Genetic Assessment of Potential Source Populations for the Reintroduction of Northern Leopard Frogs (*Rana pipiens*) to Sites in Alberta
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Genetic Assessment of Potential Source Populations for the Reintroduction of Northern Leopard Frogs (*Rana pipiens*) to Sites in Alberta

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Population persistence is often dependent upon immigration. As habitat loss and fragmentation increase, movement corridors are lost and movement of individuals between populations is reduced or eliminated. Populations may then be extirpated without the possibility of re-establishment through natural means. In such cases, human-mediated translocation may be required to reintroduce a species to parts of its historical range where appropriate, unoccupied habitat exists. Despite the fact that the source population can affect reintroduction success, genetic background is rarely considered when source populations are chosen. The northern leopard frog (*Rana pipiens* Schreber, 1782) underwent a large decline in the western portion of its range during the 1960s and 1970s, and only occurs in 20% of historically occupied sites in Alberta. Many unoccupied sites still appear to offer good habitat, and the absence of northern leopard frogs may reflect their inability to disperse to these locations from surviving populations. Consequently, human-mediated translocation has been proposed. In this study we used three criteria to examine the genetic background of potential sources for translocation: diversity, similarity to the area of reintroduction, and evolutionary history. We genotyped 187 samples and sequenced 812bp of the mitochondrial NADH dehydrogenase 1 gene from 14 Canadian northern leopard frog populations. Nuclear and mitochondrial diversity were highest in frogs from Manitoba and western Ontario, and declined westward. There was no significant relationship between genetic and geographic distance, suggesting genetic drift is a driving force affecting the genetic relationships between populations, with the possibility of local adaptation. Populations separated by more than ~50 km were quite differentiated. Therefore, source populations similar to the original inhabitants of an area scheduled for reintroduction may be difficult to find or nonexistent. Mitochondrial analyses revealed all populations sampled in this study share a recent evolutionary history, belonging to the western clade of this species.

Consequently, we can make the following recommendations for source populations in reintroduction efforts: i) source populations genetically similar to the original inhabitants of a region are ideal. However, these are unlikely to be found outside of fine spatial scales (~50 km), ii) watershed-based measures of distance are not better predictors of genetic distance than linear distances, suggesting that populations within
a watershed are not better sources than those in different watersheds, iii) populations within Alberta all have similar levels of genetic diversity and differentiation, so ecological exchangeability may be the most important factor when choosing source populations, iv) choosing eggs from multiple masses will increase the initial genetic diversity in a newly founded population, v) due to their high levels of diversity, populations in Manitoba and Ontario may be good sources if ecologically exchangeable populations can be found, vi) genetic monitoring of recently founded populations will allow their genetic health to be examined through time.

**Key words:** Northern leopard frog, reintroduction, genetic diversity, genetic differentiation, DNA microsatellites, mitochondrial DNA, source population.
ACKNOWLEDGEMENTS

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# TABLE OF CONTENTS

EXECUTIVE SUMMARY.......................................................................................................... ii

ACKNOWLEDGEMENTS........................................................................................................ iv

TABLE OF CONTENTS........................................................................................................ v

LIST OF FIGURES............................................................................................................... vi

LIST OF TABLES................................................................................................................. vii

1.0 INTRODUCTION .......................................................................................................... 1
  1.1 General introduction ............................................................................................... 1
  1.2 Study objectives ...................................................................................................... 3

2.0 STUDY AREA............................................................................................................. 3

3.0 MATERIALS AND METHODS .............................................................................. 5
  3.1 General sampling methods .................................................................................. 5
  3.2 Laboratory methods ............................................................................................. 6
  3.3 Statistical analysis ............................................................................................... 7

4.0 RESULTS .................................................................................................................... 9

5.0 DISCUSSION ............................................................................................................. 14
  5.1 Genetic diversity in western Canadian northern leopard frogs ....................... 14
  5.2 Genetic differentiation ......................................................................................... 15
  5.3 Conservation implications .................................................................................. 18
  5.4 Management recommendations ........................................................................ 21

6.0 LITERATURE CITED ............................................................................................. 24
LIST OF FIGURES

Figure 1. Locations of northern leopard frog populations used in this study. ............... 4
Figure 2. Linear regression between genetic diversity and longitude. ...................... 10
Figure 3. Plot of genetic and geographic distances between all frog populations. ..... 13
LIST OF TABLES

Table 1. Genetic diversity observed in northern leopard frog populations................. 5
Table 2. $F_{ST}$ distances between northern leopard frog populations, their significance after sequential Bonferroni adjustment, and linear geographic distance separating populations ...................................................... 12
Table 3. Population groups estimated with Bayesian clustering software. ............... 13
1.0 INTRODUCTION

