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RESEARCH ARTICLE

Limited genetic structure in a wood frog (*Lithobates sylvaticus*) population in an urban landscape inhabiting natural and constructed wetlands

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Abstract Urbanization has been linked to amphibian population declines globally. Habitat fragmentation can negatively impact gene flow among populations but what role artificially constructed wetlands have in maintaining gene flow in urban amphibian populations remains uncertain. We assessed gene flow in a population of wood frogs (*Lithobates sylvaticus*) inhabiting both constructed and natural wetlands located in Edmonton, Alberta, Canada. We genotyped 10 microsatellite loci in 182 frogs from eight wetlands and tested for genetic differentiation between wetlands. We tested if bottlenecks had occurred at constructed wetlands and if there were differences in allelic richness between natural wetlands and constructed wetlands. We found no evidence of bottlenecks, no differences in allelic richness among subpopulations, and no genetic structure within the population. Although significant differences in pair-wise comparisons of allele frequency distributions between wetlands were detected, all F_{ST} values were low (0.003–0.053) and Bayesian clustering indicated the presence of a single genetic cluster. Despite extensive urbanization within our study area, genetic homogeneity has been preserved indicating that it may be possible to use constructed wetlands to maintain amphibian populations and preserve gene flow among subpopulations living within urbanized landscapes.

Keywords Wood frog · Microsatellite · Genetic structure · Gene flow · Urbanization · Habitat fragmentation · *Lithobates*

Introduction

Urban development can rapidly divide animal populations. Barriers, such as roads and buildings that prevent dispersal and/or movement between breeding aggregations (demes), decrease gene flow and cause genetic isolation of subpopulations (Munshi-South and Kharchenko 2010). The strength of genetic drift increases in small isolated subpopulations leading to genetic differentiation among them. Isolation can lead to increased levels of inbreeding, and increased occurrence of homozygous genotypes and detrimental recessive alleles. Expression of detrimental alleles ('inbreeding depression') can result in reduced fitness of individuals and extinction of local subpopulations (Mech and Hallett 2001). However, subpopulations that remain large following fragmentation may have sufficient diversity to counter genetic differentiation (Newman and Squire 2001) even with low levels of migration. Thus, management and conservation strategies that aim to maintain large subpopulations may temporarily preserve genetic diversity, even in highly fragmented landscapes (Segelbacher et al. 2010).

Amphibian populations are known to be extremely sensitive to habitat fragmentation (Hels and Buchwald 2001; Gibbs and Shrivers 2005; Noel et al. 2007;

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Eigenbrod et al. 2008) and urbanization is particularly problematic because it represents a permanent loss of habitat. Many amphibian species in North America and Europe are negatively affected by urbanization (Elzanowski et al. 2008; Hammer et al. 2008; Scheffers and Paszkowski 2012, and references within). For example, urban road networks increased the probability of mortality in two ranid species (*Rana temporaria*, *R. arvalis*) and limited dispersal (a key determinant of genetic differentiation; Hels and Buchwald 2001). Loss of natural vegetation and the creation of habitat edges by urban development also prevent dispersal, which can isolate subpopulations (Rothermel and Semlitsch 2002; Gagné and Fahrig 2007; Lehtinen et al. 1999). Amphibian populations inhabiting wetlands in urban areas can have lower levels of gene flow and genetic diversity compared to populations located within natural areas with higher connectivity between wetlands (Goldberg and Waits 2010; Hitchings and Beebee 1997). However, urban amphibian populations do not always exhibit genetic structure. Several species of pond-breeding amphibian have been documented to maintain overall genetic homogeneity among subpopulations following exposure to various degrees of disturbance caused by urban and agricultural development (*Lithobaetes sylvaticus*: Crosby et al. 2008; Newman and Squire 2001; *Ambystoma maculatum*: Purrenhage et al. 2009; *Bufo calamita*: Rowe et al. 2000; *Rana temporaria*: Safner et al. 2011), suggesting that the broad effects of urbanization may not be reflected in the genetics of all amphibian species at the subpopulation/population level.

In this study, we present a genetic analysis on a wood frog (*Lithobates sylvaticus*) population, breeding in both natural and artificially constructed wetlands in the city of Edmonton, Alberta, Canada. The objective of this study was to assess if constructed wetlands are adequately contributing to overall gene flow and genetic diversity of this population. Wood frogs are aggregate breeders (Howard and Kluge 1985) and adults show a high degree of philopatry to breeding ponds (Berven and Grudzien 1990; Green and Bailey 2015). Due to this philopatric behaviour, wood frogs are expected to show genetic structure among subpopulations (Crosby et al. 2008). Juvenile wood frogs are the dispersing life stage (Semlitsch 2008), with 20 % of individuals not returning to natal ponds and moving to new locations up to 1.5 km away in natural ecosystems (Berven and Grudzien 1990), although most return to natal ponds (Green and Bailey 2015). Wood frogs also have had a rapid post-glacial range expansion, contributing to an overall lack of genetic divergence across the species range (Lee-Yaw et al. 2008). The presence of wood frog populations is positively correlated with the amount of forest cover within urban ecosystems (Rubbo and Kiesecker 2005; Scheffers and Paszkowski 2013). Although this species is sensitive to urban development (Rubbo and Kiesecker 2005; Scheffers and Paszkowski 2013), maintaining natural terrestrial habitat and creation of breeding habitat within urban landscapes may help maintain populations. Recent studies suggest that adequate gene flow can be maintained among subpopulations in urban areas. For example, a study investigating genetic differentiation in an urban population of wood frog in Ontario, Canada found little genetic structure among subpopulations, possibly resulting from high rates of juvenile dispersal (Crosby et al. 2008). Minimal genetic differentiation has also been detected in populations occurring in prairie and woodland habitats in North Dakota and Minnesota, USA (Newman and Squire 2001; Squire and Newman 2002, respectively).

