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RESEARCH

Genetic assessment and monitoring of wild, captive, and reintroduced northern leopard frog populations

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Abstract

The northern leopard frog (*Lithobates pipiens*) has undergone dramatic declines in population size and range over recent decades in western Canada and the United States. In British Columbia, only a single population remains at the Creston Valley Wildlife Management Area. Yet, the continuing viability of this population is uncertain. In this paper, the current genetic structure of northern leopard frog populations in western Canada was assessed using microsatellite markers. Historical samples from the extinct population of Fort Steele in British Columbia were compared with the Creston Valley population to understand changes in population genetic parameters over time. Genotypic data from four populations (Creston Valley, Drain K, Prince Spring, and Cypress Hill) sampled in 2004 and 2019 were compared. To evaluate changes in the genetic diversity of the Creston Valley population over time, allelic richness and expected heterozygosity of the population were compared at three time points using genotypes from 2000, 2004, and 2019. Northern leopard frog populations in western Canada showed high genetic differentiation, with genetic diversity decreasing from east to west. Although there weren't notable changes in genetic parameters between 2004 and 2019, there was evidence of a decline in diversity between 2000 and 2019. The extinct population of Fort Steele had private alleles, while the current Creston Valley population did not, suggesting a genetic bottleneck in the Creston Valley population. Therefore, genetic rescue, specifically for the endangered Creston Valley population, can be considered as an action to support recovery. Additionally, continued genetic monitoring will help in the effective management of the species by providing information on the success of conservation actions.

Keywords Genetic assessment · Genetic monitoring · *Lithobates pipiens* · Northern leopard frog · Population genetic structure

Introduction

Northern leopard frog (*Lithobates pipiens*) has experienced declines in abundance and has disappeared from many parts of its western range (Fig. 1; Leonard et al. 1999; Werner 2003; Wilson et al. 2008; COSEWIC 2009). These declines are likely due to a combination of factors such as habitat loss and fragmentation, disease outbreaks (e.g., chytridiomycosis and ranavirus), invasive species, pollution, and climate change (Kendell and Prescott 2007; Wilson et al. 2008; Green et al. 2020). The two main eastern and western clades of the northern leopard frog have been separated by the Mississippi River and the great lakes of North America for approximately two million years (Hoffman and Blouin 2004 a; O'Donnell and Mock 2012). Furthermore, there is also a major split between east and west in North Dakota, divided by the Missouri River (Waraniak et al. 2019). These

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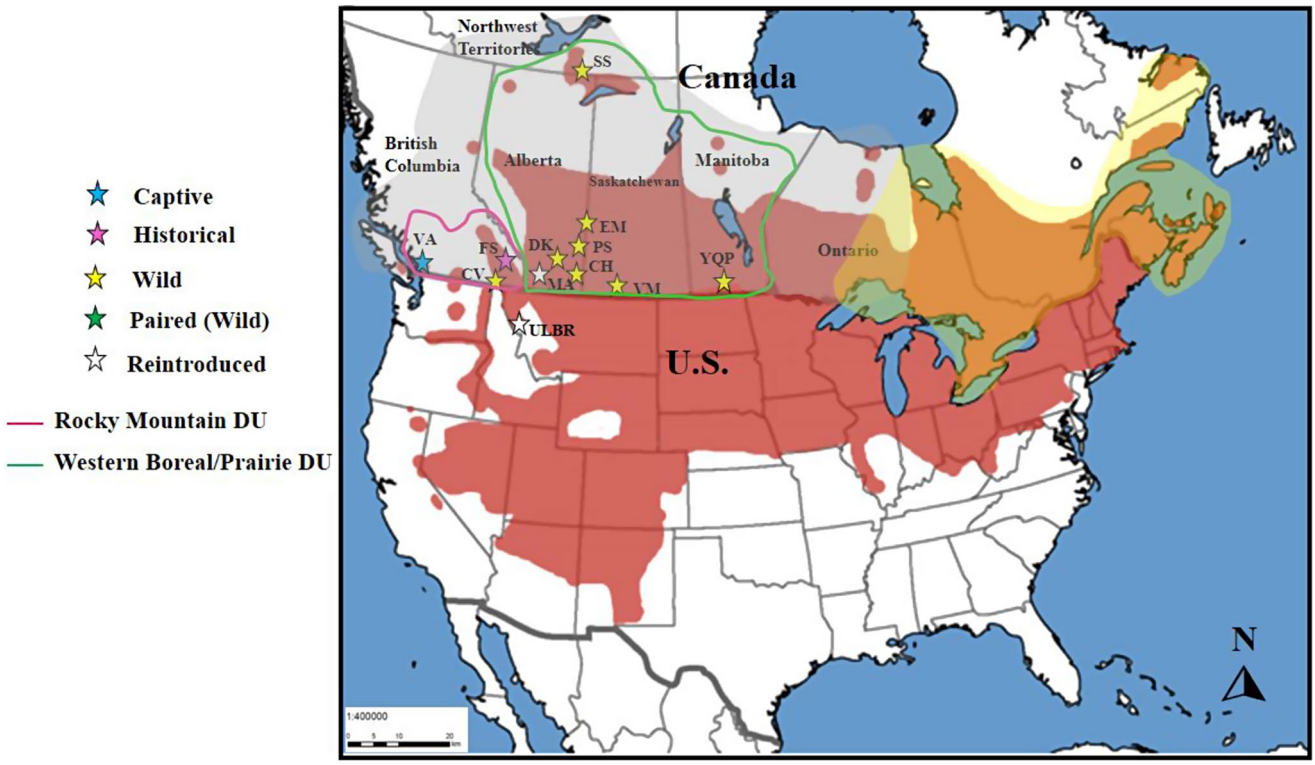