1.1 General introduction

Habitat loss and fragmentation are major threats to an increasing number of species throughout the world. Fragmentation can result in the isolation and extinction of populations by eliminating or reducing movement corridors and the possibility for a “rescue effect” through immigration (Gonzalez et al. 1998). This can result in vacant areas within a species’ historical range. In species with limited vagility like many amphibians (Blaustein et al. 1994), translocations or reintroductions may be required to repopulate areas where extirpation has occurred. Reintroductions are increasingly used as conservation tools (Griffith et al. 1989; Wolf et al. 1996; Fischer and Lindenmayer 2000), and their success depends on a number of factors, both pre- and post-release. Reintroduction success can be affected by various attributes of the source population, such as its demography, ecological similarity, and genetic characteristics. The source of individuals for translocation efforts, however, is seldom rigorously evaluated.

The genetic characteristics of a potential source population and its relationship to the population originally inhabiting a region are factors that should be considered when identifying appropriate source populations. One factor in determining ideal source populations should be genetic diversity, which can affect population viability. Populations with low levels of genetic diversity are more likely to suffer from inbreeding effects (Packer et al. 1991; Roelke et al. 1993; Fitzsimmons et al. 1995; Halverson et al. 2006), and may be less able to adapt to selection pressures in their new environment (Lacy 1997; Saccheri et al. 1998; Reed and Frankham 2003). Consequently, using genetically diverse populations should, in general, increase the likelihood of a successful reintroduction. Another consideration in the choice of source population is the level of genetic differentiation between the source and populations inhabiting the area of reintroduction. Populations may be genetically similar because they have adapted to comparable environments. Therefore, genetically similar populations may be better able to survive in the area of reintroduction (Waples 1991; Hedrick et al. 2000). The introduction of founders quite distinct genetically from surrounding populations may also result in genetic swamping, where local genotypes can be replaced by
introduced genotypes with a numerical advantage, even if they are less fit (Lenormand 2002). Finally, the evolutionary history of a source population and an area’s original inhabitants should be considered. The hybridization of unique lineages due to translocation may result in the dilution or loss of any unique genetic or phenotypic characteristics that may have evolved in this location (Dobzhansky 1970; Rhymer and Simberloff 1996; Latch et al. 2006).

The northern leopard frog (Rana pipiens Schreber, 1782) is one of the most widespread anurans in North America. However, in the 1960s and 1970s it experienced a rapid and dramatic decline in population size throughout the western two-thirds of its range, and has been extirpated from a number of locations (Hine et al. 1981; Roberts 1981; Corn and Fogleman 1984; Wagner 1997; Seburn and Seburn 1998; Leonard et al. 1999; Werner 2003; Kendell 2004). As in many anuran species, the primary cause of this decline is unclear (Stuart et al. 2004). Some contributing factors likely include habitat loss and fragmentation (Lannoo et al. 1994), disease outbreaks such as chytridiomycosis and ranavirus (Daszak et al. 1999), drought (Merrell 1977; Corn and Fogleman 1984), the introduction of predatory non-native fish species (Bradford 1989; Vredenburg 2004), and environmental contamination (James et al. 2004; La Marca et al. 2005; Metts et al. 2005). This decline continues in some areas, and few populations have recovered. In Alberta, where the northern leopard frog is considered Threatened under Alberta’s Wildlife Act, as few as 20% of historical sites are occupied (Kendell 2002; Kendell et al. 2007). As a consequence, many of the surviving northern leopard frog populations are isolated, which increases their vulnerability to further declines and extirpations.

Many uninhabited sites still appear to offer good quality habitat for northern leopard frogs. However, natural recolonization has likely been limited by poor dispersal ability exacerbated by population fragmentation. As prospects for natural recolonization appear to be minimal, the repopulation of these sites depends on human-mediated translocations (Roberts 1987). Successful translocations of northern leopard frogs have previously been performed in some areas (Fisher 1999), including Alberta (Romanchuk and Quinlan 2006). Translocations have also proven to be effective tools for the conservation of numerous amphibian species (Conant 1988; Denton et al. 1997; Fisher 1999; Sarrazin and Legendre 2000). The successful reintroduction of northern leopard
frogs to areas of their range in Alberta would decrease the fragmentation and isolation of surviving populations, thereby reducing their risk of extirpation.

1.2 Study objectives

Our study examined the genetic diversity within and relationships among northern leopard frog populations across western Canada to identify possible sources for reintroduction efforts. We used information from microsatellite and mitochondrial DNA to evaluate the relative merits of potential source populations with respect to their genetic diversity, relationship to frogs near potential reintroduction sites, and the evolutionary history of the species.