Artificial wetlands in Edmonton were constructed mid 1990s within housing developments to retain storm water runoff. Residual biological benefits from wetland construction also include the maintenance of urban amphibian populations (Scheffers and Paszkowski 2013), yet nothing is known about the genetic structure and the long-term viability of subpopulations. Such an analysis can offer additional insight into the long-term prospects of conserving urban amphibian populations. Using a well-studied urban amphibian population, we sampled individuals from a subset of the ponds surveyed by Scheffers and Paszkowski (2013), to explore if fine-scale genetic structure exists among wood frog subpopulations within the city of Edmonton. We test if individual constructed wetlands harbour subsets of the total genetic diversity found among Edmonton subpopulations of wood frogs, indicating isolation, or if individuals are genetically homogenous among wetlands, possibly indicating a high level of gene flow between ponds. To achieve this goal, we compare the levels of total genetic diversity of individuals living in constructed versus natural wetlands (located in Edmonton's extensive North Saskatchewan River valley system). In addition, we calculate the degree of genetic differentiation among wetlands, conduct Bayesian genetic clustering analysis to assess gene flow between ponds and determine if genetic differentiation is related to geographic distance between wetlands.

Methods

Study sites

We selected eight ponds containing wood frogs within the city limits of Edmonton, Alberta, Canada (population >1.1 million, 123 people/km^2; http://www.statcan.gc.ca/). These ponds were a subset of the randomly selected ponds studied by Scheffers and Paszkowski (2013) that were scattered throughout the city and had been closely monitored via trapping and visual surveys across multiple years.

Three were natural wetlands (signified by “N”; 310N, 314N, and 316N) located within the Whitemud ravine (part of the North Saskatchewan River valley system that runs through the city; Fig. 1). Four of the sites were constructed wetlands (signified by “C”; 106C, 108C, 302C, and 405C), used for storm water retention and built within housing developments in the mid 1990s (City of Edmonton, Drainage Department). A final pond was a natural wetland that was modified to act as a storm water retention pond but retained 3–4 m of its original riparian zone (signified by “M”; 308M). Based on analysis of images from Google Earth, the average pair-wise distance between wetlands was 7.9 km (range 1.9–18.9 km; measured between pond water centers).

Natural ponds were surrounded by a narrow riparian zone of grass ($\leq \sim 3$ m) and shrubs (*Cornus stolonifera*, *Salix* spp.) and by continuous forested river valley habitat (closest urban area ranged from 140 to 250 m from the pond edge) with trembling aspen (*Populus tremluoides*), balsam popular (*Populus balsamifera*), and white spruce (*Picea glauca*) as the dominant tree species. All three natural sites were oxbow ponds and ranged in surface area from 3977 to 6111 m^2. The median proportion of human modified features (including paths, paved surfaces, houses, mowed grass and planting beds) in 100 m buffers around these wetlands was 0.01, and non-modified features (uncut grass and shrubs, coniferous forest, bare ground, mixed forest and floating vegetation) constituted 0.91 of the landscape (modified from Taylor 2013, remaining 0.08 constituted natural and constructed water features). Constructed wetlands (surface areas equalled 4454–27,077 m^2) were surrounded by a 2–4 m wide riparian zone of dense cattail (*Typha* spp.) and planted trees and shrubs (mostly *Salix* spp.). The median proportion of human modified features within 100 m was 0.73, along with 0.13 unmodified landscape features (modified from Taylor 2013 remaining 0.14 constituted water features). All constructed ponds (with the exception of 405C) were roughly 20–100 m from the edge of the forested North Saskatchewan River valley system. Pond 405C was $\sim$100 m from what was agricultural land, which included aspen stands surrounding a cultivated field, cleared $\sim$2 years prior to the study for a housing development. Pond 308M also had a narrow riparian zone surrounded by mowed grass but this pond was more isolated from the river valley (separated by roads and houses by $\sim$190 m). A stand of natural vegetation ($\sim$20 m^2 of shrubs and aspen) stood at one edge of the wetland.