Fig. 1 Collection locations for northern leopard frog (Lithobates pipiens) genetic samples (stars). Codes (ID) for population identification are given in Table 1. Star colors represent the population type (blue = captive; pink = historical; yellow = wild; green = paired wild populations for which genetic monitoring analyses were compared to Wilson et al. (2008); white = reintroduced). Rocky Mountains and Western Boreal/Prairies designatable units are shown by pink and green lines, respectively. Red shading represents the species' historical indigenous range. Yellow shading shows the eastern clade, while the gray shading indicates the western clade in Canada

two clusters differentiated ~ 13–18 kilo years ago, during a period of glacial retreat in the northern Great Plains, with prehistoric climate and landscape features, such as major rivers and lakes, creating physical barriers to gene flow and driving east-west population differentiation (Waraniak et al. 2019). In western Canada, the western clade is divided into two evolutionary significant units: (1) the Western Boreal/Prairie (Alberta, Saskatchewan, Manitoba, and Northwest Territories), which is currently designated as Special Concern under the federal Species at Risk Act (SARA) in Canada, and (2) the Rocky Mountain designatable unit (Fig. 1) which is designated as Endangered under SARA and Critically Imperiled in British Columbia (BC Conservation Data Centre 2021). There is only one non-reintroduced population in the Rocky Mountain designatable unit and reintroduction activities have met with limited success to date (Randall et al. In press).

In a previous study, Wilson et al. (2008) revealed that northern leopard frog genetic diversity declines from western Ontario westward, and the only existing wild population in British Columbia, located in the Creston Valley, had the lowest genetic variation and was genetically distinct from other populations in North America (Wilson et al. 2008). In Washington, the last remnant population inhabits the Potholes Reservoir area of the Columbia Basin Wildlife Area (Germaine and Hays 2009), and recent genetic data revealed the presence of three subpopulations within this population (Seaborn and Goldberg 2020). Like the Canadian northern leopard frog populations, the overall genetic diversity of the western clade of northern leopard frog in the United States is low (Phillipsen et al. 2011; Seaborn and Goldberg 2020).

In addition to measuring genetic diversity in populations of conservation concern, monitoring genetic changes over time may be critical to restoring and maintaining genetic diversity, adaptive potential, and long-term survival in changing environments of northern leopard frog populations (Frankham 2019). Conservation translocation is an important wildlife management tool for establishing viable and self-sustaining populations (Dodd and Seigel 1991; Griffiths and Pavajeau 2008) which can also play a key role in conserving at risk northern leopard frog populations. Conservation translocation refers to the intentional, human-mediated, wild-wild or captive-wild transfer of living organisms from one area to another, either inside their natural range (i.e., restoration) or outside of it (i.e., introduction/assisted colonization) (Armstrong and Seddon 2008; IUCN/SSC 2013; Germano et al. 2015). Genetic rescue

as a type of restoration is the introduction of new alleles into a population that increases genetic diversity, fitness, and adaptability in it (Frankham 2019). Reintroduction is another important type of restoration, with the main purpose of establishing self-sustaining populations in habitats within the historic indigenous range of rare, at-risk species (Conant 1988; IUCN/SSC 2013). Assessing the success of rare species reintroductions requires managers to consider the processes contributing to rarity (Haskins 2015), including founder effects (e.g., low genetic diversity and small effective population size; Bi et al. 2013; Der Sarkissian et al. 2015) and admixture (e.g., hybrid breakdown; Díez-Del-Molino et al. 2018; La Haye et al. 2017).

In this study, we compared the genetic structure of current (2019) northern leopard frog populations in western Canada to a historical (1973), extirpated Rocky Mountain population. Additionally, we examined changes in genetic diversity in three populations in Alberta at three different time points to monitor over time changes in population genetics parameters. We compared genetic diversity, allelic richness, and expected heterozygosity in the Creston Valley population in 2000 (Hoffman and Blouin 2004b), 2004 (Wilson et al. 2008), and 2019. A better understanding of changes in genetic diversity and effective population sizes is critical to effectively managing and recovering endangered populations.

Materials and methods

Sample/data collection

During spring and summer 2019–2020, we collected buccal swabs (MW 113 dry) from adult and juvenile northern leopard frogs and stored swabs in 95% ethanol. Swabs were collected from eight wild populations (Yellow Quill Property, Manitoba (YQP), Val-Marie, Saskatchewan (VM), Empress, Alberta (EM), Cypress Hill, Alberta (CH), Prince's Spring, Alberta (PS), South Slave, Northwest Territories (SS), Drain K, Alberta (DK), and Creston Valley, British Columbia (CV), one captive population(Vancouver Aquarium (VA) in British Columbia), and two reintroduced populations (Magrath in Alberta (MA) Upper Little Bitterroot River in Montana, U.S. (ULBR) (Appendix, Table S1; Fig. 1). Adults and juvenile frogs were preferentially swabbed under the assumption that they were less likely to be siblings than young of year frogs (Wilson et al. 2008). However, when this was not possible, we swabbed young of year frogs. We collected buccal swabs from captive frogs from different clutches at the Vancouver Aquarium to avoid sampling siblings. In addition to 90 swabs collected, we collected five tadpoles from each of six egg masses ($n = 30$) from the CV population in 2019 to determine the effective population. Tadpoles were euthanized using a buffered 1.0% w/v solution (10 g per L) of MS222 (CCAC 2021; Appendix, Table S1; Fig. 1). Additionally, we obtained 10 tissue samples of northern leopard frog from South Slave (Northwest Territories), which were collected by researchers in 2009 (Schock 2010; Appendix, Table S1; Fig. 1).

To better understand the current population genetic differentiation of the species and monitor population genetic metrics over time, we compared genotypes from our sampling efforts with genotypes of the same populations sampled by Wilson et al. 2008. Furthermore, we obtained samples of 10 historical (1973) skins of northern leopard frogs from a now extinct Rocky Mountain population near Fort Steele, British Columbia (Appendix, Table S1; Fig. 1). These historical samples were accessible via the Amphibian Collection of the Canadian Museum of Nature (Khidas and Torgersen 2022). The species conservation status restricted our sampling ability, leading to uneven sample sizes. Therefore, we corrected diversity measures for sample size whenever it was necessary (Waples and Yokota 2007).