2.0 STUDY AREA

Samples were collected from throughout the Canadian range of the northern leopard frog from northern Ontario westward (Figure 1). Efforts were made to collect samples from at least one population from each major drainage system within the species’ range in Alberta to examine differentiation between drainages (Table 1) as rivers may have served as routes for post-Pleistocene colonization. The Leland Lake population is separated from the rest of the range in Alberta by approximately 700 km, and historical evidence suggests this is not a recent phenomenon (Kendell 2002). Northern leopard frogs have declined in numbers in Manitoba, western Ontario, and Saskatchewan, although little is known about current and historical numbers in the latter province (Seburn and Seburn 1998). Our goal was to obtain samples from at least one population in each of these provinces. Northern leopard frogs are quite rare in British Columbia, and only exist in the Creston region (Seburn and Seburn 1998), from which we obtained samples.
Figure 1. Locations of northern leopard frog populations used in this study. Codes for population identification are given in Table 1.
Table 1. Genetic diversity observed in northern leopard frog populations, including population identification (ID), sample size for genotypes ($N_G$) and haplotypes ($N_H$), mean allelic richness (AR) and expected heterozygosity ($H_e$) for microsatellite loci, and number of different mitochondrial haplotypes observed ($H$). Drainage abbreviations: BR = Bow River, SSR = South Saskatchewan River, MR = Milk River, SR = Slave River, OR = Oldman River, RDR = Red Deer River.

<table>
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<tr>
<th>Population</th>
<th>ID</th>
<th>Drainage</th>
<th>$N_G$</th>
<th>$N_H$</th>
<th>AR</th>
<th>$H_e$</th>
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</table>

### 3.0 MATERIALS AND METHODS

#### 3.1 General sampling methods

A total of 210 northern leopard frog tissue samples were collected, 156 as toe clips from metamorphosed individuals and 44 as tailclips from tadpoles, which constituted the samples from British Columbia and Belza Pond, Saskatchewan. Metamorphosed frogs were targeted under the assumption that siblings would be less likely to be sampled than if tadpoles were taken, where sampled individuals could be from a recently
hatched egg mass. DNA was extracted from all tissue samples with a DNeasy Tissue Kit (Qiagen).

3.2 Laboratory methods

Eight microsatellite loci; Rpi100, Rpi101, Rpi103, Rpi104, Rpi107, Rpi108 (Hoffman et al. 2003), Rp193, and Rp415 (Hoffman and Blouin 2004a), were individually amplified. Each 15 μL PCR consisted of 0.27 μM each primer (one of which was dye-labeled), 120 μM dNTPs, 1.5 U Taq polymerase, 1 × PCR buffer (50 mM KCl, 0.1% Triton X-100, 0.16 mg/mL bovine serum albumin, 10 mM Tris buffer, pH 8.8) and ~40 ng genomic DNA. Each reaction contained 2.5 mM MgCl₂, except for the Rpi101 and Rpi193 amplifications, which contained 2.0 mM MgCl₂. Our amplification protocol consisted of an initial denaturing step of 94°C for 1 min, 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. PCRs were performed on an Eppendorf Mastercycler and resolved on an ABI PRISM 3100-Avant Genetic Analyzer.

Most individuals (160; Table 1) were sequenced for an 812bp fragment of mitochondrial DNA, corresponding to bases 27 - 838 of the NADH dehydrogenase subunit 1 (ND1) gene, with newly designed primers RpND1F (5’ GGT TCA AAT CCC CTT ACT A 3’) and RpND1R (5’ AGT TGG TCA TAG CGG AAT CGT G 3’). Each 25 μL reaction contained 0.4 μM each primer, 160 μM dNTPs, 1 U Taq polymerase, 1× PCR buffer, 2.5 mM MgCl₂, and ~50 ng genomic DNA. Our amplification for this fragment consisted of an initial denaturing step at 95°C for 5 min, followed by 35 cycles of denaturing at 94°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 90 s, followed by a final 5 min extension at 72°C. PCR products were purified with the QIAquick PCR purification kit (Qiagen) and directly sequenced using the forward primer and the BigDye 3.1.1 (Applied Biosystems) chemistry. Removal of unincorporated dye terminators was performed with the DyeEx 96 kit (Qiagen). Fragment resolution was performed on an Applied Biosystems 3730 DNA Analyzer, and the resultant sequences were analyzed with ABI PRISM SeqScape 2.1 software. In a previous study of northern leopard frogs (Hoffman and Blouin 2004b), two haplotypes were defined by a single variable site near the priming region. Therefore, individuals determined to have one of
these two haplotypes were also sequenced using a reverse primer RpND1R.int (5’ TTG AGG ATA CCG AGG CAG AGC 3’) for sequence confirmation near the 5’ end.

