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RESEARCH ARTICLE

Exploring the effect of 195 years-old locks on species movement: landscape genetics of painted turtles in the Rideau Canal, Canada

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Abstract

Aquatic systems have been extensively altered by human structures (e.g., construction of dams/canals) and these have major impacts on the connectivity of wildlife populations through the loss and isolation of suitable habitats. Habitat loss and isolation affect gene flow and influence the persistence of populations in time and space by restricting movements. Isolation can result in higher inbreeding, lower genetic diversity, and greater genetic structure, which may render populations more vulnerable to environmental changes, and thus to extinction. Given the ubiquity and the persistence of dams and canals in space and time, it is crucial to understand their effects on the population genetics of aquatic species. Here, we documented the genetic diversity and structure of painted turtle (*Chrysemys picta*) populations in the Rideau Canal, Ontario, Canada. More specifically, we used 13 microsatellites to evaluate the influence of locks on genetic variation in 822 painted turtles from 22 sites evenly distributed along the 202-km canal. Overall, we found low, but significant, genetic differentiation suggesting that some dispersal is occurring throughout the canal. In addition, we showed that locks contribute to the genetic differentiation observed in the system. Clustering analysis revealed two distinct genetic groups whose boundary is associated with a series of six locks. Our results illustrate how artificial waterways, such as canal systems, can influence population genetic structure. We highlight the importance of adopting management plans that can mitigate the impacts of human infrastructure and preserve gene flow across the landscape to maintain viable populations.

Keywords Conservation · Artificial waterway · Population structure · Human infrastructure · MLPE models · Freshwater turtles

Introduction

Increase and expansion of human activities have led to the loss and isolation of natural habitats which, in turn, have resulted in the loss of biodiversity worldwide (Haddad et al. 2015; WWF 2018; Su et al. 2021). Habitat loss and isolation have major impacts on the distribution, abundance and connectivity of wildlife populations that are often reflected in their population genetic diversity and structure (Fahrig 2003; Schlaepfer et al. 2018). Alterations of natural habitats by human activities reduce the area and connectivity of suitable habitats for wildlife, restricting movements of individuals that are necessary to maintain gene flow (Fahrig 2003; Lowe and Allendorf 2010; Schlaepfer et al. 2018). By reducing population size and increasing genetic drift, loss of connectivity results in higher inbreeding and lower genetic diversity, leading to greater population genetic differentiation between isolated populations (Schmidt et al. 2020). A reduction of dispersal may also limit the exchange of alleles that are potentially important for individuals to adapt to their environment (Lenormand 2002; Morjan and Rieseberg 2004). In the long term, fitness of individuals from isolated populations can decrease and populations may become more vulnerable to environmental changes and, thus, to extinction (Reed and Frankham 2003; Willi et al. 2006; Leigh et al. 2019). Therefore, to maintain gene flow and ensure the persistence of small and isolated populations in space and time, it is crucial to identify the factors that limit gene flow among these populations, especially in highly modified environments.

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Freshwater habitats have been extensively altered by humans (e.g., construction of dams/canals) to sustain diverse economic activities (e.g., transportation, energy and water use) (Nilsson 2005; Grill et al. 2015). Worldwide, water flow and connectivity of almost half of all rivers have been modified by dam construction (Grill et al. 2015). These alterations have drastically affected habitat quality and connectivity for aquatic species and, thus, the ecological integrity of ecosystems (Barbarossa et al. 2020; Lin et al. 2020; Su et al. 2021). Reduction in the natural connectivity of ecosystems can impede the life cycles of freshwater species and represents a major cause of the decline of freshwater biodiversity worldwide (Jansson et al. 2000; Perkin and Gido 2012; Fuller et al. 2015; Carvajal-Quintero et al. 2017). In comparison to dams, canals are more complex systems where both barriers (e.g., dams and locks) and new connections (e.g., excavated channels) are created (Lin et al. 2020). This duality makes it difficult to predict the long-term ecological effects of canals on aquatic ecosystems (Lin et al. 2020). The balance between possible negative and positive effects of canals on connectivity can also depend on their design and permeability (i.e., length and number of locks and dams), the original characteristics of the aquatic and surrounding terrestrial habitats, the extent of changes made, and the management history of the canal (Lin et al. 2020). There is currently a lack of research on how genetic population structure of aquatic species is affected by artificial waterways, such as canal systems, especially at large spatial scales (Koschorreck et al. 2020; Lin et al. 2020; Bergman et al. 2021).

To determine how freshwater species are affected by anthropogenic changes, it is crucial to assess how their genetic structure is related to human-made barriers in these artificial systems (Selkoe et al. 2015). To do so, a landscape genetics approach can be used to determine the permeability to gene flow and the occurrence of genetic discontinuities in artificial waterways (Selkoe et al. 2015). In particular, by using landscape genetics modelling, one can quantify the relationship between genetic differentiation (e.g., genetic distance such as F_{ST}) and landscape features (e.g., number of barriers) (Van Strien et al. 2012; Row et al. 2017). Landscape genetics can also inform management decisions for vulnerable species by identifying areas of conservation concern (e.g., populations with low genetic diversity and high genetic structure, and identification of barriers responsible for the evolution of distinct genetic groups; Manel et al. 2003; Holderegger and Wagner 2008).