Laboratory methods

We extracted genomic DNA using DNeasy Blood and Tissue Kits and included a negative for each set of extracted samples. We used PCR to amplify ten microsatellite loci previously published for northern leopard frog Rpi100, Rpi101, Rpi103, Rpi104, Rpi106, Rpi107, Rpi108 (Hoffman et al. 2003), RP193 (Hoffman and Blouin 2004 b), Rasp09, and Rasp20 (McKee et al. 2011) (Table 1). Our amplification protocol consisted of an initial denaturing step of 94 °C for 60 s (sec) followed by three cycles of denaturing at 94 °C for 30 s, annealing at 44 °C for 20 s and extension at 72 °C for 5 s, followed by 30 cycles of denaturing at 94 °C for 15 s, annealing at 45 °C for 20 s, and extension at 72 °C for 1 s followed by a final 30 min extension at 72 °C. All PCRs were run with a negative control to test for reagent contamination. PCR products were run on a 1.5% w/v agarose gel using a standard gel electrophoresis protocol and visualized using a DigiDOC IT electrophoresis gel imager (UVP Inc.). Samples showing clearly defined bands representing the DNA fragment PCR products of the specified microsatellite loci were then analyzed. To assess genotype scoring consistency, we randomly reran 10% of the samples. Microsatellite alleles were scored using GeneMapper v.5.0 (Applied Biosystems, Inc.). We used MICRO-CHECKER v. 2.2.3 to assess the presence of null alleles, allelic dropout, and false alleles (Van Oosterhout et al. 2004).

Table 1 Primer information

Locus	Sequences (5'-3')	Annealing temp. (°C) (From literature)	Size	No. alleles
RPi-100-F	GGACTGGGGAGTTTCATCC	62 (Hoffman et al. 2003)	174–222	9
RPi-100-R	AAGTCCTATCCCTAGTATGATACAC			
RPi-101-F	AACGCACAGCAAAGGAGTAA	62 (Hoffman et al. 2003)	161–201	9
RPi-101-R	CAAGGGATGACTTAGAAAGGG			
RPi-103-F	TTGAACAGGTATATCTAATAAAGT	56 (Hoffman et al. 2003)	135–211	10
RPi-103-R	TGCTTCCATTTTAATTGTGTC			
RPi-104-F	CAGGGCAATGTGGAATGTGGA	62 (Hoffman et al. 2003)	226–230	2
RPi-104-R	AGGACCACTCAGGTACAAAATGTTCT			
RPi-106-F	ACAGGGGTAAACAAAAATACTT	50 (Hoffman et al. 2003)	307–607	11
RPi-106-R	GGGCTAAAAAGGACATCAA			
RPi-107-F	GTGGTCTTATTACATTTCTTAC	57 (Hoffman et al. 2003)	161–223	8
RPi-107-R	GCCAGTGAGTGTAGATAGAT			
RPi-108-F	AAATAACTCCTGGGAAATGT	57 (Hoffman et al. 2003)	272–298	7
RPi-108-R	CATCCCAAAGAGTCATATC			
Rasp09-F	GGTGAAACCCTGGAGACGTA	58 (Hoffman and Blouin 2004 b)	316–364	*
Rasp09-R	CATGGCCAACAGAGTGGAAA			
Rasp20-F	TGATGGTCAGGTCCACAAACT	65 (McKee et al. 2011)	140–283	*
Rasp20-R	CCTTATCCTGTTGGCAGCAAT			
RP193-F	CCATTTTCTCTCTGATGTGTGT	50 (McKee et al. 2011)	143–203	*
RP193-R	TGAAGCAGATCACTGGCAAAGC			

Data analysis

To estimate genetic diversity and inbreeding for the CV population in British Columbia, we included in the analysis genotypes obtained from the 90 buccal swabs and from one randomly selected tadpole from each of the six egg masses. We tested for linkage disequilibrium (LD) and deviations from the Hardy–Weinberg equilibrium (HWE) using GENEPOP version 4.7.5 (Rousset 2008). Levels of significance for both LD and HWE tests were adjusted using non-sequential Bonferroni corrections (Rice 1989). For all current and historical populations, we calculated expected and observed heterozygosity in GenAlEx v. 6.503 (Peakall and Smouse 2006) and corrected for sample size in HP-Rare v.1.0 (Kalinowski 2005). We calculated the fixation index (FIS, inbreeding coefficients) indicating deviations from Hardy–Weinberg equilibrium and its significance using FSTAT version 2.9.4 (Goudet 1995).

After removing alleles with frequencies lower than 5% (*P*crit = 0.05) and omitting singleton alleles, we calculated the effective population size for each sampling site using the LD method in the NeEstimator software version 2.1 (Do et al. 2014). Samples from endangered species or museums can be biased in the estimation of effective population size due to the relatively low number of available samples. In these calculations, we used all the individuals (including siblings) sampled from CV population to increase the precision of estimates. The effect of siblings on effective population size estimation is not well investigated (Waples and Anderson 2017; Sládkovičová et al. 2022) but we decided to include potential siblings to increase the sampling size to improve the accuracy and avoid downward bias of estimate (Waples and Yokota 2007; Sved et al. 2013; Sládkovičová et al. 2022).