3.3 Statistical analysis

Individuals were considered to be from a single population if they were collected from within ~1 km of each other in the same wetland complex. In some instances, populations were defined as individuals occurring within the same drainage if these post hoc populations were in Hardy-Weinberg equilibrium (data not shown). This grouping was justified by the relatively large dispersal distances observed in northern leopard frogs relative to other anurans (with upper dispersal distances of at least 5 - 8 km, Dole 1971; Seburn et al. 1997). Groups that consisted of fewer than eight individuals were not included in microsatellite analyses. The 31 tadpole samples from Belza Pond, Saskatchewan (SK), consisted of full- and half-sib groups (Wilson et al. 2008). Consequently, a single individual was chosen at random from each full-sib group, resulting in a sample size of eight. We therefore analyzed microsatellite data for 187 northern leopard frogs from 14 populations (Figure 1; Table 1).

All loci were examined for deviations from Hardy-Weinberg and linkage equilibria using GENEPOP 3.4, which implements a Markov chain method (Raymond and Rousset 1995). One thousand batches were run with 10,000 iterations per batch. Allelic richness standardized for population size using rarefaction (AR) and expected heterozygosity ($H_e$) were used as estimators of genetic diversity for each population. Allelic richness was rarified to a population size of seven and calculated with the program CONTRIB (Petit et al. 1998). Expected heterozygosity was calculated with GENALEX 6.0 (Peakall and Smouse 2006).

Genetic differentiation between populations was examined using a traditional genetic distance measure ($F_{ST}$) calculated with FSTAT 2.9.3 (Goudet 1995) using the nested ANOVA method (Weir and Cockerham 1984). In order to determine if populations showed isolation by distance, genetic distance as represented by $F_{ST} / (1 - F_{ST})$ was linearly regressed against linear geographic distance, calculated with ESRI ArcMap 9.1, using S-PLUS 8.0 (Insightful). A Mantel test was performed to determine the relationship between genetic and geographic distance with IBDWS (Jensen et al. 2005),
where significance was determined by randomizing rows and columns in the geographic distance matrix. As northern leopard frogs prefer riparian areas, particularly in dry prairie landscapes, linear distance between sites may not be the ideal predictor of genetic distance. Therefore, distances along riparian corridors between the seven sampled populations in southern Alberta were also calculated with ESRI ArcMap 9.1 and linearly regressed against genetic distance.

Population differentiation was explored with STRUCTURE 2.1 (Pritchard et al. 2000), BAPS 4.14 (Corander and Marttinen 2006), and Geneland 1.0.8 (Guillot et al. 2005). STRUCTURE implements a Bayesian clustering method to estimate the number of genetic units represented in a sample of individuals. An iterative approach was taken in selecting the best STRUCTURE run. Three independent STRUCTURE replicates were performed for \( K = 1 \) to 21. Each chain was run for 1,000,000 iterations, with a burn-in of 100,000. No prior information about the potential source populations was implemented and allele frequencies were assumed to be correlated. We then calculated \( \Delta K \) (Evanno et al. 2005) to detect the highest level of population structure. \( \Delta K \) was highest when the number of populations was eight. However, this grouping made little biological sense, and the \( \Delta K \) for \( K = 8 \) was inflated by low standard deviations between runs at this value, so we chose \( K = 2 \), which had the second highest \( \Delta K \). At this level of structure, populations from Saskatchewan, Manitoba and Ontario (SK, HI, PB, LF, ON) formed one group, while Alberta and British Columbia (BR, MH, OC, SC, LL, TC, FF, PS, BC) formed the second group. Both of these groups were analyzed further for population structure using the settings listed above, except replicates were performed for \( K = 1 \) to 10 for the first group and \( K = 1 \) to 16 for the second group.

Geneland is another technique for examining spatial structure. It is similar to STRUCTURE, but can take into account individual geographic locations. Analyses were conducted from \( K = 1 \) to 17 with 100,000 iterations and a thinning interval of 100 using the Dirichlet spatial model. BAPS implements a Bayesian stochastic partitioning approach to genetic mixture modeling, and can incorporate spatial information. As most individuals representing a population were sampled at the same location, genetic mixture modeling was performed at the population level using BAPS. The population was then considered the sampling unit for this analysis, as opposed to the methods implemented by Geneland and STRUCTURE, which consider the individual the
sampling unit. As such, for this analysis, BAPS provides posterior probabilities of population (not individual) clusters occurring. Analyses incorporating and not incorporating spatial information were performed separately.