Turtles are among the most vulnerable taxa to anthropogenic changes (Gibbons et al. 2000; Buhlmann et al. 2009; Böhm et al. 2013) given their life-history traits (e.g., late sexual maturity, low juvenile survival) that make populations less resilient to reductions in adult survival (Brooks et al. 1991; Congdon et al. 1994; Midwood et al. 2015). In Canada, six out of 10 native freshwater turtles are considered at risk by the Committee on the Status of Endangered Wildlife in Canada (Species at risk public registry: www.canada.ca/en/environment-climate-change/services/species-risk-public-registry). Previous studies have evaluated the impact of landscape changes (e.g., expansion of road networks and agricultural/urban conversion of natural habitats) on genetic patterns of turtle populations (see Laporte et al. 2013; Willoughby et al. 2013; Reid and Peery 2014; Reid et al. 2017 for examples). While some studies found no genetic effects of landscape changes (Laporte et al. 2013; Willoughby et al. 2013), others indicated that anthropogenic changes were associated with higher genetic differentiation and lower genetic diversity (Reid and Peery 2014; Reid et al. 2017). To our knowledge, however, no study to date has assessed the effects of large-scale aquatic landscape alterations (e.g., constructions of canals) on genetic population structure of freshwater turtles [see Bennett et al. (2010) for a study at a small spatial scale].

In this study, we characterize the genetic diversity and genetic population structure of painted turtles (*Chrysemys picta*) in the Rideau Canal, Ontario, Canada. More specifically, we assess how landscape features, especially the presence of locks, are related to genetic differentiation throughout the system. We hypothesize that the construction of locks for the Rideau Canal has impeded movements of painted turtles and thus reduced gene flow between populations. Specifically, we predict that: (i) the number of locks will be a better predictor of genetic structure than other landscape features (i.e., historical features of the landscape prior to canal construction and geographic distance) and (ii) populations isolated by many locks will be more genetically distant than populations separated by few or no locks. Our study provides a rare example of how a canal system can influence the landscape genetics of a long-lived species.

The Rideau Canal is a slackwater canal located in southeastern Ontario, Canada, that connects the Ottawa River to Lake Ontario. This 202-km continuous waterway is a network of rivers, lakes, and excavated channels that were connected through the construction of 23 lockstations (45 locks), many of which were built with water-control dams (Legget 1986). Construction started in 1826 and the canal officially opened in 1832. The main purpose of this canal was to provide a supply route for military activities to protect Canadian British colonies against the United States (Tulloch 1981). Since its construction, however, the Rideau Canal has been mainly used for economic and recreational activities (Parks Canada 2006). Given that the Rideau Canal is the oldest continuously operated canal in North America and that the effect of its construction on landscape connectivity remains completely unexplored, it is a valuable study system to evaluate the long-term effects of such constructions on genetic composition of turtle populations.

Materials and methods

Study species

The painted turtle is a long-lived generalist species (i.e., generation time ~ 30–45 years; COSEWIC 2018) widely distributed across North America that lives in various aquatic habitats (e.g., swamps, marshes, rivers and lakes; Ernst and Lovich 2009). Painted turtles can disperse overland between wetlands and colonize new and artificial wetlands (Bowne 2008; Dupuis-Desormeaux et al. 2018). Even if painted turtles are considered stable over their range by the IUCN (Van Dijk 2011), populations in southeastern Ontario are considered of Special Concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2018).

Study system and sample collection

We sampled painted turtles at 22 sites distributed approximately every 10 km along the entire Rideau Canal in Ontario, Canada (Fig. 1a; Table 1). There was wide variation in the number of locks between pairs of sites (range = 0–33, mean = 11.3, SE = 0.5). We sampled in suitable habitats for painted turtles characterized by shallow water, weak current, abundant aquatic vegetation, and presence of structures for basking (e.g., rocks and stumps).

We captured painted turtles with fyke nets between May and August in 2018–2020. We deployed fyke nets for one week at each site and checked them every 24 h. We sampled some sites for more than one year to evaluate their temporal genetic stability (10/22 sites; Table 1; see Genetic diversity and differentiation section). Each painted turtle we captured was marked with the North American code (Nagle et al. 2017) to ensure unique sampling. We took blood samples from the coccygeal, jugular, or supracarapacial vein using a U-100 insulin syringe with 28G × 12.7 mm microfine needle (BD Medical). We dried the blood samples on a qualitative P8 grade filter paper (Thermo Fisher Scientific) prior to DNA extraction.