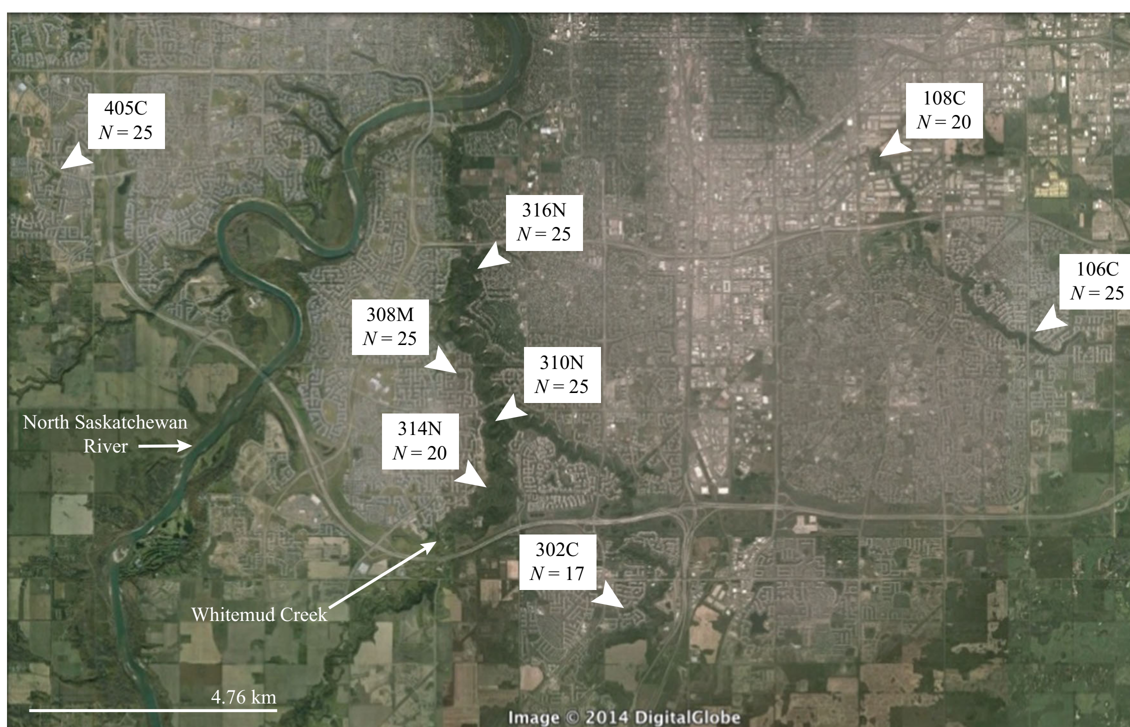

Fig. 1 A satellite image of Edmonton, Alberta, Canada with study sites and sample sizes (number of wood frogs genotyped for each location) indicated in the adjacent *white box*. “N” refers to the site being a natural wetland; “C” indicates a constructed storm water retention pond; Pond 308M (“M” is a mixed history site) is a site that was natural, but has been surrounded by urban development and used as a storm water retention pond

Collection and laboratory procedures

Tissue collection was performed between April 2008 and August 2011 in conjunction with other studies of wood frog populations. We captured wood frogs either by hand or with pitfall traps. We took toe clips, consisting of two phalanges from a single digit, throughout the spring and summer from both adults and juveniles, and stored them in 75 % ethanol. All procedures adhered to the University of Alberta animal health and welfare protocol (#581805).

We selected 17–25 individuals from each site (either all the available tissue samples from a pond or a random subset of the total available samples; 182 samples total; Fig. 1) for genetic analysis and isolated DNA with Qiagen DNEasy Blood and Tissue Extraction kits (Qiagen Inc., Valencia, California). Initially, we genotyped all individuals for 21 microsatellite loci using published primers for wood frogs: 1A11 and 2B02 (Zellmer and Knowles 2009); RsyC11, RsyC23, RsyC41, RsyC52, RsyC63, RsyC83, RsyD20, RsyD25, RsyD32, RsyD33, RsyD40, RsyD55, RsyD70, RsyD77, RsyD88 (Julian and King 2003); RsAAT1, RsAAT23, RsAAT46, RsAAT134 (Newman and Squire 2001). Primers were ordered from Integrated DNA technologies (Coralville, Iowa, USA). We tailed forward primers with M13 sequences to facilitate fluorescent labelling with either FAM, VIC, NED or PET dyes (Schuelke 2000). We amplified the samples using hot start PCR and analysed the amplifications using an ABI 3730 sequencing platform (see Online Appendix A for detailed information on genetic work). Genotypes were visually assessed using GENEMAPPER v.4.1 (Applied Biosystems). Ten loci were either monomorphic, did not amplify, or produced inconsistent results, so we excluded them from further work (RsAAT134, RsyC41, RsyC52, RsyD25, RsyD33, RsyD40, RsyD55, RsyD77, 1A11, RsyC83). One primer pair co-amplified two non-overlapping loci (referred to as RsyD32A and RsyD32B), however RsyD32A also produced inconsistent results and was excluded.

Genetic analysis

Within pond variation

For the 10 usable loci, we determined genotypes for all 182 individuals (with <1 % missing data). Observed and expected heterozygosities were calculated using the Microsatellite Tool Kit in Excel (Park 2001). We used GENEPOP web v.4.0.10 (Raymond and Rousset 1995a) to determine the presence of null alleles by examining F_{IS} values for each locus in each subpopulation (1000 burnin, 1000 interactions and 100 batches). F_{IS} values were calculated by an exact P-value test outlined by Haldane (1954) accompanied by a Bonferroni correction (Rice 1989). Linkage disequilibrium tests with a Bonferroni correction (Rice 1989) were carried out in GENEPOP to ensure that the loci used are assorting randomly. We also used FreeNA (Chapuis and Estoup 2007; Chapuis et al. 2008) to investigate the presence of null alleles.

We determined if any bottlenecks (severe reductions in genetic diversity, due to a decline in the effective population size) had occurred at any of the wetlands using program BOTTLENECK v.1.2.02 (Cornuet and Luikart 1997). We used 1000 iterations, assuming a step-wise allele mutation model (SMM), and evaluated the results with a Wilcoxon test (two tails for heterozygote excess and deficiency). We chose to use a SMM model as it provided the most conservative test for bottlenecks (Cornuet and Luikart 1997) and studies have shown that most intraspecific variation is best fit by a SMM model, whereas two phase models generally add unnecessary parameters and can lead to poor estimates (Bhargava and Fuentes 2010; Sainodiin et al. 2004). We did perform bottleneck tests with a two phase model, but the results were inconsistent and depended heavily on the parameter settings necessary for performing analyses with two-phase models (i.e. the values chosen for the priors controlling the amount of SMM and variance of the geometric distribution). It could be that the extra parameters in this model were too much for our data to estimate. We employed HP-RARE (Kalinowski 2005) to calculate a rarefied estimate of allelic diversity at the study ponds. Rarefaction is a statistical procedure that, in this case, estimates a value of allelic richness based of the smallest number of samples taken among the study sites (pond 302C, 17 individuals) correcting for unequal samples sizes (as increased sampling effort often yields increased richness).