To assess evolutionary history of our sampled populations, observed mitochondrial haplotypes were analyzed with those described in Hoffman and Blouin (2004b), which were retrieved from GenBank. Our sequences were longer than those obtained by Hoffman and Blouin (2004b); thus, sequences were trimmed to be fully overlapping, giving a final matrix of 603 characters. Maximum parsimony (MP) tree searching was performed with the program PAUP* 4.0.b10 (Swofford 2003) using 10 random sequence addition replicates. One thousand bootstrap pseudoreplicates were also performed, with each random addition replicate limited to saving a maximum of 500 trees. Two closely related Rana species from Hoffman and Blouin (2004b) were used as outgroups (AY157644, AY157645).

4.0 RESULTS

Six of 104 locus-population comparisons showed heterozygote deficiency (P < 0.05). After sequential Bonferroni adjustment for multiple tests (Rice 1989), only the PB population showed a significant deficiency of heterozygotes at locus Rpi193 (P < 0.00048). Thirteen of 342 locus-locus comparisons showed evidence for linkage disequilibrium (P < 0.05). However, none of these were significant after Bonferroni adjustment (P < 0.00015).

Levels of genetic diversity tended to be higher in the eastern populations of Manitoba and Ontario, with a decline in diversity in the western populations with all measures (Table 1). Linear regressions between longitude and all three measures of diversity were significant (P < 0.05; Figure 2). Highest nuclear diversity was seen in the ON population (AR = 5.23, He = 0.739), and the lowest in BC (AR = 2.36, He = 0.396). The LF population contained the most mitochondrial haplotypes, with four observed in a sample of 10 individuals. All populations in Alberta were fixed for a single haplotype that was also observed in Saskatchewan and Manitoba. The haplotype fixed in British Columbia was not observed in any other population.
Global $F_{ST}$ for this study was 0.248. The 95% CI for global $F_{ST}$ bootstrapped over loci was (0.212, 0.288). Pair-wise $F_{ST}$ values ranged from 0.063 (between ON and HI) to 0.417 (TC - BC; Table 2). Most $F_{ST}$ values (85 of 91 comparisons) were significant after Bonferroni correction ($P < 0.05$). Non-significance was likely the result of small sample size. For example, four of the six non-significant $F_{ST}$ values involved SK, which had a sample size of eight. There were moderate to very great levels of differentiation between all population pairs (where $F_{ST}$ values of 0.05 - 0.15 indicate moderate differentiation, 0.15 - 0.25 indicate great differentiation, and $F_{ST}$ values above 0.25 reveal very great differentiation (Wright 1978)). BC was very greatly differentiated from all other populations. Populations within Saskatchewan, Manitoba, and Ontario were all moderately differentiated from one another. Except for SC - PS ($F_{ST} = 0.144$), Alberta populations were greatly to very greatly differentiated from one another and all other populations. Geographic distance explained very little variation in genetic

Figure 2. Linear regression between genetic diversity and longitude. Diversity is measured by (A) allelic richness: $y = 19.65 - 0.15x$, $r^2 = 0.91$, (B) expected heterozygosity: $y = 2.25 - 0.016x$, $r^2 = 0.91$, and (C) haplotype number: $y = 14.14 - 0.12x$, $r^2 = 0.70$. 
differentiation, and the regression between $F_{st}/(1 - F_{st})$ and linear geographic distance was not significant ($P > 0.05$; Figure 3). The regression between distance along riparian corridors in southern Alberta and associated $F_{st}/(1 - F_{st})$ values was also not significant ($P > 0.05$; data not shown).

Measures of population differentiation performed with STRUCTURE, Geneland, and BAPS suggested that the northern leopard frogs sampled in this study were highly structured (Table 3). However, there were some differences in how the populations were clustered among these methods. As described in Section 3.3, an iterative approach was used for the STRUCTURE analysis, resulting in two initial groups. In the eastern STRUCTURE group, $\Delta K$ was highest when $K = 3$. All populations in the east assigned to a single group except for HI, which was split into all three eastern groups. One of these groups contained both populations LF and SK. The western STRUCTURE group had the highest $\Delta K$ for $K = 5$. In the west, BR, LL, and TC grouped together, as did SC and PS, and MH and OC. Population groups generated with Geneland, which incorporates spatial information, were similar. The major differences were that HI assigned to a single group (with PB), and SC and PS formed their own groups. The population-level analysis performed with BAPS, both with and without spatial information, assigned a single population to most groups. The exceptions were a group consisting of PB and HI, and one of MH and OC. These two groups contained the populations that were geographically closest, separated by 45.6 and 29.5 km, respectively.

