for waterway distance and the value for that particular reach was used for stream gradient and temperature matrices. In this way, elements of gradient and temperature

matrices characterized mostly the stream corridor between a pair of individuals (when they were geographically apart) but also characterized stream habitat in which a pair of individuals was collected (when they were found in the same reach).

Mantel tests and multiple regression on distance matrices (MRM) were used to examine the effect of riverscape variables on individual genetic differentiation. Mantel tests assess the correlation between two matrices, and MRM simultaneously examines the effect of a group of explanatory matrices on the response matrix (Legendre & Legendre 1998; Lichstein 2007; Goslee 2010). Mantel tests were performed between the genetic distance matrix and each riverscape matrix, and MRM were run using all riverscape matrices in each study area. In addition, the same sequence of analyses was performed for the two different subsets of continuous reaches that were not influenced by seasonal barriers (i.e. Spruce Brook arm and Jefferson Hill Brook arm), identical to the spatial autocorrelation analysis. Mantel tests and MRM were carried out using the ecodist package (Goslee & Urban 2007) available in R. Statistical significance (P-value) was obtained by 10 000 permutations for both analyses, and statistical significance of riverscape variables was declared at $\alpha = 0.05$ for MRM, and $\alpha = 0.05$ with the Bonferroni correction method for Mantel tests (i.e. 0.05/number of riverscape variables tested in each study segment). We report two-tailed P-values (null hypothesis: $r_M = 0$ for Mantel tests and $\beta = 0$ for MRM). However, recall that our ecological prediction was for positive correlation between genetic and riverscape matrices (Table 1), and we expected that few, if any, would have a negative relationship.

Results

Field sampling and descriptive statistics

Both study areas were typical of small, headwater streams characterized with high stream gradient. Mean stream gradient across 50-m reaches was 3.4% (range: 0.6–11.6%) in Jefferson Hill Spruce Brook and 4.0% (range: 1.1–13.4%) in Kent Falls Brook. Mean stream temperature in late-July was 18.7 °C (range: 17.7–19.3) in Jefferson Hill-Spruce Brook and 19.0 °C (range: 16.5–21.0) in Kent Falls Brook, and these values approached the upper thermal range for brook trout (Hartman & Cox 2008). The size class of brook trout that was genotyped (81–140 mm total length) was continuously distributed across the watershed (Fig. S1, Supporting information), but larger trout were more unevenly distributed and were associated with deep pool habitat (Kanno 2010).

A total of 1,732 adipose fin clips was collected from brook trout of 81–140 mm total length in the two study sites (953 in Jefferson Hill-Spruce Brook and 779 in Kent Falls Brook). A sub-sample of 740 individuals was genotyped in the two sites combined; 473 in Jefferson Hill-Spruce Brook and 267 in Kent Falls Brook. The number of trout genotyped was consistent among reaches, with a mean of 3.1 individuals (SD: 1.1) in Jefferson Hill-Spruce Brook and a mean of 3.3 individuals (SD: 1.1) in Kent Falls Brook.

All eight microsatellite loci were polymorphic (Table S1, Supporting information). There was no evidence of stutter products or large allelic dropout based on the MICRO-CHECKER results. Low frequencies of null alleles were estimated across the eight loci in the FREENA results: the mean of 2.26% (range: 0.61–4.06) in Jefferson Hill-Spruce Brook and 1.52% (range: 0.68–2.47) in Kent Falls Brook. Genetic diversity was generally lower in Kent Falls Brook than in Jefferson Hill-Spruce Brook, perhaps because of the isolation of Kent Falls Brook by the natural waterfalls located downstream of the study area. In Jefferson Hill-Spruce Brook, observed heterozygosity (H_O) was 0.514–0.871 and expected heterozygosity (H_E) was 0.537–0.922. In Kent Falls Brook, H_O was 0.195–0.835, and H_E was 0.209–0.847. Genetic linkage disequilibrium was not significant after Bonferroni corrections in any pair-wise comparison of loci in the two sites (all P-values >0.00045). None of the eight loci showed deviation from Hardy–Weinberg equilibrium after Bonferroni correction in Kent Falls Brook, but three loci showed a sign of heterozygote deficiencies in Jefferson Hill-Spruce Brook (P-value <0.006) (Table S1, Supporting information). Given the low occurrence of null alleles and small positive values of inbreeding coefficient across the eight loci, departures from Hardy–Weinberg equilibrium indicated a first sign of fine-scale population within the latter watershed.

Movement of large brook trout

Large brook trout were sedentary and were most typically found in the same 50-m reaches during summer (Fig. 2a). Of the 63 individuals recaptured in both study sites in late summer (mean total length = 184 mm; range = 136–258 mm), 47 individuals were collected from the same reaches in which they had been tagged and released in early summer. The longest upstream movement distance observed within summer was 450 m, and the longest downstream movement was 500 m.

From summer to fall, it was still common to recapture brook trout in the same 50-m reaches (47 of 105 individuals in both study sites combined), but some trout