Population differentiation

We used GENEPOP to assess genetic differentiation among subpopulations by evaluating the homogeneity of allele distributions with pair-wise comparisons of subpopulations. GENEPOP examines whether allelic distributions among individuals from a pair of ponds were drawn from a common distribution of alleles by comparing the observed allelic differences between the pair to a random distribution of possible allelic arrangements, and calculates a P-value following a Markov chain Monte Carlo process (Raymond and Rousset 1995b).

We used GENEPOP again to calculate F_{ST} values based on Weir and Cockerham's (1984) Analysis of Variance method. We also tested five different Analysis of Molecular Variance (AMOVA) models using ARLEQUIN v3.5 (Excoffier and Lischer 2010). These five models tested different landscape attributes and how they may be contributing to population differentiation. Model 1 tested if the

existence of large tracts of urban/suburban area were limiting gene flow and ponds were thus divided into three groups: West Edmonton (405C), middle of the city (310N, 302C, 314N, 308M, 316N), East Edmonton (106C, 108C). Model 2 was the same as Model 1, except natural wetlands in the middle of the city were put into their own group as, presumably, these are less affected by urban development given their location in the river valley: West (405C), middle constructed (302C, 308M), middle natural (310N, 314N, 316N), East (106N, 108N). Model 3 tested if natural wetlands were genetically different from constructed wetlands and thus natural wetlands composed one group and each of the constructed wetlands was assigned its own group (six groups total). Model 4 was similar to Model 3, except that all of the constructed wetlands were put into a single group, to test if total genetic variation in the constructed wetlands equalled that of the natural wetlands. Models 1 through 4 assess if urbanization contributes to genetic structuring and/or if subpopulations inhabiting constructed wetlands are genetically differentiated. Finally, Model 5 tested if the North Saskatchewan River, as a major natural barrier, produced genetic differentiation, splitting the ponds into two groups: North of the river (405C), South of the river (310N, 302C, 314N, 308M, 316N, 106C, 108C). For all models, we tested four hierarchical levels of variation: within individuals, among individuals within subpopulations, among subpopulations within groups, and among groups. All analyses were done with 1000 permutations to test for significance and used locus-by-locus analyses, following recommendations in the ARLEQUIN manual.

As a naive approach to test for population structure, we used TESS v.2.1. (Chen et al. 2007). This analysis is naïve in the sense that it will attempt to find genetic differentiation without any a priori specification of potential groups (unlike the F_{ST} and AMOVA analyses above). TESS operates using a Bayesian clustering algorithm to assign individuals (based on their unique genotype) probabilistically to K number of clusters, which have been characterized based on a set of allele frequencies, incorporating sampling location, but not predefined populations (unlike F_{ST}, which uses a priori defined populations; Chen et al. 2007). The incorporation of sampling information allows TESS to penalize admixture between distant samples, and allows for samples located physically closer to one another to have increased admixture, if that is supported by the data. By specifying different numbers of clusters ($K = 2$, 3, 4, etc.), the program will determine the proportion of an individual's genotype that matches the characteristics of each cluster in the analysis, working to maximize linkage equilibrium and Hardy–Weinberg equilibrium within K populations. TESS returns the deviance information criterion statistic (DIC) for which higher values indicate stronger support for a given K. We ran the simulation for $K = 2$–10, with 10 repetitions per K, a burn-in of 10,000, followed by 100,000 Markov-chain steps. The output was passed through CLUMPP v.1.1.2 (Jakobsson and Rosenberg 2007) to correct for label switching and average ambiguous cluster assignment across independent TESS runs of a given K. The D statistic was calculated to determine the similarity algorithm to use (CLUMPP manual) and when the Greedy or LargeK algorithm was used, this was followed by 1000 repetitions to find maximal similarity across runs.

To assess whether or not pairwise genetic distance between ponds was related to pairwise geographic distance between ponds, we performed a regression analysis of pairwise $F_{ST}/(1 - F_{ST})$ against pairwise geographic distance (Rousset 1997) using the ISOLATION BY DISTANCE WEB SERVICE v.3.23 (Jensen et al. 2005). A Mantel test with 5000 permutations was used to assess significance of the relationship between the two variables.

Results

Within pond variation

The number of alleles for the 10 loci examined ranged from four to 29 per locus (Fig. 2a). Two of the 10 loci analyzed appeared to have null alleles and were dropped from further analyses; RsyD20 and RsyC83 were the only loci that showed significant F_{IS} values (post Bonferroni correction) in nearly all ponds. In the ponds where these two loci did not have significant F_{IS} values after the correction, F_{IS} values were significant prior to the correction (Table 1). Significant F_{IS} values were seen for three other loci but only sporadically across subpopulations, thus it is unlikely that null alleles were the cause. Results were identical for the FreeNA analysis (results not shown).

Only two pairs of alleles were found to display significant linkage disequilibrium after Bonferroni corrections; each case occurred within different ponds (316N: RsyC63 and RsyD88, $P < 0.0002$; 405C: 2B02 and RsyD32(B), $P < 0.0002$). Given that 224 tests were performed, some pairs were expected to show significant disequilibrium by chance alone, thus, we assumed linkage disequilibrium to be absent among the eight loci examined. The rarefied estimate of allelic diversity based on eight loci indicated a similar level of allelic richness in each pond (Fig. 2b). The bottleneck analysis indicated that no subpopulations had suffered a bottleneck within recent history (P values >0.05 for each pond).