A total of seven different mitochondrial haplotypes were observed, four of which had not been previously described. LF had the highest number of haplotypes, with four (Table 1). All populations in Alberta and British Columbia were fixed for a single haplotype, although the haplotype in British Columbia differed from that seen in Alberta. Fifteen equally most parsimonious trees were obtained (MP score = 191), all of which recovered the eastern and western haplotypes as distinct clades. The western haplotype group was supported by 78% MP bootstrap support and the eastern haplotype group by 99% MP bootstrap support. All newly obtained haplotypes were recovered as part of the western haplotype group described by Hoffman and Blouin (2004b; data not shown).
Table 2. $F_{st}$ distances between northern leopard frog populations (above diagonal), their significance after sequential Bonferroni adjustment, and linear geographic distance (km) separating populations (below the diagonal). $P$ values are not significant (NS), or $0.01 < P < 0.05$ (all others). Codes for population identifications are given in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>ON</th>
<th>LF</th>
<th>PB</th>
<th>HI</th>
<th>SK</th>
<th>BR</th>
<th>MH</th>
<th>OC</th>
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Figure 3. Plot of genetic \( (F_{ST} / (1 - F_{ST})) \) and geographic (linear distance in km) distances between all frog populations.

Table 3. Population groups estimated with Bayesian clustering software. Populations are only listed as members of clusters if their proportion of membership in that cluster exceeded 20%. Subscripted numbers refer to the proportion of each population assigned to that cluster. As the BAPS analysis was performed at the population level, this value is not applicable to this scenario. Codes for population identifications are given in Table 1.

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5.0 DISCUSSION

5.1 Genetic diversity in western Canadian northern leopard frogs

Genetic diversity in many anuran species tends to be low, in part owing to characteristics of pond breeding anurans that can reduce genetic diversity. These include large variance in reproductive success (Merrell 1968; Scribner et al. 1997; Wilson et al. 2008), highly skewed operational sex ratios (Vieites et al. 2004; Lode et al. 2005), natural fluctuations in population size (Berven 1990; Seppa and Laurila 1999), and low vagility (Blaustein et al. 1994). The range of genetic diversity seen in our study is similar to (Beebee and Rowe 2000; Newman and Squire 2001; Monsen and Blouin 2003; Funk et al. 2005) or higher than (Seppa and Laurila 1999; Rowe and Beebee 2007) that observed in other anurans, although a direct comparison of microsatellite diversity levels between studies is difficult due to ascertainment bias, as the microsatellite loci used in each study are chosen in a non-random fashion (usually to favour diverse loci). Mean heterozygosities between our study and a study of northern leopard frogs in the Pacific Northwest (Hoffman and Blouin 2004a), where the same loci were used, were not significantly different (Mann-Whitney U, P > 0.05).

The most striking result concerning genetic diversity in our study was the decline in diversity from Manitoba westward observed with both microsatellite and mitochondrial DNA (Figure 2). There are two possible explanations for this pattern: 1) western populations were as variable as those in the east, but lost genetic diversity due to the population bottleneck that occurred in this region in the 1960s and 1970s, or 2) leopard frogs in western Canada originated in the east after the last glaciation, and lost diversity as they moved westward due to a series of founder events. Species undergoing rapid range expansions are expected to exhibit a loss of genetic diversity (and an increase in population structure) due to serial founding events, especially after long-range dispersal (Hewitt 1996; Ibrahim et al. 1996; Hewitt 1999). In a previous study of northern leopard frogs, Hoffman and Blouin (2004a) showed that populations in the western United States had similar levels of genetic diversity before and after the population bottleneck of the 1960s and 1970s. They attributed the low level of genetic diversity in this region to the founding events that created these populations. Relatively low levels of genetic diversity at range edges have also been observed in other anurans (Beebee and Rowe 2000; Newman and Squire 2001). Therefore, a loss of
diversity via a series of founding events is a plausible explanation for the decline in diversity from east to west observed in our study, although this may have been exacerbated by population bottlenecks. Attempts to identify bottleneck events in our sampled populations using common techniques (Cornuet and Luikart 1996; Garza and Williamson 2001) were equivocal, likely due to small sample size from some populations.