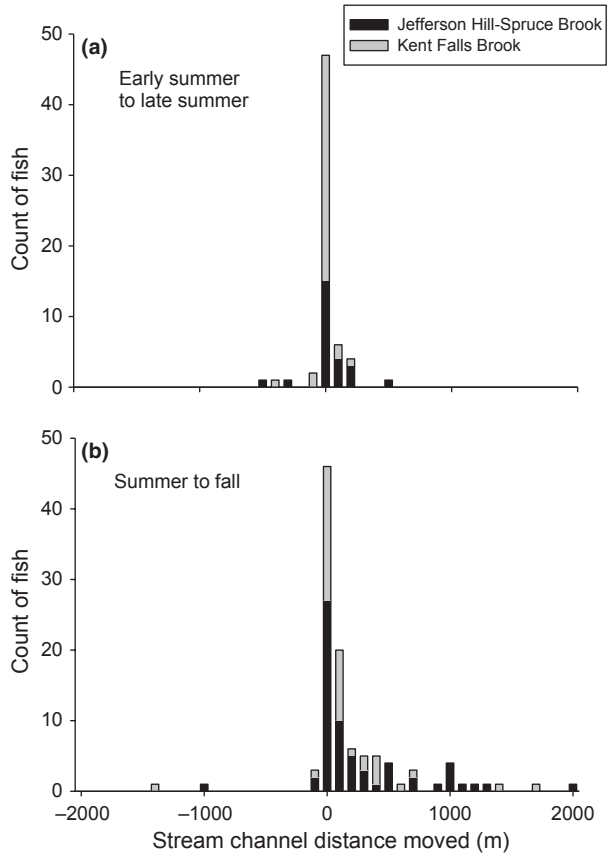

Fig. 2 Movement (+: upstream, −: downstream) of brook trout individuals tagged with Visible Implant Alpha tags, from (a) early summer to late summer [mean total length = 184 mm (range: 136–258 mm)], and (b) summer to fall [mean total length = 176 mm (range: 132–252 mm)]. Recapture events from Kent Falls Brook and Jefferson Hill-Spruce Brook are differentiated by grey and black shading. Movement distance of 0 indicates that an individual was marked and recaptured in the same stream reach.

moved and the direction of movement was predominately upstream (Fig. 2b). The longest upstream movement was 1950 m by a 210 mm male tagged in the lower part of Jefferson Hill Brook (750 m above the Spruce Brook confluence) during summer and recaptured in a mainstem reach located upstream of the JT3 confluence during fall (Fig. 1). The mean total length of recaptured trout during fall was 176 mm with a range of 132–252 mm.

Genetic clusters and local dispersal

The presence of a hierarchical population structure was evident in Jefferson Hill-Spruce Brook, but the evidence of genetic clusters was weak at best in Kent Falls Brook for which STRUCTURE and DAPC provided equivocal results. In Jefferson Hill-Spruce Brook, the estimated log probability of the data generally increased with an increasing number of K in STRUCTURE, but $K = 2$ was an apparent choice following the approach of Evanno *et al.* (2005) (Fig. 3a). This division corresponded with the presence of a seasonal barrier in Spruce Brook (Figs 1 and 3b). In addition, subsequent STRUCTURE runs using only the Jefferson Hill Brook data inferred the presence of four genetic clusters within it. The population structure was weak, as indicated by the prevalence of admixture individuals (i.e. vertical bars with more than one colour) in the STRUCTURE output (Fig. 3b). However, the four genetically distinguishable clusters could be attributed to each of the three tributaries ('JT 1–3') and Spruce Brook, and the main stem of Jefferson Hill Brook harboured a mixture of individuals assigned to these clusters. No finer-level clusters were identified for reaches in Spruce Brook above the seasonal barrier ($K = 1$ had the best support) (Fig. 3b).

DAPC similarly identified a hierarchical structure in Jefferson Hill-Spruce Brook. In successive K-means clustering, the initial sharp decline in Bayesian Information Criterion (BIC) values continued up to $K = 5$–7 (Fig. 4a). When using a group ($K = 5$) of spatially close individuals that accounted for the locations of seasonal barriers and tributaries (Fig. 4b), DAPC separated Spruce Brook from Jefferson Hill Brook along the first principal component axis (eigenvalue = 212.49) (Fig. 4c). Along the second axis that represented much less variability of data (eigenvalue = 65.90), brook trout in a semi-isolated tributary ('JT1') was plotted distant from other genetic clusters in Jefferson Hill Brook (Fig. 4c). Overlapping distributions of genetic clusters on the ordination plot indicated a low degree of genetic differentiation. Pair-wise F_{ST} values among genetic clusters ranged between 0.004 ('JeffDown' vs. 'JT2+') and 0.085 ('JT1' vs. 'Spruce') (Table S2, Supporting information).

Analysis of local movement rates provided additional evidence of genetic clusters connected by varying degrees of dispersal in Jefferson Hill-Spruce Brook. Using the five genetic clusters identical to DAPC analysis, estimates of movement rates among them were derived from BAYESASS with small 95% confidence intervals (Table 2 and Fig. 5). Fish movement was primarily unidirectional towards the downstream direction when seasonal barriers were present (Fig. 5). In the absence of such physical barriers, movement could be bidirectional such as the movement pattern between 'JT2+' and 'JT3+'. The downstream reaches of Jefferson Hill Brook ('JeffDown') were characterized with a mixture of immigrants from other parts of the watershed (Fig. 5), and this result was in line with the STRUCTURE and DAPC results. Despite the asymmetric pattern of gene flow, the five genetic clusters were