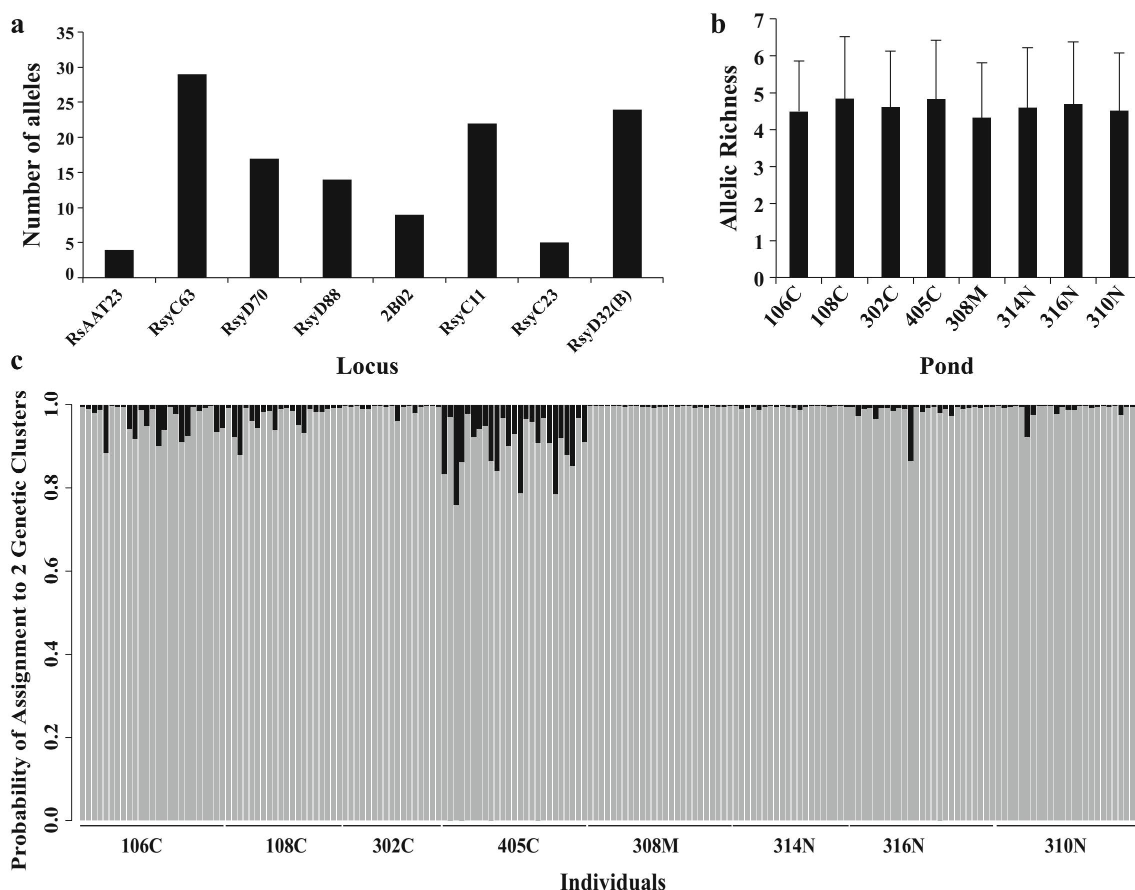

Fig. 2 **a** Allele counts for each locus for all 182 wood frogs sampled in this study. **b** A rarefied estimate of allelic richness (*error bars* are standard deviation) of all individuals at each wetland. **c** TESS genetic structure analysis for the probability of assignment of each individual (represented by a *single vertical bar*) to either of two genetic clusters ($K = 2$) averaged over 10 independent runs. Individuals from all ponds assign mostly to the same cluster (*gray colour*), indicating a lack of any genetic structure between wetlands (indicated *below the individual bars*)

Population differentiation

Significant differences in the homogeneity of allele frequencies were observed for almost all pairs of subpopulations, but these were associated with very low F_{ST} values (Table 2). P-values for homogeneity of allele frequencies had an average $P = 0.010$ (range = 0–0.157) suggesting that differentiation exists among wood frogs among all the ponds with the exception of one pair (314N and 316N: $P = 0.157$), but the degree of differentiation (F_{ST}) is very low (average $F_{ST} = 0.020$; range = 0.003–0.053).

The AMOVA analyses suggested that urban development is not strongly contributing to genetic structuring of the wetlands (Table S1). For all models, >96 % of the variation was within individuals (i.e. differences between the two alleles of a diploid individual). Model 1, which compared groups of wetlands in the East vs Middle vs West of the city (a grouping scheme that maximizes the amount of urban development between groups), found no significant differentiation among groups (among groups: 0.12 % variation explained, $P_{among} = 0.38$). Model 2, similar to Model 1 but with natural wetlands separated into in their own group, returned a significant result (at $\alpha = 0.05$) for the among groups component (among groups: 0.73 % variation explained, $P_{among} = 0.04$), but with minimal total variance explained. Model 3, with natural wetlands placed in one group and each constructed wetland in its own group, suggested perhaps there is some degree of differentiation among these groups (among groups: 1.0 % variation explained, $P_{among} = 0.064$), but the effect was not significant at $\alpha = 0.05$ and explained minimal total variance. When all constructed wetlands were grouped together (Model 4), there was no support for differentiation among groups (among groups: 0.12 % variation explained, $P_{among} = 0.35$). Finally, no support was found for the North Saskatchewan River contributing to genetic differentiation (among groups: 0 % variation explained, $P_{among} = 0.68$).