5.2 Genetic differentiation

Substantial population differentiation is the norm for amphibians (Driscoll 1998; Barber 1999; Shaffer et al. 2000; Monsen and Blouin 2003; Jehle et al. 2005a). This has been attributed to the high site fidelity and low vagility of these animals, as seen in many capture-mark-recapture studies (Blaustein et al. 1994). Population differentiation in amphibians is often seen even at fine scales (Shaffer et al. 2000; Lampert et al. 2003; Monsen and Blouin 2003; Jehle et al. 2005b), although this is not always the case (Seppa and Laurila 1999; Newman and Squire 2001; Squire and Newman 2002). In most instances, populations separated by more than a few kilometres are genetically distinct (Shaffer et al. 2000; Jehle et al. 2005b).

Genetic distances observed in our study, as measured by $F_{ST}$, tended to be larger than those reported for other anurans (Monsen and Blouin 2003; Funk et al. 2005; Rowe and Beebee 2007). This is likely a consequence of the large geographic distance separating the populations in our study, which can cover discontinuous habitat and shifts between major ecoregions. For example, the populations of Creston, British Columbia and Black Sturgeon, Ontario, were separated by over 1,500 km, whereas in a study of British natterjack toads ($Bufo calamita$ Laurenti, 1768) by Rowe and Beebee (2007), populations were separated by a maximum of 60 km. Only three pairs of populations in our study were separated by distances less than this value. Similarly, a study of montane ranid frogs found a global $F_{ST}$ value of 0.16 between populations separated by a maximum of ~250 km (Monsen and Blouin 2003). The global $F_{ST}$ value in our study was 0.248, and 63 of 91 pairwise distances were greater than 250 km. This is also larger than the global $F_{ST}$ found in another study of northern leopard frogs (0.043), where populations were separated by a maximum of 400 km (Hoffman et al. 2004). This may be due to the
relatively large effective population sizes observed in the previous study, which would reduce the effect of genetic drift (Hoffman et al. 2004).

Using Wright’s (1978) classification, all northern leopard frog populations showed moderate to very great differentiation. High levels of genetic differentiation have also been observed in northern leopard frogs from other areas of their range (Kimberling et al. 1996; Hoffman and Blouin 2004a). This pattern is generally interpreted as resulting from a long period of time since population divergence. This is the likely scenario in our study area where populations may have been relatively isolated after post-Pleistocene range expansion into this region. Genetic distances between eastern populations in Manitoba and Ontario ($F_{ST}$ ranged between 0.063 and 0.117) were smaller than those observed between similarly spaced Alberta populations (0.144 - 0.348). This pattern could be the result of interplay between the original founding events and subsequent population bottlenecks. As described above, the genetic variability and resultant differentiation present in northern leopard frog populations were likely affected by a series of founding events as northern leopard frogs expanded their range westward after the last glaciation. Genetic drift would have been a driving force determining how diversity was compartmentalized in newly founded populations, especially when population sizes were small. If gene flow between regions is small, genetic differentiation would remain high among the western populations. Also, differentiation in western Canada may have increased after the population bottlenecks in the 1960s and 1970s. A further compounding factor could be differences in the permeability of the habitat matrix surrounding northern leopard frog populations in southern Alberta and populations farther east. The grasslands of southern Alberta tend to be fairly dry, which may not be amenable to long-distance movements by northern leopard frogs.

Genetic distances tended to be larger between the northern Leland Lake population and other Alberta populations ($F_{ST}$ ranged between 0.235 and 0.365) than among the Alberta populations when Leland Lake was not considered (0.144 - 0.348). However, it is surprising that Leland Lake was not more differentiated given that it was separated from the other Alberta populations by an average of about 900 km. By comparison, the largest distance separating the southern Alberta populations was ~250 km. This suggests that the Leland Lake population has remained relatively large, and has not
been greatly affected by genetic drift. However, little is known about the history of the Leland Lake population, so more work must be done to explain this observation.

Hutchison and Templeton (1999) outlined four different relationships between genetic (as measured by $F_{ST}$) and geographic distance that result from varying the relative strengths of gene flow and genetic drift. In case III, there is a lack of regional equilibrium and the driving force affecting the relationship between genetic and geographic distance is genetic drift. This can be graphically represented by an absence of significant linear regression and high degree of scatter (Figure 1 in Hutchison and Templeton 1999). The relationship between genetic and geographic distance observed between northern leopard frog populations (Figure 3) resembles this pattern, as there is no significant correlation between genetic and geographic distance (as measured by linear distance or distance along riparian areas) and a high degree of scatter. According to Hutchison and Templeton (1999), this scenario is expected to arise following the invasion of an area where populations are isolated and allele frequencies drift independently of geographic distance. The random sampling of alleles each generation via genetic drift results in a high variance in $F_{ST}$ values irrespective of geographic distance. Therefore, genetic drift seems to be a strong force dictating the genetic relationships among northern leopard frog populations in western Canada. This suggests that migration events are rare at our study scale, which seems logical given the high site fidelity and low mobility typical of anurans (Blaustein et al. 1994). The isolation of populations is likely exacerbated by habitat loss in Alberta through habitat alteration and climate change (Kendell 2002; Kendell et al. 2007). A similar pattern of weak isolation by distance has been seen in a number of other anurans (Seppa and Laurila 1999; Monsen and Blouin 2003). Isolation by distance may occur in northern leopard frogs at a finer geographic scale than used in this study, as has been seen in the montane frog (Monsen and Blouin 2004).