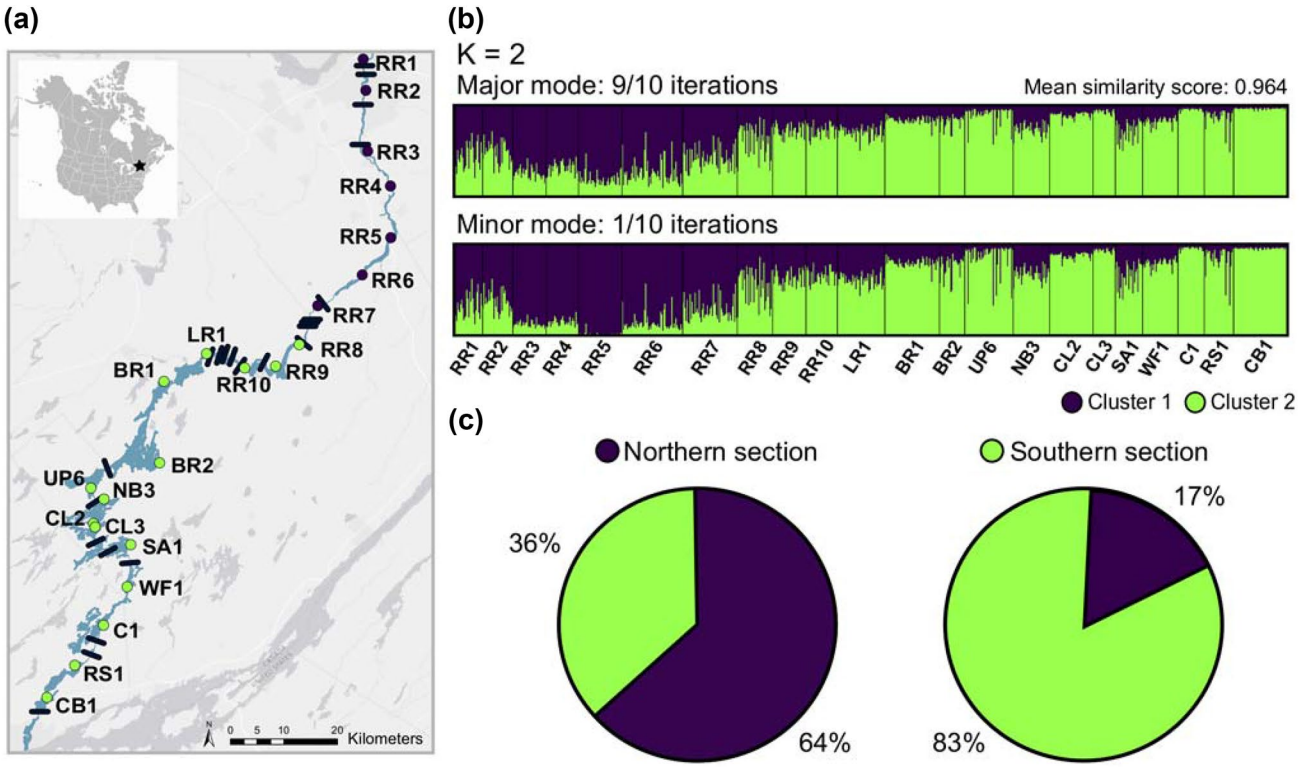

Fig. 1 **a** Map of the Rideau Canal, Ontario, Canada and the 22 sites (dots) sampled from 2018 to 2020: Dark dots (purple) represent the sampling sites of the northern cluster, light dots (green) represent the sampling sites of the southern cluster. Solid bars (dark blue) along the waterway indicate the location of the lockstations. The map was built using ArcGIS® software by Esri (www.esri.com). **b** Major and minor modes obtained from STRUCTURE analyses using K = 2 with the LOCPRIOR function for 822 painted turtles (*Chrysemys picta*) sampled throughout the canal. Vertical lines show the proportional membership for individuals to each cluster. Figures were generated with CLUMPAK (Kopelman et al. 2015). **c** Percentage of membership to each cluster identified by STRUCTURE for each section of the canal, based on cluster assignment of individuals

Table 1 Population genetic statistics for each of the 22 sampling sites (Site) of the Rideau Canal, Canada: Number of painted turtles sampled (N), the number of private alleles (P_A), observed heterozygosity (H_O), expected heterozygosity (H_E), allelic richness (A_R) and inbreeding coefficient (F_{IS}) averaged over all loci with 95% confidence intervals [95% CI]

Site	Sampling year	N	P_A	A_R [95% CI]	H_O	H_E	F_{IS} [95% CI]
RR1[a]	2019 and 2020	27	2	8.15 [7.23–8.92]	0.721	0.737	0.026 [−0.071 to 0.089]
RR2	2020	29	2	8.89 [8.00–9.69]	0.759	0.745	0.002 [−0.076 to 0.045]
RR3[a]	2019 and 2020	33	1	9.20 [8.23–10.08]	0.774	0.752	−0.044 [−0.106 to −0.014]
RR4	2019	32	2	8.86 [8.00–9.62]	0.765	0.736	−0.042 [−0.108 to −0.008]
RR5	2019	43	0	9.19 [8.39–9.92]	0.719	0.74	0.017 [−0.040 to 0.051]
RR6[a]	2019 and 2020	60	2	9.56 [8.69–10.39]	0.765	0.75	−0.029 [−0.068 to −0.006]
RR7[a]	2019 and 2020	54	1	9.45 [8.54–10.31]	0.719	0.739	0.014 [−0.031 to 0.041]
Northern cluster	–	278	12	16.65 [16.15–17.08]	0.745	0.756	0.008 [−0.012 to 0.024]
RR8	2019	35	0	9.43 [8.46–10.31]	0.731	0.757	0.029 [−0.043 to 0.074]
RR9[a]	2019 and 2020	34	0	8.87 [7.92–9.69]	0.726	0.745	0.03 [−0.034 to 0.063]
RR10	2019	31	0	8.86 [7.92–9.69]	0.759	0.744	−0.028 [−0.094 to 0.004]
LR1	2019	47	2	9.48 [8.54–10.31]	0.738	0.733	−0.019 [−0.066 to 0.008]
BR1[a]	2019 and 2020	54	1	9.18 [8.23–10.00]	0.712	0.727	0.024 [−0.029 to 0.059]
BR2	2020	25	0	8.79 [7.85–9.62]	0.711	0.739	0.077 [−0.045 to 0.126]
UP6	2018 and 2019	48	1	9.57 [8.54–10.46]	0.759	0.761	−0.009 [−0.061 to 0.023]
NB3	2018	36	4	9.35 [8.31–10.31]	0.718	0.744	0.029 [−0.043 to 0.076]
CL2	2018 and 2020	43	3	9.65 [8.69–10.54]	0.744	0.745	−0.014 [−0.071 to 0.022]
CL3	2018	22	3	8.22 [7.31–9.00]	0.721	0.728	0.006 [−0.085 to 0.046]
SA1	2018 and 2019	27	1	9.15 [8.15–10.08]	0.707	0.713	0.000 [−0.062 to 0.029]
WF1[a]	2019 and 2020	35	3	8.70 [7.69–9.62]	0.716	0.721	0.006 [−0.058 to 0.043]
C1[a]	2019 and 2020	26	2	8.98 [8.08–9.77]	0.751	0.759	0.008 [−0.071 to 0.048]
RS1[a]	2019 and 2020	29	2	8.88 [8.00–9.69]	0.772	0.741	−0.058 [−0.121 to −0.025]
CB1[a]	2019 and 2020	52	4	9.44 [8.54–10.23]	0.75	0.752	−0.003 [−0.056 to 0.029]
Southern cluster	–	544	50	18.37 [17.37–19.00]	0.735	0.755	0.021 [0.007–0.033]
Overall	2018 to 2020	822	1.63 ± 0.27	9.08 ± 0.09	0.738 ± 0.005	0.741 ± 0.003	0.018 [0.009–0.027]