The TESS analysis indicated that only a single population exists. Inspection of the $K = 2$ assignment plot reveals that all individuals maximally assign to the same cluster, a

Table 1 Genetic variability of 10 loci for wood frogs at eight study ponds in the city of Edmonton

Pond	RsAAT23	RsyC63	RsyD20	RsyD70	RsyD88	2B02	RsyC11	RsyC23	RsyC83	RsyD32(B)
106C										
H_o/H_e	0.440/0.598	0.800/0.852	0.520/0.771	0.960/0.887	0.760/0.834	0.840/0.771	0.840/0.820	0.360/0.440	0.240/0.350	0.960/0.922
F_{IS}/P value	0.269/0.051	0.063/0.763	**0.331/0.003**	−0.084/0.974	0.090/0.011*	−0.091/0.339	−0.024/0.804	0.185/0.423	0.319/0.013*	−0.042/0.2826
108C										
H_o/H_e	0.600/0.637	0.850/0.949	0.400/0.778	0.850/0.909	0.800/0.834	0.750/0.751	0.900/0.896	0.300/0.347	0.000/0.097	0.800/0.936
F_{IS}/P value	0.060/0.234	0.107/0.300	**0.493/0.001**	0.075/0.370	0.033/0.290	0.002/0.404	−0.004/0.300	0.140/0.127	1.000/0.026*	0.149/0.228
302C										
H_o/H_e	0.824/0.620	0.765/0.900	0.412/0.836	1.000/0.909	0.706/0.834	0.647/0.647	0.882/0.841	0.588/0.587	0.235/0.492	0.882/0.932
F_{IS}/P value	−0.341/0.354	0.155/0.248	**0.515/0.001**	−0.103/0.048*	0.158/0.201	0.000/0.155	−0.050/0.866	−0.003/1.000	0.026/0.034*	0.055/0.105
308M										
H_o/H_e	0.600/0.517	0.960/0.926	0.400/0.769	0.880/0.842	0.960/0.848	0.720/0.717	0.680/0.816	0.458/0.489	0.042/0.263	1.000/0.887
F_{IS}/P value	−0.165/0.269	−0.038/0.290	**0.485/0.000**	−0.046/0.251	−0.135/0.057	−0.005/0.921	0.169/0.455	0.063/1.000	**0.845/0.000**	−0.130/0.240
314N										
H_o/H_e	0.700/0.589	0.895/0.905	0.550/0.882	0.850/0.921	0.900/0.853	0.526/0.613	0.950/0.909	0.500/0.501	0.000/0.277	0.950/0.903
F_{IS}/P value	−0.196/0.664	0.011/0.698	**0.383/0.001**	0.079/0.359	−0.057/0.941	0.145/0.893	−0.046/0.525	0.003/0.288	**1.000/0.000**	−0.054/0.172
316N										
H_o/H_e	0.640/0.555	0.917/0.918	0.360/0.840	0.920/0.884	0.880/0.863	0.560/0.714	0.880/0.922	0.480/0.496	0.320/0.438	0.960/0.926
F_{IS}/P value	−0.157/0.912	0.001/0.403	**0.577/0.000**	−0.042/0.246	−0.020/0.090	0.220/0.046*	0.046/0.563	0.032/0.612	0.273/0.059*	−0.038/0.971
405C										
H_o/H_e	0.440/0.597	0.920/0.938	0.440/0.764	0.920/0.892	0.760/0.843	0.640/0.772	0.880/0.901	0.480/0.458	0.160/0.519	0.920/0.936
	0.267/0.228	0.020/0.239	**0.429/0.002**	−0.032/0.753	0.101/0.449	0.174/0.213	0.024/0.674	−0.049	**0.696/0.000**	0.017/0.324
F_{IS}/P value	0.720/0.623	0.840/0.902	0.417/0.806	0.920/0.891	0.708/0.849	0.800/0.655	1.000/0.889	0.600/0.520	0.200/0.528	0.840/0.907
310N										
H_o/H_e	−0.160/0.808	0.070/0.170	**0.488/0.000**	−0.034/0.729	0.169/0.328	−0.228/0.538	−0.128/0.975	−0.158/0.423	**0.626/0.000**	0.075/0.456
F_{IS}/P value	0.440/0.598	0.800/0.852	0.520/0.771	0.960/0.887	0.760/0.834	0.840/0.771	0.840/0.820	0.360/0.440	0.240/0.350	0.960/0.922

Bold values remain significant after Bonferroni correction following an exact *P* value test on F_{IS} values. A * values which were only significant prior to the correction for multiple tests. RsyD20 and RsyC83 were considered to have null alleles and were dropped from all analyses

H_o heterozygosity observed, H_e heterozygosity expected

Table 2 Pairwise comparisons of the heterogeneity of allele frequencies (top diagonal) and F_{ST} values (bottom diagonal) for eight microsatellite loci sequenced for 182 wood frogs

	106C	108C	302C	314N	316N	308M	310N	405C
106C		0.004	0	0.001	0.001	0	0	0
108C	0.019		0.032	0.021	0.008	0	0.001	0.001
302C	0.022	0.011		0.049	0.004	0	0	0.006
314N	0.016	0.011	0.007		0.157	0	0	0.006
316N	0.019	0.013	0.005	0.005		0	0	0
308M	0.053	0.032	0.018	0.04	0.022		0	0
310N	0.034	0.015	0.028	0.018	0.014	0.043		0
405C	0.024	0.007	0.008	0.003	0.014	0.034	0.024	

These analyses include three constructed wetlands (C), three natural wetlands (N) located in the North Saskatchewan River valley of Edmonton, and one natural pond surrounded by urban development (M)

pattern that was consistent with all other values of K (Fig. 2c). Though the DIC values declined from $K = 2$ through $K = 10$ (Fig. 3a), the bar plot changed minimally, indicating convergence of clustering. The regression analysis and associated Mantel test assessing the level of isolation by distance (IBD) using pairwise $F_{ST}/(1 - F_{ST})$ versus pairwise geographic distance between ponds found no relationship ($r = -0.162$, one-sided $P = 0.694$) (Fig. 3b).