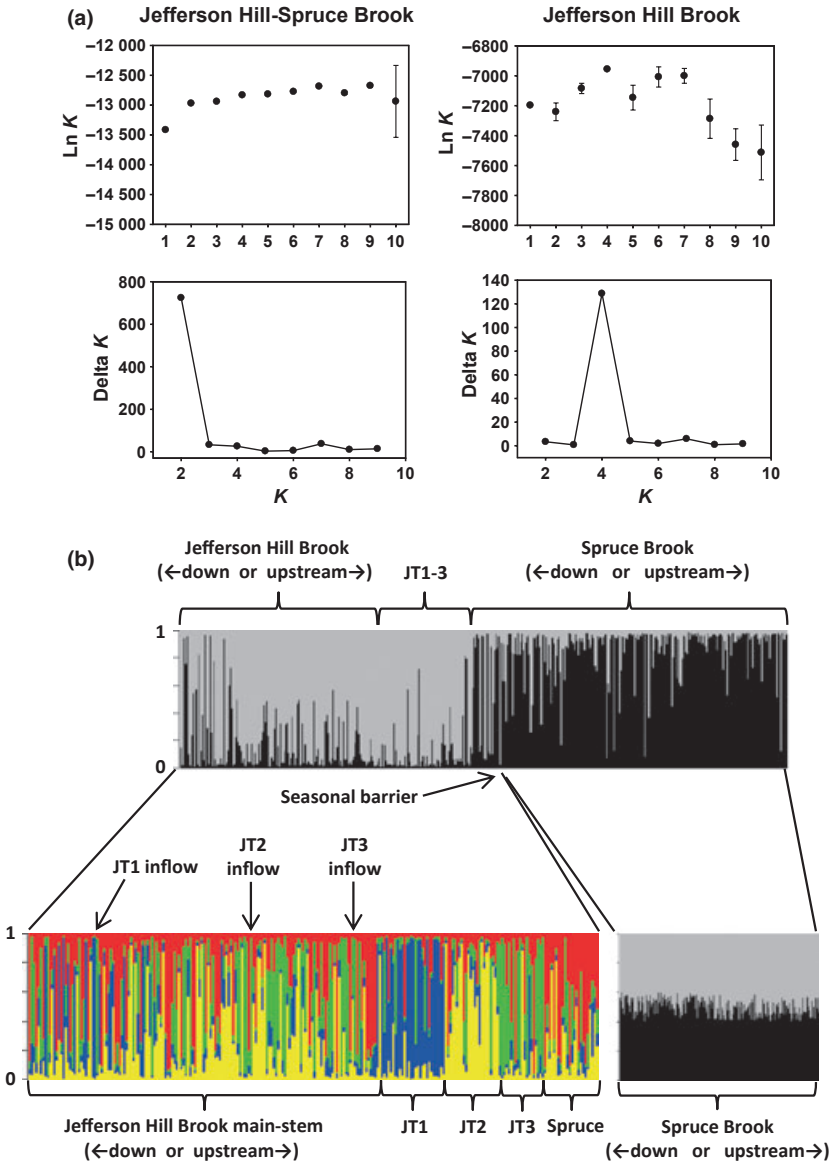

Fig. 3 STRUCTURE results in Jefferson Hill-Spruce Brook showing a hierarchical genetic structure: (a) Mean log likelihood over 10 runs (error bars = standard deviations) and ΔK, the second order rate of change in the likelihood, at each K. (b) Bar plots showing individual genotype membership to K clusters (each cluster is represented by a different colour and each vertical bar represents an individual). STRUCTURE was initially run with all samples in Jefferson Hill-Spruce Brook (K = 2 shown), followed by subsequent runs for Jefferson Hill Brook (K = 4 shown) and Spruce Brook (K = 2 shown) individually.

characterized by similar values for H_E (range: 0.720–0.764) and H_O (range: 0.716–0.736) (Table S3, Supporting information).

In Kent Falls Brook, evidence for genetic clusters was not identified by STRUCTURE (i.e. K = 1) (Fig. 6). The log probability of data *decreased* between K = 1–4 (Fig. 6a) and genetic clusters were not discernible even at K = 2 (Fig. 6b). To the contrary, the initial sharp decline in BIC values continued up to K = 4–5 in DAPC (Fig. 7a), indicating the potential presence of genetic clusters. However, when using four genetic clusters delineated by tributary confluences (Fig. 7b) (see the following paragraphs for the importance of confluences in this study site), the four clusters were plotted with much overlap in the ordination space produced by DAPC (Fig. 7c): eivenvalues; Axis 1 = 53.52, Axis 2 = 29.18. Pair-wise F_{ST} values among genetic clusters were small ranging between 0.007 ('KentMiddle' vs. 'KentUp') and 0.027 ('KentDown' vs. 'KentUp') (Table S4, Supporting information). Thus, population structuring, in terms of genetic clusters, was weak at best in Kent Falls Brook.

Isolation-by-distance

Spatial autocorrelation analysis showed a weak isolation-by-distance pattern in all stream segments tested (Fig. 8). Genetic similarity between individuals gradually decreased with geographic distance in all stream segments. Autocorrelation coefficients (r) were significantly positive up to 400 m in both Jefferson Hill Brook and Spruce Brook, and 700 m in Kent Falls Brook. Significantly negative coefficients were first detected at 2200 m in Jefferson Hill Brook, 1900 m in Spruce Brook and 1600 m in Kent Falls Brook.

Riverscape genetics analysis

Mantel tests and MRM identified at least one riverscape variable that affected individual genetic differentiation for each study area or stream segment (Table 3). The Mantel statistic, r_M (ranges −1 and 1), was statistically significant at small absolute values (−0.20 to 0.20), a phenomenon often reported in other studies (Dutilleul *et al.* 2000; Goslee 2010). Not surprisingly, some riverscape variables were not statistically significant in MRM, although they were significant when their effect was assessed individually in Mantel tests.

Waterway distance (i.e. isolation-by-distance) was the most consistent influence across stream segments. It was statistically significant in all tests, except MRM for Kent Falls Brook (Table 3). Consistent with the cluster analyses, the presence of seasonal barriers increased genetic differentiation in Jefferson Hill-Spruce Brook and it had the highest value of the Mantel statistic among the riverscape variables tested ($r_M = 0.158$, $P = 0.0001$).

The influence of other riverscape variables was less consistent across stream segments. Stream gradient was positively related to genetic differentiation in Jefferson Hill-Spruce Brook in Mantel tests ($r_M = 0.106$, $P = 0.0001$), but it was not statistically significant in MRM ($P = 0.1626$). Significance of stream gradient appeared to be related to the distinct habitat differences between Jefferson Hill Brook and Spruce Brook. Jefferson Hill Brook, which is characterized by the presence of genetic clusters (Figs 3–5), was consistently steeper and colder than Spruce Brook (Fig. S2, Supporting information); so, a pair of individuals in the former was typically associated with higher stream gradient and colder temperature than that in the latter. This may explain the *negative* coefficient for stream temperature in Jefferson Hill-Spruce Brook (Table 3), contrary to the original hypothesis of stream temperature effect (Table 1).