Mean value ± SE for overall P_A, A_R, H_O, H_E

[a]Sites used for the hierarchical AMOVA

DNA extraction and amplification

We extracted DNA from a ≥ 7-mm punch of a blood-soaked filter paper with an overnight proteinase K digestion followed by a salt extraction (Aljanabi and Martinez 1997). We assessed extraction quality by running extraction products on 1% agarose gels (stained with ethidium bromide) and revealing the band under UV light. We then normalized each DNA sample to a final concentration of 10 ng/µl prior to PCR amplification. We genotyped all DNA samples using 15 microsatellite loci previously described and tested on painted turtles (Online Resource 1—Table S1). We optimized PCR conditions by adapting original PCR protocols and by examining amplified products on 2% agarose gels (Online Resource 1—Table S2 and S3). All forward primers were labelled with a fluorescent dye (Online Resource 1—Table S1). We ran amplified products using three multiplexes with an AB3500 Genetic Analyzer and using GeneScan™ 600 LIZ as a size standard (Applied Biosystems). We scored all alleles twice manually with GeneMapper™ v.6 (Applied Biosystems). We assessed genotyping error rate for each locus from repeated genotyping of 38 painted turtles (= 4.6% of samples) that were sampled more than once between years.

Genetic diversity and differentiation

We obtained population genetic statistics using different packages in R 3.6.2 (R Core Team 2019). We tested for deviations from Hardy–Weinberg equilibrium (HWE) for each locus, and for each combination of sampling sites and locus, using Chi-squared tests (Chisq) and exact tests based on 10,000 Monte Carlo (MC) permutations with the R package pegas (Paradis 2010). We estimated the proportion of loci that deviated from HWE for each sampling site and estimated the proportion of sites out of HWE for each locus using a false discovery rate correction based on the significant deviations previously tested [based on MC and Chisq

tests with and without Bonferroni correction; Benjamini and Yekutieli (2001)]. We tested all loci for linkage disequilibrium with the R package poppr (Kamvar et al. 2014). We also assessed the frequency of null alleles for each locus with the R package PopGenReport (Adamack and Gruber 2014). We excluded loci with (i) a high frequency of null alleles (i.e., > 10%), (ii) a significant deviation from HWE across several sampling sites, or (iii) a high genotyping error rate (i.e., > 10%) from further analyses (see Sample collection and genotyping in Results section).

From the dataset with the remaining loci, we estimated observed and expected heterozygosity (H_O and H_E), allelic richness (A_R) and inbreeding coefficients (F_{IS}) for each locus and sampling site using the R package diveRsity (Keenan et al. 2013). We then determined the number of private alleles (P_A) for each sampling site with the PopGenReport package (Adamack and Gruber 2014). We also used the diveRsity package to calculate the global value of F_{IS}, F_{ST}, and G_{ST} and the pairwise F_{ST} (Weir and Cockerham 1984) and G_{ST} (Hedrick 2005) values between each pair of sites. We estimated confidence intervals at 95% (95% CI) for A_R, F_{IS}, F_{ST}, and G_{ST} with a bias-corrected bootstrapping method implemented in the DiveRsity package (10,000 bootstraps). We also estimated H_O, H_E, A_R, F_{IS}, and F_{ST} values by grouping data according to the genetic cluster identified in the clustering analysis (see Genetic clustering analysis sections). We calculated the probability of identity (PI) and PI among siblings (PISibs) with the Excel extension of GenAlEx (Peakall and Smouse 2012) to assess if our genetic parameters were biased by the sampling of close relatives. We performed a hierarchical AMOVA on data from 10 sites sampled in 2019 and 2020 (Table 1) by grouping sampling sites per year of sampling to determine the temporal stability of genetic diversity with the ade4 package (10,000 permutations; Thioulouse et al. 2018). We estimated the presence, degree, and direction of asymmetric gene flow among sampling sites using the relative migration network method developed by Sundqvist et al. (2016) and implemented in the diveRsity package. We calculated estimates of significant relative migration rates with G_{ST} and Nm [i.e., effective number of migrants based on a statistic that incorporates information from Nei's G_{ST} and Jost's D; Alcala et al. (2014)] based on a bootstrap method with 50,000 replications.

Genetic clustering analysis

We used STRUCTURE v 2.3.4 (Pritchard et al. 2000) to estimate the most likely number of genetic clusters (K) in the system. We performed 10 runs for each number of potential clusters (K = 1–22) by assuming an admixture model with correlated allele frequencies (length of burn-in period set to 100,000 repetitions; number of MCMC replicates: 250,000; sufficient for each run to converge). We evaluated the most likely number of clusters with two metrics: the mean log likelihood probability (LnP(K)) of the data for each K (Pritchard et al. 2000) and the delta K based on the rate of change in probability between successive K values (Evanno et al. 2005). We used CLUMPAK to compile and combine all runs for each K and to visualize the likelihood values (Kopelman et al. 2015). We then performed 10 additional runs with the selected most likely number of K applying the LOCPRIOR function that uses sampling sites as additional information in the analysis. The LOCPRIOR parameter provides a more definite distinction between clusters previously determined by the unsupervised model when population structure is relatively weak (Porras-Hurtado et al. 2013).