Discussion

Constructed wetlands

Constructed wetlands did not appear to harbour subsets of the regional genetic diversity of wood frogs (Fig. 2c). Site-specific allelic richness estimates indicated that natural and constructed wetlands contain comparable levels of allelic diversity (Fig. 2b) and AMOVA models reveal that most (>96 %) genetic variation is within individuals. Assuming immediate colonization by surviving animals previously living in the mixed agricultural landscape that existed prior to the initiation of housing developments, wood frogs in constructed ponds have likely undergone only 5 or so generations, as the ponds were built within the last 20 years. Thus, there may have not been sufficient time for these populations to diverge from the natural sites. For this to be the case, however, wood frogs would had to have existed in these areas prior to development, recolonized the ponds after construction and experienced no immigration since. Removal of all topsoil is a common practice during construction of these sites, and the surrounding houses were either being built or had been built prior to wetland construction, making survival unlikely during the construction phase. It is therefore probable that the frogs inhabiting these wetlands are not from previously existing populations, but migrants from other areas. No bottlenecks were detected in our analyses, so ongoing migration, following wetland establishment, is likely.

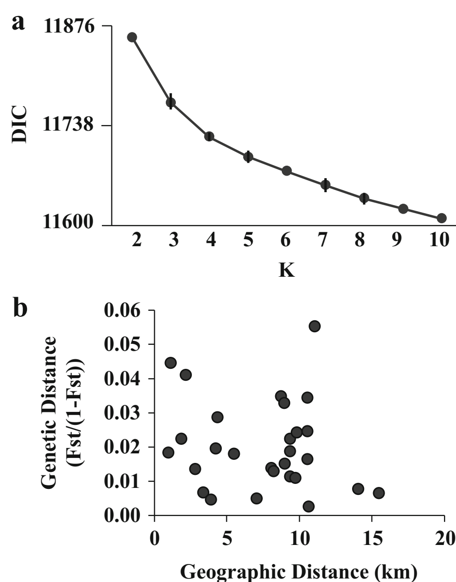

Fig. 3 **a** DIC statistic from TESS genetic structure analysis, high values indicate greater support for a given K (number of population clusters). Lower K values are preferred in the analysis. **b** Genetic isolation by distance analysis, as measured by Mantel tests and regression analysis

AMOVA analyses provided some support that impediments to gene flow between constructed and natural wetlands may exist. These models tested if urbanization was impacting gene flow broadly across the city (Models 1 and 2) or if genetic patterns reflected differences between constructed and natural wetlands (Models 2 and 3). The marginally significant results of Models 2 and 3 suggest that differences exist between the genetic structure of subpopulations in natural and constructed wetlands. But the magnitude of this effect is very small and, considering that both models placed most subpopulations in their own "group", the results may have been driven by the philopatric behaviour of the wood frog. Philopatry leads to inbreeding within each wetland and creates slight site-specific differences in genetic variation, which are not necessarily a function of constructed versus natural wetland or a function of division by urbanization (pairwise F_{ST}

analysis revealed slight genetic differences between all wetlands and most pairwise comparisons of heterogeneity in allele frequency were significantly different; Table 2). Comparing Model 3 to Model 4 reveals that when the genetic variation of all constructed wetland subpopulations are combined into a single group (Model 4), it is indistinguishable from the genetic variation within natural wetland subpopulations (no significant differences found for Model 4). The slight paucity of genetic variation of individual constructed wetlands could be attributed to their more recent origin or potentially smaller population sizes. The AMOVA models revealed that any grouping scheme accounts for little genetic variation and the Bayesian TESS analysis, lacking any predefined groupings, placed all subpopulations within a single genetic population. These analyses indicate that wood frogs at constructed wetlands are likely not genetically isolated from the rest of the Edmonton population.

In this study, IBD analysis failed to detect any significant relationship between genetic and geographic distance of wood frog subpopulations (the observed slope was not statistically different from zero; Fig. 3b and "Results" section). This pattern could reflect the dispersal capability of the wood frog (up to 1.5 km in topographically challenging terrain; Berven and Grudzien 1990). Radio tracking of wood frogs in the Edmonton area (at the wetlands included in this study and others within the city) has revealed that wetland type (constructed vs. natural) does not affect the general movement habits of adult wood frogs around wetlands (Taylor 2013). Frogs inhabiting constructed and natural wetlands had similar home range sizes, exhibited a preference for shrub/grass covered habitat features and did not significantly prefer closed canopy forest (Taylor 2013). All constructed wetlands in our study are 20–100 m from the edge of forested ravine habitat, connected by grass or shrub cover with some intervening artificial features (paved paths, mowed lawn). Although wood frogs avoid extended occupancy of human altered landscape features, they are found to cross them to reach preferred habitat (Taylor 2013). This suggests wood frogs in our study area are capable of moving between the constructed wetlands and natural areas and 10 % of the tracked adult frogs were found to move >85 m from water's edge (Taylor 2013). Although the migration distance of any individual adult frog, or dispersal of juvenile frogs, in a single event would not likely cover the smallest pairwise distance between ponds in this study (1.9 km), multi-year or multi-generational migration and dispersal, and/or other wetlands present in the city (Scheffers and Paszkowski 2013) could facilitate gene flow among the subpopulations. A lack of IBD has been found in other studies of wood frog populations over similar geographic scales (Crosby et al. 2008; Newman and Squire 2001 (excluding one distant population)). However, those results and ours could be due to a lack of statistical power. When much larger distances and/or more ponds are used (>100 km: Richardson 2012), or when genetically structured subpopulations are included (Crosby et al. 2008; Peterman et al. 2013), signals of IBD can be found. The lack of a statistical relationship in this study does not preclude the possibility of a small effect being present.