Stream temperature was positively correlated with genetic distance when only reaches above the seasonal barrier in Spruce Brook were considered (Table 3). However, stream temperature was fairly homogeneous longitudinally in Spruce Brook (late July mean temperature <1 °C), with slightly colder reaches located in an upstream area (Fig. S2b, Supporting information).

The number of tributary confluences was statistically significant in Jefferson Hill-Spruce Brook (Mantel tests) and Kent Falls Brook (Mantel tests and MRM) (Table 3). The importance of this riverscape variable in Jefferson Hill-Spruce Brook is in agreement with cluster analyses that identified Jefferson Hill Brook tributaries as genetically distinguishable (Figs 3–5). Interestingly, it was the only significant riverscape variable in MRM for Kent Falls Brook (Table 3), even though tributaries were not identified as genetically distinguishable in this study site.

Discussion

Brook trout in the study headwater stream networks were characterized by fine-scale population structure over a few kilometres. Genetic clusters were evident when seasonal barriers were present in Jefferson Hill-Spruce Brook, and a perceptible isolation-by-distance pattern was consistently observed within stream segments. Individual genetic differentiation was also explained by stream gradient, temperature and number of tributary confluences in some stream segments. In the following sections, we discuss our results of fine-scale population structure and riverscape influence by highlighting our research questions (see Introduction).

Fine-scale population structure

Genetic clusters. The presence of genetic clusters was evident in Jefferson Hill-Spruce Brook, but weak at best in Kent Falls Brook. This discrepancy is because of the presence of seasonal barriers in the former study site (note that no fish were found above the permanent barrier in 'KT3' in Kent Falls Brook shown in Fig. 1). Two genetic clusters located above the seasonal barriers in Jefferson Hill-Spruce Brook (i.e. 'JT1' and 'Spruce') were characterized with an asymmetrical pattern of gene flow (i.e. biased downstream). In fact, we did not recapture any tagged trout that had ascended the two seasonal barriers in our field season. However, weak genetic clusters were identified even in the absence of apparent physical barriers (i.e. 'JT2' and 'JT3'). It should be noted that the delineation of the inferred clusters was not clear-cut, as would be expected from a fine-scale genetics study. Highly polymorphic markers can detect genetic clusters even when the movement rate is high, and clusters may maintain demographic dependence until the movement rate reaches 10% (Waples & Gaggiotti 2006). Because some movement rates among

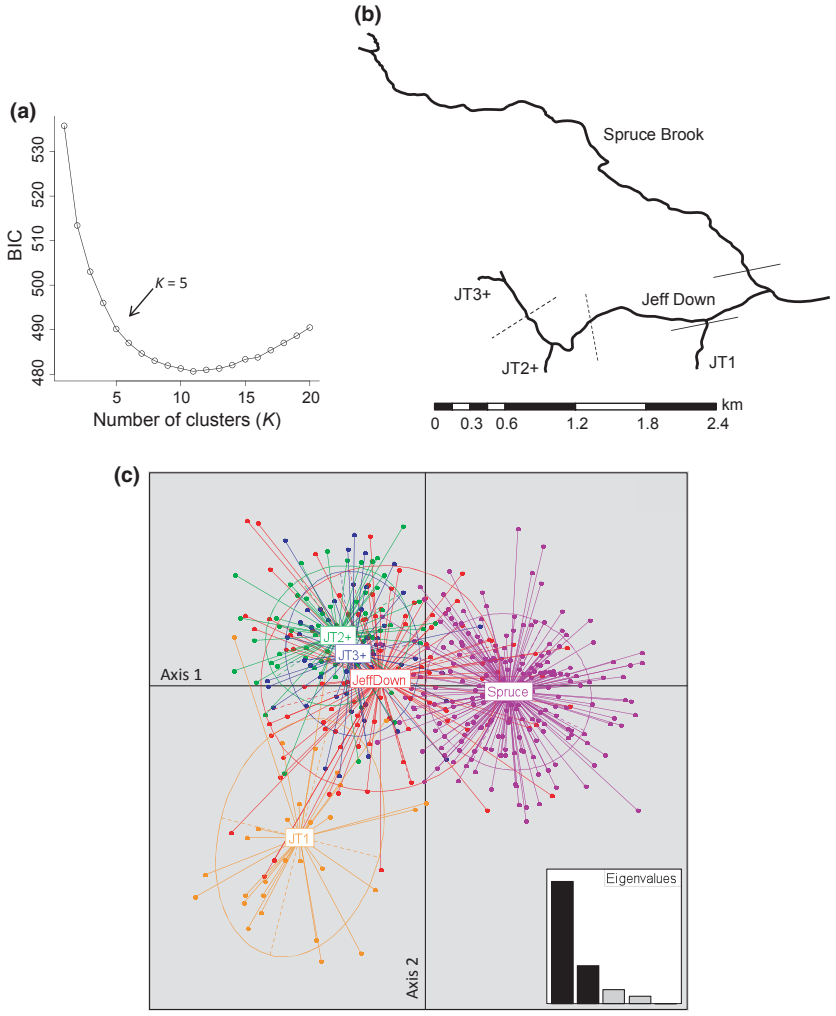

Fig. 4 Summary of discriminant analysis of principal components (DAPC) for Jefferson Hill-Spruce Brook: (a) Changes in mean Bayesian Information Criterion (BIC) values in successive K-means clustering. Ten runs were executed at each *K* value (the standard deviation across the runs was so small that it could not be shown here). (b) Spatial boundaries of genetic clusters used for DAPC. Solid lines indicate locations of seasonal barriers, and dashed lines indicate arbitrary boundaries of two genetic clusters (JT2+ and JT3+). (c) Ordination plot of DAPC for the five genetic clusters. Genetic clusters are shown by different colours and inertia ellipses, and dots represent individuals. The bottom-right inset shows eigenvalues of the four principal components in relative magnitude.

clusters in Jefferson Hill-Spruce Brook were near this value, it is most likely appropriate to treat the entire study watershed as a single management unit from a conservation perspective.