We used a nested ANOVA (Weir and Cockerham 1984) to quantify genetic differentiation between Canadian populations using a measure of genetic distance (F_{ST}) in FSTAT version 2.9.4 (Goudet 1995). We excluded the reintroduced population of ULBR (Randall et al. 2021) from F_{ST} and further population genetic structure analysis as it is a reintroduced population which is an unsuitable source for the potential rescue of the CV population. To test genetic isolation by distance throughout the study area, we performed a regression between pairwise genetic distances (FST/(1-FST) and linear geographical distances between non-reintroduction sampling sites (Rousset 1997) in GENEPOP version 4.7.5 (Rousset 2008). For geographical distances, we measured the shortest route between two sampling sites using the distance tool in GoogleEarth version 4.2.1.

To explore population differentiation and the number of genetic clusters in Canada (with the historical FS population included), we used a Bayesian clustering method (STRUCTURE version 2.3.4; Pritchard et al. 2000). We performed ten independent runs for k = 1 to 12. Each chain was run with a burn-in of 100,000 and an additional 1,000,000 iterations. To identify the most likely number of genetic clusters, we performed the Delta K method (Evanno et al. 2005) in Structure Harvester (Earl and VonHoldt 2012). We plotted the log probability of the data [LnP (D)] as a function of the number of clusters (K) to determine the optimal configuration (Pritchard et al. 2000). We compiled and visualized results of all runs from STRUCTURE using CLUMPAK (Kopelman et al. 2015). To determine the genetic structure of the historical FS

Table 2 Measures of genetic diversity including rarefied allelic richness (AR), rarefied private allelic richness (PR), observed heterozygosity (H_o), expected heterozygosity (H_e), and Inbreeding coefficient (F_{is}) at each sampling site. Sampling sites in each section are ordered from east to west

Population type	Sampling site	ID	*n*	AR (SE)	PR (SE)	H_o (SE)	H_e (SE)	F_{is} (SE)
Wild	Yellow Quill Property, Manitoba	YQP	19	4.99 (0.47)	0.88 (0.27)	0.74 (0.05)	0.75 (0.04)	0.05 (0.05)
	Val-Marie, Saskatchewan	VM	9	4.44 (0.48)	0.10 (0.24)	0.65 (0.08)	0.65 (0.06)	0.06 (0.08)
	Empress, Alberta	EM	5	3.30 (0.30)	0.32 (0.18)	0.60 (0.07)	0.59 (0.04)	0.09 (0.08)
	Cypress Hill, Alberta	CH	31	3.86 (0.30)	0.45 (0.14)	0.68 (0.05)	0.68 (0.04)	0.01 (0.07)
	Prince's Spring, Alberta	PS	32	2.90 (0.38)	0.38 (0.21)	0.50 (0.09)	0.50 (0.09)	0.07 (0.06)
	South Slave, Northwest Territories	SS	10	2.9 (0.47)	0.11 (0.05)	0.42 (0.11)	0.44 (0.09)	0.10 (0.09)
	Drain K, Alberta	DK	36	2.76 (0.24)	0.03 (0.02)	0.50 (0.07)	0.47 (0.06)	−0.05 (0.06)
	Creston Valley, British Columbia	CV	96	1.76 (0.22)	0.00 (0.00)	0.23 (0.07)	0.24 (0.07)	0.07 (0.04)
	Total Number		**250**	-	-	-	-	-
Captive / reintroduced	Magrath, Alberta	MA	18	2.56 (0.25)	0.11(0.07)	0.49 (0.08)	0.46 (0.07)	-0.04 (0.07)
	Upper Little Bitterroot River, Montana	ULBR	25	3.40 (0.39)	0.36 (0.28)	0.57 (0.08)	0.57 (0.08)	0.03 (0.07)
	Vancouver Aquarium	VA	40	1.81 (0.23)	0.00 (0.00)	0.27 (0.08)	0.26 (0.07)	−0.02 (0.07)
	Total Number		**83**	-	-	-	-	-
Historical	Fort Steele	FS	10	2.24 (0.34)	1.78 (0.39)	0.38 (0.09)	0.36 (0.09)	−0.01(0.07)

population without the influence of the current British Columbia populations (VA and CV), we conducted STRUCTURE analysis using only historical samples from the historical FS population and populations from the Western Boreal/Prairie DU. In this analysis, the model parameters were identical to those in previous STRUCTURE analyses.

Results

Genetic diversity and structure of current northern leopard frog populations

We found no deviations from HWE, no evidence for linkage disequilibrium, and few null alleles (2 out of 10 genotyped loci). Thus, all data were retained for further analysis. The mean number of alleles in this study was 4.32 ± 0.45 for the non-captive populations (wild and reintroduced) of Canada and 2.24 ± 0.36 for the historical samples from Fort Steele. Rarefied allelic richness (± SE) for sampling sites ranged from 1.76 ± 0.21 to 4.99 ± 0.47, and rarefied private allele richness varied from 0 to 0.88 ± 0.27 SE for the CV and YQP, respectively (Table 2). The CV population had the lowest genetic diversity (rarefied allelic richness and expected heterozygosity), while the Yellow Quill Property population in Manitoba had the highest genetic diversity (Table 2). There was a significant relationship between the genetic diversity measures (allelic richness and H_e) and longitude with an east-to-west decline ($r^2 = 0.64$, $p = 0.017$ for allelic richness and $r^2 = 0.72$, $p = 0.007$ for H_e; Fig. 2a and