Landscape genetic analyses

We used maximum likelihood population effect models (MLPE models; Van Strien et al. 2012) with the R lme4 package (Bates et al. 2015) to assess how genetic differentiation was influenced by landscape features that could act as barriers to turtle movement. This approach allows to consider the non-independence of pairwise data by using a residual covariance structure with a mixed modelling approach (Clarke et al. 2002). We used pairwise F_{ST} measures previously calculated between each pair of sampling sites as the genetic distance matrix and different types of landscape features as pairwise predictor matrices. We considered four landscape features that were calculated between each pair of sampling sites: (i) the shortest aquatic distance (in meters) based on the geographic location of each sampling site, to represent a scenario of isolation by distance, (ii) the number of locks, (iii) the number of human-made constructions (e.g., mill dams) between 1783 (i.e., beginning of European settlement in the Rideau Canal region) and 1826 (i.e., beginning of Rideau Canal construction), and (iv) the sum of permeability values of historical features [i.e., waterfalls, rapids, and land barriers based on the work of Watson (2006)] previously located on the current path of the canal before any human-made alterations were made (Fig. 2a; Online Resource 2). We included the number of human-made constructions between 1783 and 1826, and historical features to disentangle the genetic effects of the canal construction from those of the original (prior to canal construction) landscape features. We also included the identity of sampling site pairs, used to calculate the genetic distance and landscape features matrices, in all models as a random effect.

We generated different combinations of permeability values for each historical feature (see Online Resource 2) given that each feature may not have the same permeability to turtle movement. We compared the influence of each combination of permeability values on the genetic distance

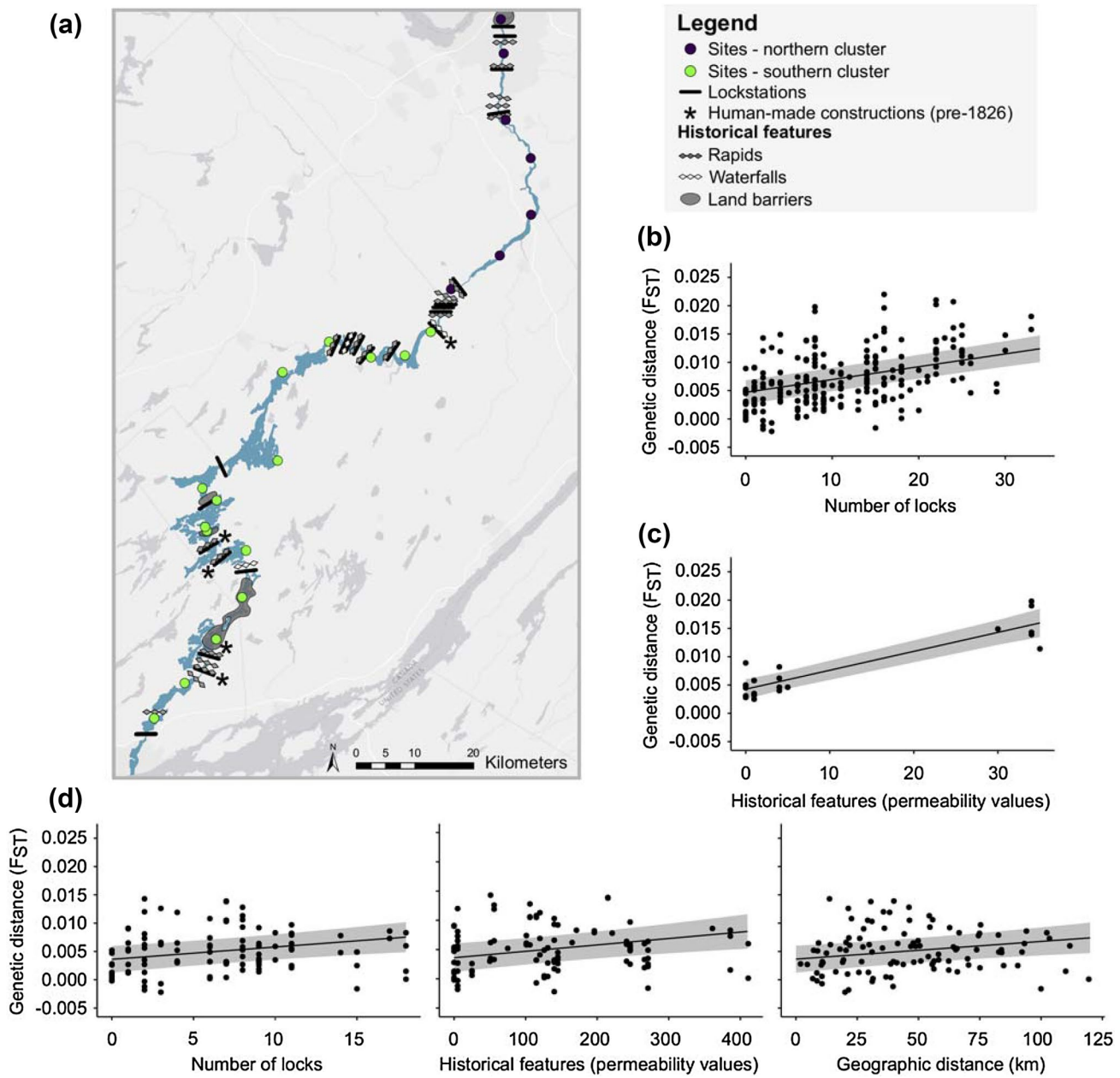

Fig. 2 **a** Map of the Rideau Canal, Ontario, Canada with the locations of lockstations (solid black bars), human-made constructions prior to Rideau Canal construction (e.g., mill dams; asterisks), and historical features (rapids: gray diamond bars; waterfalls: white diamond bars; land barriers: gray zones) in relation to sampling sites (dots). Dark dots (purple) represent the sampling sites from the northern cluster, light dots (green) represent the sampling sites from the southern cluster. **b**–**d** Relationship between the genetic distance (pairwise F_{ST}) and landscape features in painted turtles (*Chrysemys picta*) from the best selected MLPE (maximum likelihood population effect) models of each section of the Rideau Canal: **b** entire system, **c** northern cluster, **d** southern cluster. Black dots are observed genetic distances. Grey areas represent the 95% confidence intervals of predictions (black line)

matrix by generating a set of univariate MLPE models. We used information criteria metrics (i.e., AICc) to identify the best model from the set of candidate models. We retained the combination of permeability values with the lowest AICc, among the set of candidate models, for further analyses (Online Resource 2).