Asymmetric dispersal indicated spatial habitat heterogeneity in Jefferson Hill-Spruce Brook. The most downstream cluster (i.e. ''Jeff Down'' in Table 2 and Fig. 5), characterized by low population abundance (Fig. S1, Supporting information) and few age 0+ trout (data not shown), received immigrants from other parts of the watershed. BAYESASS assumes a low immigration rate that cannot exceed one-third of a population (Wilson & Rannala 2003), and the immigration rate into this cluster approached the threshold (1−0.681 = 0.319: Table 2). This result implied that immigration rates into this cluster might be potentially even higher. Downstream-biased dispersal is probably an important mechanism to maintain the spatial genetic structure in this watershed; if dispersal had been upstream-biased, tributaries might have been more genetically homogenized to one another. Downstream-biased gene flow has also been observed for other stream organisms (Hänfling & Weetman 2006; Chaput-Bardy *et al.* 2008). Our tagging data provided complementary information in understanding spatial population ecology of brook trout because it showed upstream-biased movement by large trout during fall. The fall movement is presumably related to spawning, and small tributaries are typical spawning and rearing habitat for brook trout (Witzel & MacCrimmon 1983; Petty *et al.* 2005). Taken together, we

Table 2 BAYESASS estimates of movement rates (with 95% confidence interval) among genetic clusters in Jefferson Hill-Spruce Brook

	Movement from				
	Jeff Down	JT1	JT2+	JT3+	Spruce
Movement into					
Jeff Down	0.681 (0.667–0.706)	0.067 (0.036–0.102)	0.087 (0.044–0.138)	0.058 (0.025–0.104)	0.107 (0.067–0.146)
JT1	0.011 (0.000–0.054)	0.935 (0.856–0.993)	0.030 (0.000–0.091)	0.011 (0.000–0.048)	0.013 (0.000–0.053)
JT2+	0.008 (0.000–0.036)	0.005 (0.000–0.024)	0.852 (0.774–0.921)	0.126 (0.058–0.207)	0.009 (0.000–0.032)
JT3+	0.095 (0.049–0.147)	0.009 (0.000–0.037)	0.088 (0.025–0.154)	0.798 (0.735–0.868)	0.010 (0.000–0.034)
Spruce	0.001 (0.000–0.007)	0.001 (0.000–0.005)	0.001 (0.000–0.007)	0.001 (0.000–0.004)	0.996 (0.985–1.000)

Diagonal elements refer to the proportions of individuals derived from their own clusters. Spatial locations of the five clusters are shown in Fig. 5.

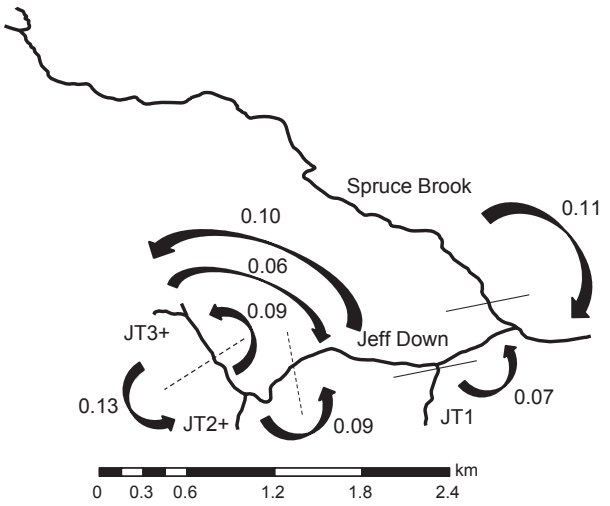

Fig. 5 BAYESASS estimates of movement rates among genetic clusters in Jefferson Hill-Spruce Brook. Only movement rates over 5% are shown here (see Table 2 for movement rates between all pairs of genetic clusters). Solid lines indicate locations of seasonal barriers, and dashed lines indicate arbitrary boundaries of two genetic clusters (JT2+ and JT3+). The five genetic clusters are identical to those used in DAPC (Fig. 4b).

conclude that (i) tributaries and upstream reaches of Jefferson Hill Brook support spawning and rearing habitat, (ii) some individuals dispersed into downstream reaches of Jefferson Hill Brook at early life stages (i.e. before age 1+), and (iii) genetic structure among tributaries is maintained either because a good portion of individuals remain in their tributaries or because those that dispersed into downstream reaches have high fidelity of return to their natal tributaries.

The dual use of STRUCTURE and DAPC was important for analysing genetic clusters in the current study. The standard application of STRUCTURE analysis would have missed any sign of clustering in Kent Falls Brook. In fact, the 'clusters' in Kent Falls Brook may be an artefact of isolation-by-distance, given the seemingly high ability to identify genetic clines in the recently developed DAPC (Jombart *et al.* 2010). Further, DAPC was more efficient than STRUCTURE when detecting a hierarchical structure in Jefferson Hill-Spruce Brook as DAPC identified the pattern in a single analysis that took a few seconds to compute, as opposed to the series of STRUCTURE runs required to identify spatial hierarchy. A difficulty with identifying the number of genetic clusters using DAPC is the absence of an accepted quantitative method, as opposed to those frequently used in STRUCTURE (Pritchard *et al.* 2000; Evanno *et al.* 2005); we used a visual approach in successive *K*-means clustering (Jombart *et al.* 2010). In this study, using two different clustering methods (Bayesian vs. multivariate analysis) in an exploratory manner led to a less biased assessment of data, and we recommend such practices be followed in other landscape genetics studies.