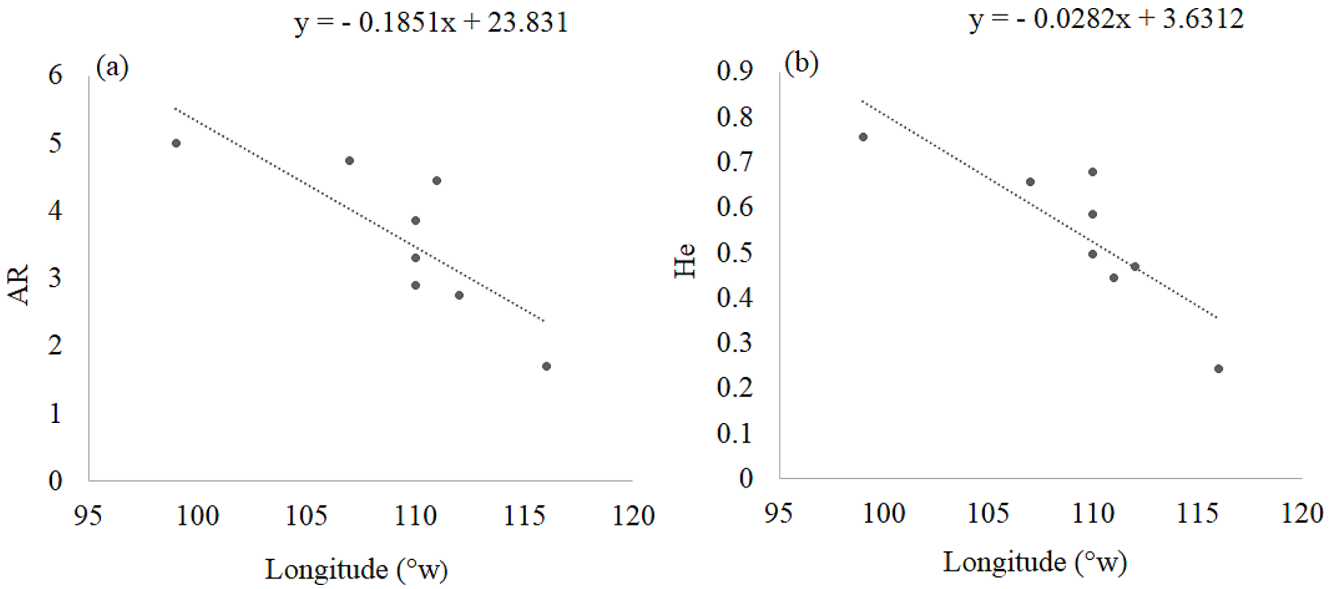

Fig. 2 Linear regression between genetic diversity and longitude of wild northern leopard frog populations in Canada. Diversity is measured by (**a**) allelic richness ($r^2 = 0.64$, $p = 0.007$), and (**b**) expected heterozygosity ($r^2 = 0.72$, $p = 0.007$)

Table 3 The effective population size (N_e) of sampling sites based on linkage disequilibrium after omitting alleles with frequencies lower than 5%. Codes for population identification (ID) are given in Table 1. Sampling sites in each population type are ordered from east to west

Population type	ID	*n*	N_e	95% CI
Wild Populations	YQP	19	14.3	10.3–20.9
	VM	9	305.1	16.9-Infinite
	EM	5	61.2	1.9-Infinite
	CH	31	18.2	12.5–27.9
	PS	32	29.9	14.8–95.2
	SS	10	6.8	2.2–40.9
	DK	36	20.6	11.7–41.1
	CV	120	2.0	1.2–2.9
Captive and reintroduced populations	MA	18	14.9	5.1–118
	ULBR	25	18.1	10.7–36.1
	VA	40	3.5	1.9–10.4

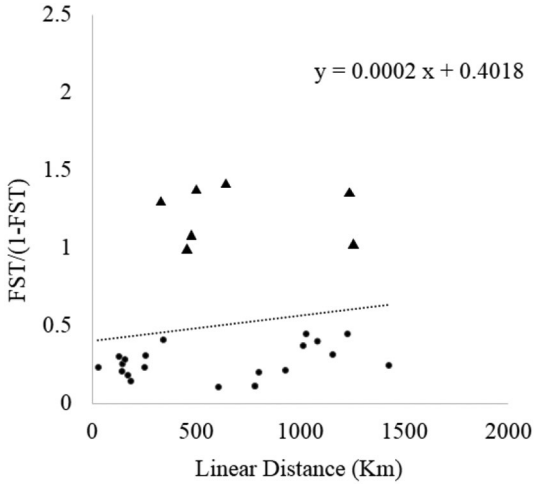

Fig. 3 Correlation of genetic (FST/ (1- FST) and geographical distances (Km) between wild northern leopard frog populations ($p = 0.42$), triangle dots are Creston Valley

b). The inbreeding coefficient ($F_{IS} \pm SE$) within populations ranged from -0.04 ± 0.05 to 0.10 ± 0.093 for DK and SS, respectively. Standard errors overlapped among inbreeding coefficients of sampled populations making the comparison between them difficult (Table 2).

Effective population size (N_e) ranged from 2.0 to 305.1, with CV and VM having the lowest and highest N_e, respectively, although the 95% confidence intervals for the estimated N_e of the populations overlapped (Table 3).

Genetic distance between all sampled populations in this study varied between moderate (0.05–0.15), great (0.15–0.25), and very great differentiation (above 0.25; Wright 1978; Table 4). The lowest F_{st} for the current non-captive Canadian populations (wild and reintroduced, assessment with 10 loci) was 0.097 between VM and YQP, while the highest F_{st} was 0.625 between CV and MA (Table 4). In fact, CV was highly different from all other wild and reintroduced frog populations in Canada but showed very little genetic differentiation (0.024) with the captive population from the Vancouver Aquarium (Table 4).

Populations from Alberta were mostly different from each other, with the reintroduced population of MA showing the highest F_{st} with DK in the province (0.27, Table 4). The SS population (Northwest Territories) was highly differentiated from other populations except the CH and YQP populations located in Alberta and Manitoba, respectively, to which it was still greatly differentiated. The VM population (Saskatchewan) had a moderate genetic differentiation from the only sampled population in Manitoba (YQP) and the CH population in Alberta.

There was no relationship between geographic and genetic distance across the wild populations (P-value = 0.42; Fig. 3), even after the CV population was removed from the analysis (P-value = 0.14).