We fitted MLPE models for the different combinations of landscape features and the model with the lowest AICc (and $\Delta AICc < 2$) was considered as the model that best fitted the genetic structure observed in the system (Online Resource 1—Table S4). Given that most landscape variables were highly correlated with each other ($r > 0.8$), we could not use

them in the same model (Online Resource 1—Table S5). Following the results from STRUCTURE, we performed MLPE analyses separately for each genetic cluster identified to assess the role of landscape features on the genetic structure within each cluster (see Genetic clustering analysis in Results section). We verified model assumptions for each candidate model and calculated confidence intervals (95%) of estimates from the best models. R codes used for MLPE models were adapted from codes available in R LandGen-Course package (Wagner 2020). We obtained model predictions and built figures with the R ggeffects (Wickham 2016) and ggplot2 packages (Lüdecke 2018). We only present results from F_{ST} pairwise values given that analyses conducted with pairwise G_{ST} values and with Slatkin's linearized F_{ST} ($F_{ST}/[1-F_{ST}]$) gave similar results (Online Resource 1—Table S6).

Results

Sample collection and genotyping

We collected blood samples from 822 painted turtles across the Rideau Canal (mean = 37 individuals/site, SE = 2.3; Fig. 1a; Table 1). We excluded two microsatellites from the analyses (Online Resource 1—Table S1): GmuD87 had a high genotyping error rate (14%; Online Resource 1—Table S7), a high frequency of null alleles (11%; Online Resource 1—Table S8), and deviated from HWE in 14% of the sampling sites (Online Resource 1—Table S9); CpGT124 had a high genotyping error rate (18%; Online Resource 1—Table S7). We found no evidence of linkage disequilibrium among the 13 retained loci. PI and PIsibs were under 0.01 when a minimum of 2 loci and of 6 loci, respectively, were combined (Online Resource 1—Table S10). For the 13 retained loci, the missing data were 0.21% and the mean genotyping error rate was 2.3%.

Genetic diversity and differentiation

Population genetic statistics indicated a relatively high variability for the 13 retained loci (e.g., H_O ranging from 0.28 to 0.93; see Online Resource 1—Table S1) and a homogenous genetic diversity between sampling sites with A_R values ranging from 8.2 to 9.7 with overlapping 95% confidence intervals (Table 1). Overall, we found a low, but significant, genetic differentiation throughout the canal ($F_{ST} = 0.007$, 95% CI 0.005–0.009) and a relatively low inbreeding level ($F_{IS} = 0.018$, 95% CI 0.009–0.027) (Table 1, Online Resource 1—Table S11).

The hierarchical AMOVA revealed that the majority of genetic variance occurred within samples (97.6%; $p < 0.001$) with the remaining variance partitioned between samples within sampling sites (1.6%; $p = 0.01$) and between sampling sites (0.8%; $p < 0.001$). We detected no significant genetic variance between years within sampling sites ($p = 0.31$) and no significant asymmetric migration rates between sampling sites.

Genetic clustering analysis

STRUCTURE analyses identified two genetic clusters throughout the Rideau Canal (Fig. 1b, Online Resource 1—Table S12). The probability of having a single genetic cluster (K = 1), however, is close second [mean LnP(k) for K = 1: − 43,164.93; K = 2: − 43,159.16; Online Resource 1—Table S12). The split between the two clusters occurred between sites RR7 and RR8 (Fig. 1a). We estimated that individuals from sites in the northern section of the canal (i.e., sites RR1, RR2, RR3, RR4, RR5, RR6 and RR7) had a likelihood of membership of 64% to cluster 1, while individuals from sites in the southern section (i.e., sites RR8, RR9, RR10, LR1, BR1, BR2, UP6, NB3, CL2, CL3, SA1, WF1, C1, RS1, CB1) had a likelihood of membership of 83% to cluster 2 (Fig. 1b, c). Probability of assignment was stronger in the southern section of the canal where individuals from five sampling sites had a likelihood of membership over 90% to cluster 2 (i.e., UP6, CL2, CL3, C1, CB1; Fig. 1b). A few sites were characterized by a lack of definitive assignment to a specific cluster (mean membership to each cluster ~ 50%, e.g., sites RR1, RR2, RR7, RR8, Fig. 1b).

Comparison between genetic clusters

We found a pairwise F_{ST} of 0.004 (95% CI 0.002–0.005) between the two identified genetic clusters. We observed a lower A_R in the northern cluster ($A_R = 16.7$, 95% CI 16.2–17.0; N = 278) than in the southern cluster ($A_R = 18.4$, 95% CI 17.4–19.0; N = 544; Table 1). The mean number of P_A per site was also lower in the northern cluster (mean = 1.43, SE = 0.30) than in the southern cluster (mean = 1.73, SE = 0.37; Table 1). On the other hand, F_{IS} was 0.021 (95% CI 0.007–0.033) in the southern cluster, while in the northern cluster the 95% CI overlapped with zero ($F_{IS} = 0.008$, 95% CI − 0.012 to 0.024; Table 1). Finally, while we detected high relative migration rate among the two clusters (G_{ST} and Nm: North to South = 0.84; South to North = 1.00), we found significant asymmetric gene flow only from the southern cluster to the northern cluster based on a bootstrap method with 50,000 replications.