Isolation-by-distance. Brook trout were mating non-randomly at the watershed scale and the weak pattern of isolation-by-distance was consistently found in all stream segments. Spatial autocorrelation coefficients in our study were small ($r < 0.05$), but similar values have been commonly reported in previous studies conducted at fine spatial scales (i.e. distances comparable to individual organisms' dispersal capability) (Vignieri 2005; Neville *et al.* 2006b; Jones *et al.* 2007; Gomez-Uchida *et al.* 2009). Brook trout, at least large individuals, were potentially capable of moving in the studied high-gradient headwater stream channels. For example, the 210-mm male trout that moved the longest upstream distance (1950 m) was able to ascend the high-gradient stream channel of Jefferson Hill Brook. The high mobility of large brook trout has also been reported from other streams (Gowan *et al.* 1994; Adams *et al.* 2000). However, it was still most common to find large

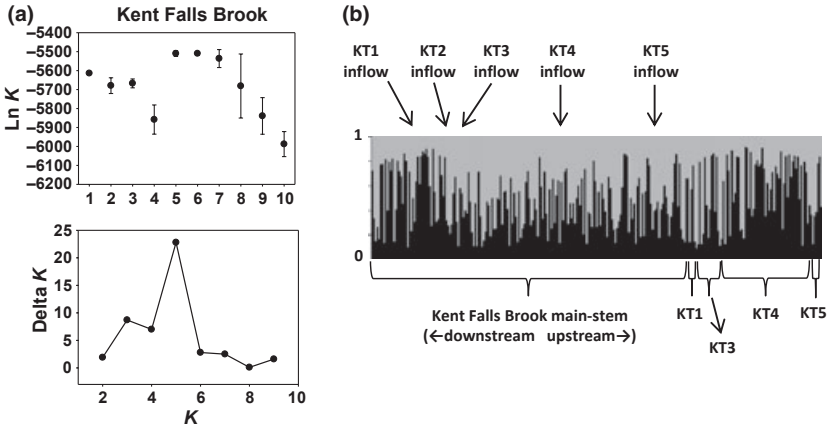

Fig. 6 STRUCTURE results in Kent Falls Brook showing the absence of evident genetic clusters: (a) Mean log likelihood over 10 runs (error bars = standard deviations) and ΔK, the second order rate of change in the likelihood, at each K. (b) Bar plots showing individual genotype membership to $K = 2$ clusters (each cluster is represented by a different colour, and each vertical bar represents an individual).

individuals in the same reaches during the recapture events within a single field season (summer to fall). In addition, limited dispersal was also inferred from the size class of brook trout genotyped (81–140 mm) by recording spatial distributions of genetically inferred full-sib individuals in the study areas (Kanno *et al.* 2011). Hudy *et al.* (2010) similarly reported restricted movement of brook trout from a Virginia headwater stream, where genetically related brook trout were spatially clustered and their inferred parents were typically collected nearby. These results are in agreement with previous findings that stream salmonid populations are composed of 'movers' and 'non-movers' (Gowan *et al.* 1994; Rodríguez 2002), leading to the common but weak isolation-by-distance pattern identifiable by informative genetic markers.

Fine-scale genetic differentiation is often attributed to large variation in reproductive success between individuals (Barr *et al.* 2008; Tatarenkov *et al.* 2010). However, sibship reconstruction based on the same genetic data did not reveal such highly skewed reproductive success between individuals in our study streams (Kanno *et al.* 2011). In addition, both males and females were typically inferred to be polygamous (Kanno *et al.* 2011), which should help buffer against genetic drift. These results provide additional evidence that the isolation-by-distance pattern may have resulted from restricted movement of brook trout.

Brook trout in stream habitat are known to be territorial, defending the most profitable microhabitat that maximizes the net energy intake (Nakano *et al.* 1998; Gowan 2007). Dominant individuals may force subordinate individuals to leave optimal habitat and disperse to suboptimal habitat (Gowan & Fausch 2002). Rodríguez (2002) reported that such competitive exclusion of subordinate individuals was common among lotic populations of brook trout, but their displacement distance was typically short (<50 m). This movement model of brook trout is in agreement with the observed isolation-by-distance pattern in this study; one should expect the absence of fine-scale spatial population structure if long-range dispersal by displaced individuals were to be common.

Riverscape influence on genetic differentiation

Number of confluence. In both study sites, the number of confluence was an important riverscape variable to explain genetic differentiation between individuals. The more dendritic pattern of Jefferson Hill Brook, compared with Spruce Brook, may be partly responsible for the more structured genetic pattern in the former, particularly because total stream network lengths are similar between them. A dendritic habitat pattern associated with a downstream-biased gene flow (just like Jefferson Hill Brook) is theoretically shown to promote genetic differentiation between branch populations and increase genetic diversity in a downstream direction (Morrissey & de Kerckhove 2009). The discovery of genetic clusters centred around tributary locations in Jefferson Hill Brook fits into such predictions. However, genetic diversity as measured by observed and expected heterozygosity was similar among the five genetic clusters. In our study, measures of genetic diversity alone would not have revealed asymmetry of gene flow, and the quantification of local dispersal rates in spatially extensive sampling was the key to finding the spatial population structures.