Structure analysis revealed a strong signal at K = 2 and K = 10 (Supplementary material, Fig. S1, and Fig. S 4). The VA and CV populations constituted one cluster (Fig. 4a and b). The historical FS population and the other eight Canadian populations were grouped together in a second genetic cluster (Fig. 4b). However, the historical population of FS showed evidence of some admixture with the remnant CV population (Fig. 4a). After removing VA and CV from the

Table 4 F_{st} distance (above diagonal) and linear geographical distance (Km; below diagonal) between non-captive northern leopard frog populations in Canada.* represents a reintroduction site, and ^{H} represents the historical population of Fort Steele. Acronyms for the population are given in Table 1

	CV	MA*	DK	SS	PS	CH	EM	VM	YQP	FS^H
CV	-	0.625	0.560	0.573	0.523	0.500	0.579	0.585	0.507	0.698
MA*	272.5	-	0.275	0.339	0.255	0.201	0.224	0.275	0.213	0.514
DK	339.4	109.1	-	0.286	0.206	0.221	0.155	0.290	0.175	0.523
SS	1237.3	1181.8	1082.0	-	0.309	0.241	0.272	0.308	0.199	0.525
PS	483.3	239.4	144.6	1027.3	-	0.231	0.188	0.236	0.167	0.494
CH	469.6	196.9	160.2	1155.0	127.4	-	0.172	0.128	0.101	0.383
EM	510.8	267.7	172.5	1013.8	28.5	143.6	-	0.187	0.101	0.489
VM	649.4	375.9	344.6	1228.6	258.3	186.2	253.1	-	0.097	0.415
YQP	1253.8	980.5	929.2	1425.1	801.8	784.4	780.4	607.8	-	0.347
FS^H	88.3	200.2	259.6	1182.8	400.1	392.6	427.4	575.4	1176.6	-

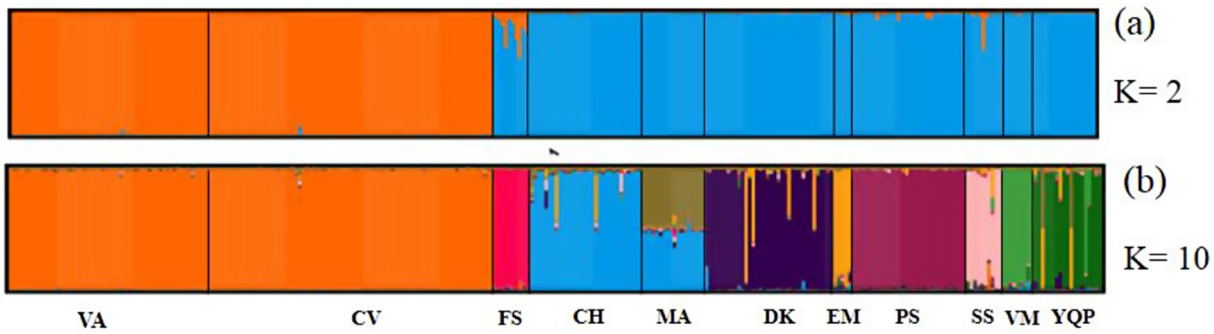

Fig. 4 Admixture plots for northern leopard frog populations (VA, CV, FS, CH, MA, DK, EM, PS, SS, VM, and YQP) in Canada showing individual membership to K clusters for K values of 2 (**a**) and 10 (**b**) inferred by STRUCTURE. Unique clusters are represented by color. Each vertical line represents an individual. Codes for population identifications are given in Table 1

analysis, FS still clustered with the Western Boreal/Prairie populations (Supplementary material, Fig. S2, Fig. S3, Fig. S5). Furthermore, while most of the populations became distinct for K = 10, CH and MA still showed similarity.

Change in population genetic metrics over time

Genetic diversity of the extinct FS population in British Columbia was higher than the current CV population as measured by the rarefied allelic richness, rarefied private allelic richness, H_o, and H_e (Table 1). However, the standard errors of these indicators of genetic diversity overlapped, as did the inbreeding coefficient of both populations (Table 1). In general, the extinct Rocky Mountain population of FS was highly differentiated from current northern leopard frog populations, including the CV population, and was more genetically similar to populations of the species east of the Rocky Mountains as compared to the current CV population (Table 3).

To determine if there had been changes in the genetic diversity of populations over time, the current genetic data for CH, PS, DK, and CV were compared to data from 2004 (Wilson et al. 2008; Supplementary material, Table S 2). Although the sample size was smaller in 2004 than in 2019, there was not a considerable change in rarefied allelic richness, rarefied private allelic richness, H_o and H_e (Supplementary material, Table S 2). Rarefied allelic richness and expected heterozygosity of the CV population decreased by 26% and 44% respectively, between 2000 and 2019 (Hoffman and Blouin 2004b; Fig. 5). The 2000 rarefied allelic richness (± SE) of the population was 2.32 ± 0.28 and the expected heterozygosity (± SE) was 0.43 ± 0.08 (Hoffman and Blouin 2004b) while the 2019 rarefied allelic richness (± SE) was 1.76 ± 0.22 and expected heterozygosity (± SE) was 0.23 ± 0.07 (Table 1). However, the temporal changes in genetic diversity for the CV population were not significant (allelic richness: $p = 0.708$ between 2000 and 2019, $p = 0.431$ between 2004 and 2019, $p = 0.401$ between 2000 and 2004; expected heterozygosity: $p = 0.349$ between 2000 and 2019, $p = 0.346$ between 2004 and 2019, $p = 0.799$, between 2000 and 2004).

Discussion

Current population genetic structure northern leopard frogs in western Canada

Northern leopard frog populations in Canada have high genetic differentiation and their genetic diversity declines from east to west. The CV population (the most western sampled population and the only extant population belonging to the Rocky Mountain DU) had the lowest genetic diversity, which might be due to historical bottlenecks or founder effects associated with either human activities or natural events such as glacial and post-glacial range expansion (Wilson et al. 2008).