Landscape genetic analyses

Across the Rideau Canal, the number of locks between pairs of sampling sites was the best predictor of the observed genetic structure (Table 2, Online Resource 1—Table S4).

Table 2 Summary statistics for the best MLPE (maximum likelihood population effect) models selected for each section of the Rideau Canal: the entire system, northern cluster (RR1, RR2, RR3, RR4, RR5, RR6, RR7), southern cluster (RR8, RR9, RR10, LR1, BR1, BR2, UP6, NB3, CL2, CL3, SA1, WF1, C1, RS1, CB1)

Variables	Estimate	SE	t value	95% CI
Entire system				
Number of locks	0.000226	0.000026	8.85	[0.000176–0.000277]
Northern cluster				
Historical features	0.000334	0.000042	7.97	[0.000252–0.000427]
Southern cluster				
Number of locks	0.000218	0.000067	3.23	[0.000086–0.000350]
Historical features	0.000011	0.000004	3.13	[0.000004–0.000018]
Geographic distance	0.000884	0.000306	2.89	[0.000284–0.001483]

In these models, pairwise F_{ST} values were used as a response matrix and different landscape features as predictor matrices. For each model, we provide the estimates, the standard error (SE), t value, and 95% confidence intervals [95% CI]

The genetic distance between pairs of sampling sites increased with the number of locks that separated them (Fig. 2b; Table 2). In the northern cluster, historical features were the best predictors of the genetic differentiation observed; the presence of historical features increased the genetic distance between sites (Fig. 2c; Table 2). In the southern cluster, models with historical features, number of locks, and geographic distance were the best models (Online Resource 1—Table S4); the genetic distance increased with the presence of historical features, the number of locks, and the geographic distance between sites (Fig. 2d; Table 2).

Discussion

Understanding how lasting human infrastructure can affect the genetic structure of aquatic wildlife is crucial to develop effective management plans for vulnerable species. This understanding is necessary to reconcile the heritage and economic value of infrastructure, such as the Rideau Canal, and the maintenance of gene flow between freshwater species populations. The main goal of this study was to assess the impact of landscape features, especially the presence of locks, on the genetic structure of painted turtle populations. We found that, while locks seem partly permeable to turtle gene flow, the number of locks was still the best predictor of the genetic differentiation between sites in the Rideau Canal. To our knowledge, this is the first documentation that locks can potentially modulate gene flow in a long-lived species.

Canal construction did not stop gene flow in the aquatic landscape

We found weak genetic structure and homogeneous genetic diversity throughout the canal suggesting that locks are at least partly permeable to turtle gene flow and, thus, rendering this system much closer to panmixia than to complete isolation. Partial permeability to gene flow was also supported by the lack of definitive assignment to a specific cluster in clustering analysis for 50% of individuals (i.e., cluster assignment below 0.8) (Porras-Hurtado et al. 2013). A previous study by Reid et al. (2008) also detected weak genetic differentiation and low level of assignment of individuals to potential source populations for freshwater fishes in a canal system, suggesting that locks facilitated species movement. The use of a slackwater system (i.e., use of dams to flood the rapids rather than canal cuts to bypass them) in the Rideau Canal may have avoided major alterations to aquatic connectivity (Watson 2006). By building locks "in the dry" (i.e., above pre-canal water level), only 10% of the length of the canal required alterations, such as excavated channels and locks, while the rest of the canal followed existing waterways or flooded lakes, which may have maintained gene flow in the system. It is also possible that the large population size (over 10,000 individuals in the Rideau Canal based on the Lincoln-Petersen index; see Online Resource 1—Table S13, S14) and the limited time scale (in terms of number of painted turtle generations) since canal construction have limited our ability to detect a decline of genetic diversity and/or an increase in genetic structure (Frankham 1996; Kuo and Janzen 2004). As it was observed in previous studies, the detection of genetic isolation caused by recent anthropogenic changes can be hampered by long generation times (Hailer et al. 2006; Lippé et al. 2006; Su et al. 2018).

Terrestrial movements and dispersal at any life stage, such as female exploration to find nesting sites and post-hatching dispersal of juveniles, could also have contributed to the maintenance of gene flow throughout the system. Terrestrial movements of painted turtles are, however, typically short: female movements and nest sites further than 1 km from aquatic habitats are rare (Semlitsch and Bodie 2003; Steen et al. 2012). Terrestrial dispersal (e.g., movements between ponds/wetlands) is usually shorter than 3 km (Bowne 2002, 2008; Bowne and White 2004), but longer movements are possible over longer time

periods (e.g., 11.5 km straight-line distance over approximately 10 years; COSEWIC 2018).

A series of six locks may cause population isolation

The clustering analysis revealed two genetic clusters within the canal. The boundary between genetic clusters occurred in the canal section with the highest number of locks per kilometer (i.e., 6 locks over 9.2 km; Online Resource 1—Table S15), suggesting that numerous locks in proximity can impede turtle gene flow. Previous studies in aquatic species have linked the presence of genetic clusters to permanent artificial barriers, such as dams (Roberts et al. 2013; Liu et al. 2020; Fraik et al. 2021). To our knowledge, however, our study is the first to suggest that several consecutive locks in proximity can have a similar impact on the genetic structure of an aquatic species.

We detected that gene flow between the two clusters was stronger from south to north, indicating a possible source-sink dynamic in which the southern cluster may act as a source (Sundqvist et al. 2016). The possibility of source-sink dynamic is also supported by the lower genetic diversity (i.e., lower A_R and P_A) in the northern cluster (Sundqvist et al. 2016; Gustafson et al. 2019). The direction of water flow is from south to north where the split between genetic clusters occurs and flow may have facilitated migration in this direction, as observed in other aquatic species (see Jonsson 1991; Alp et al. 2012; Junker et al. 2012). To our knowledge, however, there are no studies indicating a role for the direction of water flow in driving gene flow in freshwater turtles.