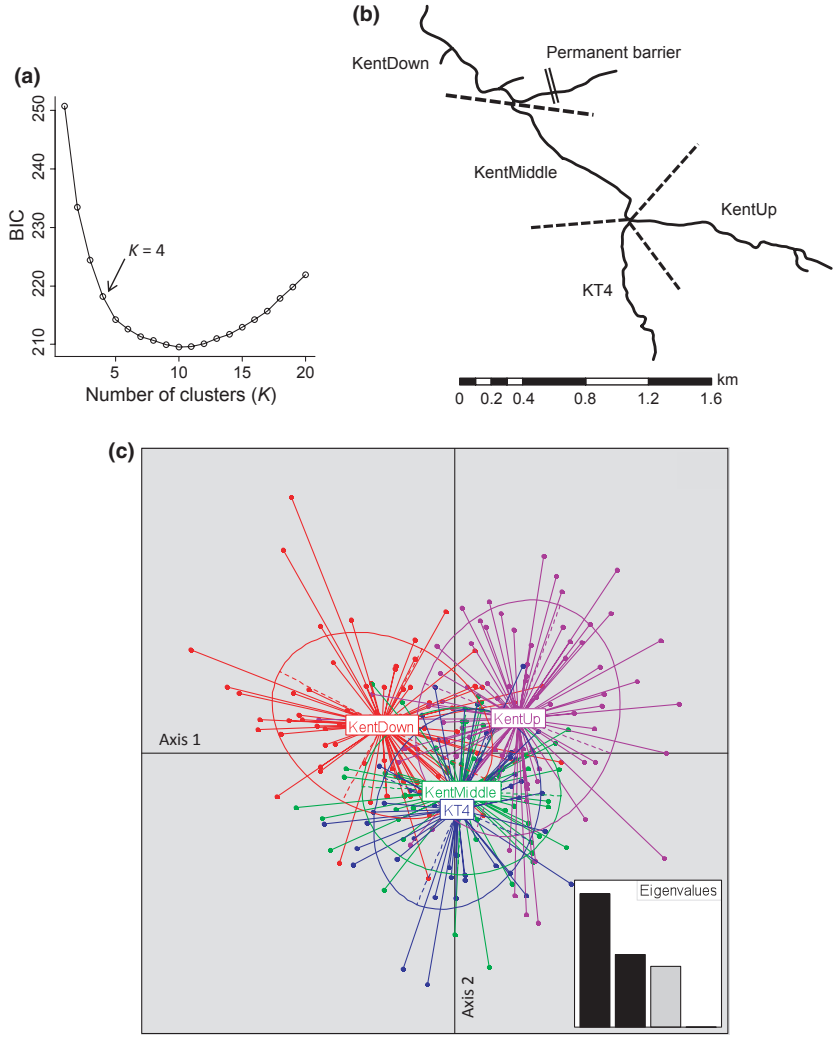

Fig. 7 Summary of discriminant analysis of principal components (DAPC) for Kent Falls Brook: (a) Changes in mean Bayesian Information Criterion (BIC) values in successive K-means clustering. Ten runs were executed at each *K* value (the standard deviation across the runs was so small that it could not be shown here). (b) Spatial boundaries of genetic clusters used for DAPC. Genetic clusters were delineated by locations of two major tributary confluences. (c) Ordination plot of DAPC for the four genetic clusters. Genetic clusters are shown by different colours and inertia ellipses, and dots represent individuals. The bottom-right inset shows eigenvalues of the three principal components in relative magnitude.

The significance of confluence number in Kent Falls Brook was a surprising result because it did not have a well-developed dendritic network pattern; its tributaries, except the largest one ('KT4'), were short (50–150 m) or warm, harbouring few brook trout individuals. Thus, Kent Falls Brook was a rather linear network system and tributaries did not harbour genetically distinguishable clusters. The discovery of spatial genetic patterns even in such a watershed sheds light on the importance of tributaries that create abrupt geomorphological changes in fluvial habitat (Benda *et al.* 2004), and tributary confluences *per se* might act to develop a spatial population structure for some aquatic organisms. Genetic patterns in dendritic habitat have recently been explored from the theoretical ground (Fagan 2002; Labonne *et al.* 2008; Morrissey & de Kerckhove 2009), and more empirical studies are warranted in other geographic locations and across various species with different longitudinal zonation and distribution (e.g. headwater species vs. mainstem species; dendritic (heart-shaped) vs. trellis (rectangular) watersheds *sensu* Benda *et al.* 2004).

Stream temperature. We predicted that warmer stream temperatures would impede gene flow and fragment brook trout populations; however, stream temperature was important only in Spruce Brook above the seasonal barrier. Plus, ecological significance is not straightforward

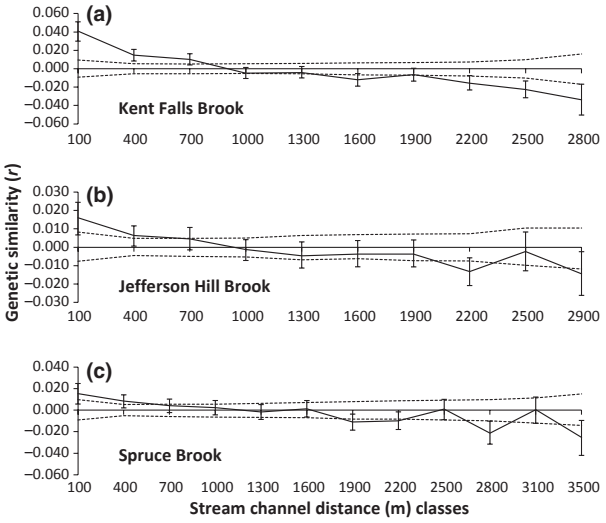

Fig. 8 Autocorrelograms of genetic and geographic distance for brook trout individuals in (a) Kent Falls Brook, (b) Jefferson Hill Brook and (c) Spruce Brook. Upper and lower 95% CI for the null hypothesis of 'no structure' are shown with dotted lines, and 95% CI of *r* are shown by error bars.

to interpret because of the limited longitudinal temperature range (<1 °C) and spatially continuous brook trout distributions. We hoped to record detailed longitudinal profiles of stream temperature possibly affected by groundwater discharge; however, such groundwater influence was not detectable by placing temperature loggers at every 150 m. Yet, brook trout are known to utilize physically restricted areas of groundwater discharge as thermal refugia during summer (Biro 1998) and spawning habitat during fall (Essington *et al.* 1998).