Effective population size provides an estimate of genetic drift and inbreeding of a population (Frankham 2005; Allendorf 2009; Sládkovičová et al. 2022). Small effective population size is an indication of inbreeding and strong genetic drift, it may be an indication of increased extinction risk (Newman and Pilson 1997). To minimize inbreeding depression in wild populations to 10% over 5 generations, an effective population size equal to or larger than 100 is required (Frankham et al. 2014). Furthermore, to maintain evolutionary potential, the effective population size needs to be equal or larger than 1000 (Frankham et al. 2014), which was greater than any estimates observed in this study. Caution should be taken when interpreting some of the effective population size estimates in this study (e.g., EM, SS, VM and YQP) because small sample size can bias estimates of true effective population size by not yielding properly weighted allele frequencies (Waples and Yokota 2007). In the case of the CV and VA populations, sample sizes were large enough that this should not have been an issue. It is possible that

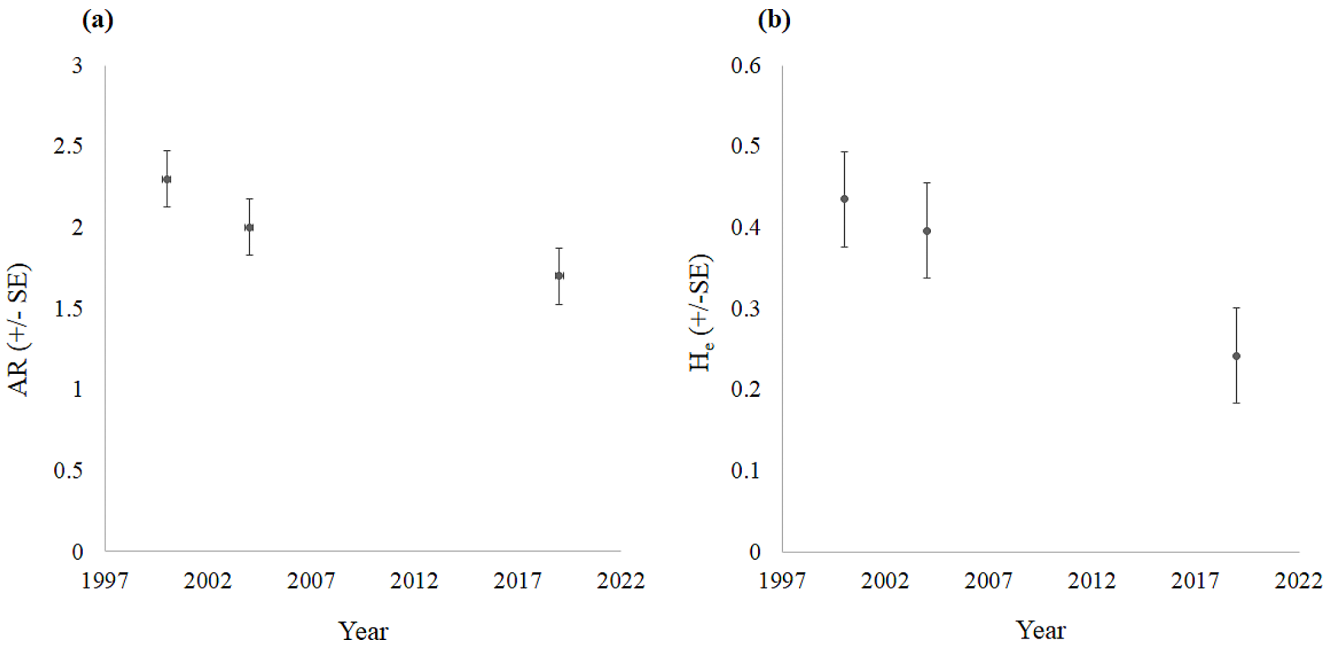

Fig. 5 Change in genetic diversity of the of the Creston Valley population at three time points in 2000 (Hoffman and Blouizn, 2004 b), 2004 (Wilson et al. 2008) and 2019. (**a**) shows change in rarefaction allelic richness (AR) and (**b**) illustrates change in expected heterozygosity (H_e) at three time points

the low genetic diversity and effective population size of the CV population resulted from a population bottleneck (Frankham et al. 2010; Frankham 2019). The small effective population size of CV and VA populations may also be due to founder effects (Arnaud-Haond et al. 2006; Waples and Yokota 2007; Ogden et al. 2020). The effective population size was smallest in the CV, the westernmost population analyzed in this study, which is also consistent with research that found that the effective population of northern leopard frogs declined from east to west (Phillipsen et al. 2011).

The genetic distance observed between northern leopard frog populations varied from moderate to very greatly differentiated but there was no relationship between genetic and geographic distance. Such differentiation has also been observed in previous genetic research conducted on the species (Kimberling et al. 1996; Wilson et al. 2008), potentially reflecting a long period since these populations have diverged. One possible reason for this genetic differentiation could be isolation due to the large geographical distance between populations and presence of physical barriers, such as the Rocky Mountains between the Rocky Mountain DU and Western Boreal/Prairie DUs, as well as discontinuous habitats and the shift between the main eco-regions (Wilson et al. 2008). In most cases, the linear distance between populations was greater than 150 km, with only 6 pairs of populations geographically closer than that. The lack of a relationship between genetic and geographic distance was consistent with the results of Wilson et al. (2008), suggesting that genetic drift resulted in greater divergence among populations than isolation caused by distance. Moreover, landscape features such as water bodies can cause genetic divergence (Silva-Arias et al. 2021). Future study to model species' dispersal across the landscape might benefit its conservation.