Locks may act as barriers to movement

We found that genetic differentiation of painted turtles in the canal increased with the number of locks and that the number of locks was a better predictor of genetic structure than other landscape features. Although recent anthropogenic changes do not always cause genetic isolation (Bennett et al. 2010; Su et al. 2018), our results suggest a relatively rapid effect of locks on genetic differentiation. Despite genetic differentiation possibly being underestimated because of the large population size, long generation time, and slow mutation rate of turtles (Avise et al. 1992; Shaffer et al. 2013), it was not sufficient to limit our detection capacity.

It is important to acknowledge the difficulty in disentangling the effects of individual landscape features given their interconnectedness. Locks were built to overcome navigational barriers. Thus, locks were usually built where waterfalls and rapids were located prior to canal construction (Watson 2006; see Fig. 2a). Therefore, we cannot exclude the possibility that the genetic structuring we observed along the canal may represent an effect of historical barriers that was exacerbated by the construction of locks.

Previous studies in migrating diadromous fishes showed that locks can impede their dispersal across waterways by reducing the number of passages (Verhelst et al. 2018; Vergeynst et al. 2019). In the Rideau Canal, lock activity varies both temporally (May to October, mean = 8.6 lockages/day, May = 2.7, June = 6.4, July = 15.4, August = 15.6, September = 7.3, October = 3.3; Online Resource 1—Table S16) and spatially (min = 2.7 for Ottawa locks, max = 19.3 for Newboro lock; Online Resource 1—Table S16). Thus, lock passage can be particularly difficult at certain times of the turtle active season and at certain locations. Even if turtles are able to enter locks [see Bennett et al. (2010)], the time window to pass through the locks can be short. Also, even if the timing is right, the use of locks could lead to disorientation, physical stress, injuries [e.g., impact by boat propellers; (Bulté et al. 2010)], and even mortality, as it was observed in migrating diadromous fishes (Verhelst et al. 2018; Vergeynst et al. 2019). Finally, if lock water filling is in the opposite direction to turtle movement, it can reduce the ability of turtles to disperse in the desired direction. Therefore, considering all these factors together, the probability to disperse through locks in the aquatic landscape can be low, especially where there is a close succession of locks.

The weaker effect of locks when we analysed the northern and southern clusters separately suggests an important role for the series of six locks on the overall genetic effect of locks we observed in the system. In the southern cluster, we were unable to distinguish the effect of locks from that of other landscape features, while in the northern cluster the best predictor of genetic structure was the historical features. In addition, the northern section of the canal contains the longest continuous section without locks (41 km without locks in the 69 km of the northern section; mean across the canal = 8 km), suggesting again that numerous locks in proximity impede gene flow more compared to distanced locks.

What can be done to maintain gene flow in the system?

Given the low genetic differentiation and the homogenous genetic diversity observed in painted turtles in the Rideau Canal, turtles in this system appear resilient to the effects of locks. The detection of two genetic clusters within the canal, however, calls to consider conservation and management actions to maintain aquatic connectivity between the southern and the northern clusters and to ensure the maintenance of gene flow. Increased connectivity could be achieved by modifying the structure or operation of the locks between sites RR7 and RR8. For example, building wildlife passages adapted for turtles (e.g., curved concrete ramp with a variety of textures: logs, rocks, and heterogeneous aquatic

vegetation, resting pools along the ramp with refuges, low water flow and limited vertical drop, as it was designed for the Gympie weir biopassage, Australia; see Sutherland 2017) on the lands around the locks and changing the timing of lock operations (e.g., leave lock valves or doors opened as often as possible) could facilitate turtle dispersal and, thus, increase gene flow. The positive impact of any potential change to canal management on turtle movement, however, needs to be carefully weighed against the increased risk of invasion by non-native species (Lin et al. 2020), such as the red-eared slider (*Trachemys scripta*) already detected in southeastern Ontario (Seburn 2015; Spear et al. 2018).

Conclusions

Overall, our study showed that locks are partly permeable to gene flow between painted turtle populations and can lead to genetic discontinuities where they are numerous and in proximity. Therefore, the effect of locks on the genetic integrity of aquatic species should be considered in conservation management plans. To our knowledge, our study is the first in the Rideau Canal, or any other similar canal system, to show how the construction of a canal can influence the genetic structure of a freshwater turtle. There is a need for additional studies on other aquatic species in the Rideau Canal and comparable canal systems, such as the Trent-Severn Waterway (386 km; connecting Lake Ontario to Georgian Bay in Canada; see Bennett et al. (2010) for a partial study) and the Erie Canal (843 km; connecting Lake Erie to the Hudson River in the USA) for a more comprehensive understanding of the long-term effects of artificial waterways on the connectivity between populations of long-lived freshwater species.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection, and data analysis were performed by AT. The first draft of the manuscript was written by AT and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

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Data availability All data collected and analysed during this study are available in the Zenodo Digital Repository: https://doi.org/10.5281/zenodo.5826150

Code availability All R codes used for this study are available in the Zenodo Digital Repository: https://doi.org/10.5281/zenodo.5826150

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval All protocols were approved by animal care committees at the University of Ottawa (protocol BL-3008) and Queen's University (protocol 2018-1836). All fieldwork was carried out under a Parks Canada Agency research and collection permit (number RIC-2018-29178) and Wildlife Scientific Collector's Authorizations from the Ontario Ministry of Natural Resources (numbers 1089358, 1092637 and 1095459).

Consent to participate and publish All authors agree to participate and to publish the produced data of this research.

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