Wood frogs have been known to inhabit constructed wetlands in appreciable abundances (Brand and Snodgrass 2010; Petranka et al. 2007; Scheffers and Paszkowski 2013). Past research in our study area reveals that wood frogs can penetrate the urban matrix, inhabit constructed habitats, and successfully breeding at these sites (Scheffers and Paszkowski 2013; Taylor 2013), and we show that this likely facilitates an on going exchange of individuals with the surrounding subpopulations, as indicated by genetic homogeneity between all wetlands. This result has also been found for wood frogs inhabiting completely natural wetlands within urban and agricultural lands of Southern Ontario, Canada (Crosby et al. 2008).

Life history traits

How life history stages and species traits interact with urban and human disturbance vary largely by species and site. Species that require two or more habitats to complete their life cycle could be more sensitive to urban development than those that do not (Rubbo and Kiesecker 2005). For example, the common frog (*Rana temporaria*) and the moor frog (*Rana arvalis*), two species that both require two distinct habitats to complete their life cycles, appear to be genetically structured in response to urban development (Hitchings and Beebee 1997; Arens et al. 2007). In addition to habitat requirements, the magnitude of the urbanization effect may also be an artefact of species movement capabilities. In a comparison of two frog species, one that moves overland more frequently than the other (*Rana pipiens* and *Rana clamitans*, respectively), population abundances of *Rana pipiens* (the species with greater overland movement) were more affected by road networks (Carr and Fahrig 2001).

Based on these examples, it would appear that wood frog populations could be negatively affected by urban development because of their complex habitat requirements, yet the species does not show strong responses (Crosby et al. 2008 and this study). Similarly, spotted salamander populations (*Abystoma maculatum*), which also require terrestrial and aquatic habitats to complete their life cycles, inhabiting fragmented urban areas of Ohio, show little genetic differentiation (Purrenhage et al. 2009; but see Richardson 2012 for a counter example). One common feature between Purrenhage et al. (2009) and our study is

the presence of a river valley corridor adjacent to most wetland sites. These river valley features likely provide migration corridors between sites, allowing animals to access both habitat types without having to travel through the urban matrix. Low resistance corridors between wetlands however, does not apply to the Crosby et al. (2008) study of wood frogs, so the use of riverine corridors is not a ubiquitous explanation for the lack of genetic structure in wood frog populations. Very few landscape features significantly impede wood frog movement, other than large roads (Crosby et al. 2008; Peterman et al. 2013; Richardson 2012). Thus, having complex life cycles does not necessarily equate to increased sensitivity to urban development.

The influence that life history traits and habitat requirements have on a species response to urbanization is difficult to predict and is not always consistent. Marsh frogs (*Pelophylax ridibundus*) spend the majority of their lives in large bodies of water and disperses through water networks and, yet, this species shows some degree of genetic differentiation in response to urban development (Mikulicek and Pisut 2012). Red-backed salamanders (*Plethodon cireneus*) in Montreal, Canada, show genetic differentiation among populations in urban fragmented areas, despite being entirely terrestrial (Noel et al. 2007). However, this species occupies extremely small home ranges, rarely moves greater than 50 m, and even minor rivers can impede their movement (Noel et al. 2007 and citations within; Marsh et al. 2007). For stream breeding salamanders, urbanization does not necessarily lead to genetic differentiation. Some populations and species show negative effects and strong genetic differentiation, such as *Desmognathus fuscus* within New York City, yet other similar species do not (see discussion in Munshi-South et al. 2013). How a particular amphibian species is affected by urban development is likely a function of its life history in combination with numerous local factors, such as the amount of connecting habitat, distance between breeding/foraging/overwintering sites, amount and severity of impervious surfaces, and natural vegetation cover. But, in general, our study in combination with the large literature of conflicting results stresses the importance of further research from geographically disparate areas. Importantly, comparative studies between species with different life history traits (such as Richardson 2012) are needed to fully understand how urbanization affects gene flow and genetic structure in amphibian populations globally.

Conclusions

Whether urbanization causes genetic structuring in populations of pond-breeding amphibians remains uncertain (Blouin et al. 2010) and the existence of genetic structure is likely species or population dependent (Johansson et al. 2005). Even when genetic divergence is found in an amphibian population fragmented by urban development, it may not have an anthropogenic cause and could be due to historic, pre-settlement patterns of gene flow and environmental variation (Measey and Tolley 2011). One result is clear—proper land management and habitat enrichment projects, such as constructing storm water wetlands, offers the best chance in supporting amphibian's life history requirements and maintaining suitable connectivity across urban landscapes in order to counter the negative effects of development, and to prevent future genetic defects. Our study suggests that constructed wetlands may serve as important habitats for conserving urban wood frog populations and their genetic diversity across wider landscapes where natural wetlands have largely been destroyed. The preservation and/or restoration of naturally vegetated areas across the broader urban landscape minimizes the risk of immediate desiccation, facilitates dispersal (Sinsch 1990; Rittenhouse et al. 2008), and is undoubtedly a critical feature that allows the long-term persistence of amphibian populations at constructed wetland sites (Angelone and Holderegger 2009; Scheffers and Paszkowski 2013).

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Compliance with ethical standards

Conflict of interest We are unaware of anything that could be considered a conflict of interest.

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