The CV and VA populations appeared as one genetic cluster, which is unsurprising since the former serves as the source population for the latter (Randall et al. 2021). Additionally, the CV population showed the highest genetic distance from other extant populations of the species. Interestingly, the CV population even had a large genetic distance from the historical FS population in British Columbia. This was contrary to the results of a mitochondrial DNA study by Hoffman and Blouin (2004 b), which showed that these two populations share the same haplotype and are clustered together. Moreover, the extinct historical population clustered with the Western Boreal/Prairie populations in Alberta, Saskatchewan, and Manitoba, although there is some evidence of genetic admixture with the CV population. Although this was contrary to our expectations, Lee-Yaw (2013) found a distinct genetic lineage of long-toed salamanders (*Ambystoma macrodactylum*), another pond-breeding amphibian in the region, that spanned both sides of the Rocky Mountains indicating that there could be unexpected connectivity among these populations. The current admixture analysis thus refines the results from Wilson et al. (2008) which only distinguished populations from British Columbia and Alberta. When more complicated genetic structures were considered, there was considerable

similarity between the CH and MA populations which is not surprising given that two of the source populations for the MA reintroduction were from Medicine Hat which is only about 50 km from the CH (Romanchuk and Quinlan 2006).

Changes in population genetic structure over time

Our results show little difference in genetic diversity, inbreeding, and effective population size between the historical population of FS and the CV population but they were genetically differentiated from one another. However, private alleles present in the former suggest the loss of genetic diversity in CV was due to a population bottleneck (Sonsthagen et al. 2017). Although the sample size is small, the low genetic diversity of the extinct population of FS shows that the historical populations of the Rocky Mountains, inhabiting the northern limits of the species range, had low genetic diversity, consistent with Hoffman and Blouin (2004 b). One possible reason for the extinction of FS population and other populations within the Rocky Mountain DU, might be due to loss or reduced diversity within the major histocompatibility complex (MHC) genes. Major histocompatibility complex is a genomic region responsible for vertebrates' adaptive immune responses to pathogens. Therefore, in amphibians this genomic region is important for immunity against diseases such as chytridiomycosis caused by *Batrachochytrium dendrobatidis* (Bd). Major histocompatibility complex heterozygosity can help explain spatial patterns of Bd prevalence, however intensity of Bd is related to environmental variables (Trujillo et al. 2021).

Genetic monitoring of the CH, DK, PS, and CV populations between 2004 (Wilson et al. 2008) and 2019 showed little change in their population genetic parameters. However, the comparison of the CV allelic richness and expected heterozygosity between 2000 (Hoffman and Blouin 2004 a), 2004 (Wilson et al. 2008) and 2019 showed that the population's genetic diversity has declined over 19 years, suggesting that it may not be possible to detect changes in genetic diversity over shorter time periods. Hoffman and Blouin (2004 b) concluded that genetic diversity was always low in these peripheral populations and therefore artificial gene flow was unnecessary; however, the results of this study reveal that genetic diversity has since declined, so perhaps this idea should be revisited. Government and conservation managers may need to revise recovery plans to improve the genetic health of these populations, particularly the CV population which has a high probability of extinction.

Considering CV population's status, genetic rescue might be a possible tool to restore the population's genetic diversity. However, the population represents a unique evolutionary lineage of northern leopard frogs and genetic rescue could result in the loss of this uniqueness by genetic swamping. Therefore, additional studies and caution should be taken in rescue programs to prevent this (Frankham et al. 2010). Since the captive VA population contains similar genetic diversity with the founded CV population, examining the captive reared individuals' fitness parameters (e.g., egg survival, and hatching rate) might help in assessing benefits and risks of potential genetic rescue.

Reintroduction from captive aquarium or zoo populations is an important type of wildlife restoration, with the main purpose of establishing self-sustaining populations in habitats within the historic indigenous range of rare (Keulartz 2015; Gilbert et al. 2017), at-risk species, but its effectiveness may be limited by inbreeding depression, genetic drift, and adaptation to captivity (Gilligan and Frankham 2003; Griffiths and Pavajeau 2008). In Alberta and British Columbia, Canada, managers have undertaken several northern leopard frog reintroduction efforts with variable success (e.g., Randall et al. 2021). Reintroduction efforts in British Columbia have met with some initial success but have not managed to produce long-term self-sustaining populations (Randall et al. 2021). The individuals used for reintroductions in British Columbia have been sourced either directly from the CV population or from captive bred frogs descended from CV frogs (e.g., Vancouver Aquarium, Calgary Zoo, or Edmonton Valley Zoo) and it is possible that low genetic diversity in these populations may be hampering recovery efforts (Wilson et al. 2008; Environment and Climate Change Canada 2017; Randall et al. 2021), although genetic monitoring has not examined this issue. Continued genetic monitoring of the northern leopard frog populations in western Canada may be helpful in tracking changes in the populations' genetic structure over time and will allow for a better evaluation of conservation actions (Schwartz et al. 2007). While microsatellites target a small number of neutral positions in the genome limiting the ability to estimate genome wide parameters, with advances in whole genome sequencing technology it is possible to sequence thousands to millions of loci in short amounts of time and at a low cost resulting in a higher number of markers than was possible in the past. As a result of this advancement, we can improve inference, reduce biases, and estimate important population genetics parameters such as genetic variation more accurately (Fuentes-Pardo and Ruzzante 2017). Furthermore, a comprehensive genetic study using modern genomic techniques across the eastern and western clades, particularly in the province of Manitoba, as well as the western United States will benefit conservation managers to better understand the recent genetic changes of the species' population and its designatable units, as well as inform the poorly understood boundary between the eastern and western clades in Canada.

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Author contributions P.KH, L.R, D.C, and D.L conceptualized the study. P.KH and L.R collected data. P. KH analyzed the data and led the writing of the manuscript. All authors contributed to editing and reviewing the final manuscript.

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Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval All research was conducted with approval from the Laurentian University Animal Ethics Committee (2017-04-04).

Competing interests The authors declare no competing interests.

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