Lack of strong evidence for stream temperature effect is also related to the spatial scale of investigation of this study. The study focused on subtle thermal heterogeneity within headwater stream channel networks where brook trout were continuously distributed. At the broader spatial scale, brook trout in the central and southern parts of the native range persist in small and fragmented headwater populations, mainly because thermal habitat is not suitable further downstream (Meisner 1990; Hudy *et al.* 2008; Kanno & Vokoun 2008). Analysis of riverscape genetics at the broader scale would likely detect a positive relationship between stream temperature and genetic differentiation, and such studies are important given the potential vulnerability of brook trout to temperature elevation owing to anthropogenic activities (e.g. groundwater withdrawals and climate change).

Table 3 Summary of Mantel tests and multiple regression on distance matrices (MRM) for examining the effect of riverscape variables on genetic differentiation between individuals. Mantel tests examine the effect of each riverscape variable individually and MRM examine the effects of all riverscape variables simultaneously

	Mantel test		MRM			
	r_M	*P*-value	β	*P*-value (β)	R^2	*P*-value (R^2)
Jefferson Hill-Spruce Brook						
Log (waterway distance)	0.101	0.0001**	0.381	0.0001**	0.040	0.0001
Seasonal barrier	0.158	0.0001**	0.801	0.0001**		
Stream gradient	0.106	0.0001**	0.110	0.1626		
Stream temperature	−0.103	0.0001**	−0.883	0.0137**		
Number of confluence	0.064	0.0003**	−0.075	0.2624		
Jefferson Hill Brook only						
Log (waterway distance)	0.043	0.0005**	0.243	0.0461**	0.002	0.4579
Stream gradient	−0.022	0.4272	−0.069	0.6210		
Stream temperature	0.018	0.4940	0.089	0.8728		
Number of confluence	0.022	0.4545	−0.014	0.9134		
Spruce Brook only						
Log (waterway distance)	0.046	0.0001**	0.200	0.0004**	0.011	0.0016
Stream gradient	−0.072	0.0209	−0.176	0.1747		
Stream temperature	0.094	0.0027**	1.314	0.0065**		
Kent Falls Brook						
Log (waterway distance)	0.053	0.0001**	−0.189	0.2605	0.012	0.0091
Stream gradient	0.044	0.1249	0.076	0.4540		
Stream temperature	−0.054	0.0544	−0.305	0.2814		
Number of confluence	0.095	0.0004**	0.339	0.0101**		

Statistical significance was based on 10 000 permutations (**two-tailed *P*-values <0.05 for MRM, and two-tailed $P < 0.05$ with Bonferroni correction for Mantel tests). In Jefferson Hill-Spruce Brook, analysis was conducted at the entire watershed scale, as well as two different subsets of continuous reaches that were not influenced by seasonal barriers.

Stream gradient. The expected influence of stream gradient was observed only when Jefferson Hill-Spruce Brook was analysed at the entire watershed scale by a Mantel test. Perhaps, the structured genetic pattern within the Jefferson Hill Brook arm is explained by its high stream gradient that may have hampered the dispersal of brook trout among tributaries because of the presence of frequent cascades and step pools. However, Adams *et al.* (2000) documented that brook trout could move through steep-gradient stream reaches comparable to our study sites. Unlike species that are less capable of negotiating steep slopes (e.g. salamanders: Spear *et al.* 2005), stream gradient may have little influence for brook trout except vertical elevation drops such as natural waterfalls. It should be noted that our study sites were nearly exclusively high-gradient stream reaches. The effect of stream gradient might be examined better by comparing the isolation-by-distance pattern between high-gradient vs. low-gradient streams.

Conclusion

This study demonstrated the utility of a riverscape genetics approach applied to linear habitat when samples were collected in a spatially continuous manner at the (headwater) watershed scale. Informative genetic markers and extensive field sampling of an obligatory riverine species and stream habitat allowed us to understand local population structure and connectivity at a scale relevant to organism's life histories and conservation (Fausch *et al.* 2002) and examine riverscape effects on genetic differentiation at the individual level (Manel *et al.* 2003).

Discovery of riverscape influence on a potentially mobile species, such as stream salmonids, even at a fine-spatial scale suggests that such riverscape influences might be common across other obligatory riverine species. Similar analytical approaches should be useful for gaining insights into local population structure of other obligatory aquatic species in stream network systems. It will be particularly interesting to investigate whether the common patterns observed in this study (e.g. isolation-by-distance and effect of dendritic geometry on genetic differentiation) are found in other regions and across other riverine organisms at a fine spatial scale.

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This work is part of Y.K.'s doctoral dissertation research at the University of Connecticut. Y.K. is interested in ecology and conservation of stream fish and habitat at multiple spatial scales. J.C.V. is a faculty member at the University of Connecticut whose research broadly encompasses fisheries conservation and management issues. B.H.L. studies lifetime fitness of stream fishes in the wild.

Data accessibility

Microsatellite data and GIS shapefiles containing spatial locations: DRYAD entry doi:10.5061/dryad.5f8s2.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Longitudinal patterns of fish count of two size classes (81–140 and >140 mm) by 50 m reach in Kent Falls Brook, Jefferson Hill Brook and Spruce Brook in late summer of 2008.

Fig. S2 Longitudinal profiles of (a) elevation and (b) late-July mean stream temperature in Jefferson Hill Brook and Spruce Brook from their confluence.

Table S1 Summary statistics for eight microsatellite loci for brook trout collected in Kent Falls Brook and Jefferson Hill-Spruce Brook.

Table S2 Pair-wise F_{ST} between genetic clusters in Jefferson Hill-Spruce Brook.

Table S3 The number of brook trout individuals genotyped (N), mean expected heterozygosity (H_E), mean observed heterozygosity (H_O), and mean allelic richness (A_R) across eight microsatellite loci for the five genetic clusters in Jefferson Hill-Spruce Brook (see Figs 4b and 5).

Table S4 Pair-wise F_{ST} between genetic clusters in Kent Falls Brook.

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