We also used modern methods based on genotypic frequencies to examine population structure (Pritchard et al. 2000; Guillot et al. 2005; Corander and Marttinen 2006). Results were similar, regardless of the technique used and whether or not geographic information was incorporated into the model (Table 3). BAPS was less likely to cluster populations together than the other analyses. This may have occurred because BAPS treats the population as an indivisible group, and clusters could only be formed if entire
populations (as opposed to some of the individuals in a population) were genetically similar. It is unsurprising that geographic information adds little to these analyses, given the absence of isolation by distance as described above. These methods all suggested high levels of differentiation among populations with little gene flow among them. The two sets of populations consistently grouped together across methods (PB - HI, and MH - OC) were also those separated by the smallest geographic distance (45 and 29 km, respectively). This suggests that beyond ~45 km the amount of migration between regions is too small to overcome the process of genetic drift, leading to a high degree of population structure across our study area.

5.3 Conservation implications

Translocations are becoming an increasingly common method for reintroducing species to regions of their range from which they have been extirpated (Griffith et al. 1989; Seddon et al. 2007). While common in parts of their range, many northern leopard frog populations in western North America underwent recent population bottlenecks (Hine et al. 1981; Roberts 1981; Corn and Fogleman 1984; Wagner 1997; Seburn and Seburn 1998; Leonard et al. 1999; Werner 2003; Kendell 2004). In Alberta, northern leopard frogs occupy as little as 20% of their former range (Kendell 2002; Kendell et al. 2007), and face increased fragmentation due to the loss of wetlands. Habitat fragmentation can put surviving populations at greater risk of extirpation (Griffith et al. 1989). While quantitative comparisons of occupied versus unoccupied habitats have not been completed, extensive anecdotal observations suggest that many previously occupied sites in the province still appear to be good-quality habitat for northern leopard frogs (K. Kendell, unpubl. data). This suggests that the reason these sites remain empty is an inability of frogs to migrate there due to low vagility exacerbated by habitat fragmentation. The high genetic differentiation and population structure among frog populations documented here support the idea that dispersal is rare. Therefore, human-mediated translocations appear necessary for the repopulation of unoccupied sites in Alberta (Roberts 1987). This will act to increase the absolute number of frogs in the province while decreasing the probability of extinction for remaining populations by reducing population fragmentation and isolation (Soule 1987).
Three criteria connected to our study can be used to evaluate northern leopard frog populations as possible sources for Alberta reintroductions; 1) genetic variability, 2) genetic similarity to the original inhabitants of a region identified for reintroduction and surrounding areas, and 3) evolutionary history. Genetic variability is an important consideration when choosing populations as sources for translocations, as populations with higher diversity also tend to have higher fitness (Packer et al. 1991; Roelke et al. 1993; Fitzsimmons et al. 1995; Halverson et al. 2006), thereby increasing the probability of long-term population viability. Based on this consideration, populations in Manitoba and Ontario are the best sources, as they displayed the highest levels of diversity with all three measures. Genetic diversity within all Alberta populations was consistently relatively low, so there was no obvious candidate to act as a source population based on this criterion.

Genetic differentiation can also affect translocation success. Genetic differences between locations can be the result of adaptation to specific environments, and populations may be genetically similar because they have adapted to comparable environments (Waples 1991; Russell et al. 1994; Hedrick et al. 2000; Palkovacs et al. 2004; Canestrelli et al. 2006). In our study, genetic differentiation among all populations separated by more than ~45 km was high with both classical and modern measures. Thus, gene flow was not strong enough to overcome genetic drift and any potential adaptive pressures to specific locales. Consequently, source populations that are genetically similar to populations close to where reintroductions are to occur are unlikely to be found.

The final criterion that we evaluated was the evolutionary history of the northern leopard frog populations. Hoffman and Blouin (2004b) described two haplotype groups in Canadian northern leopard frogs: a western group occurring in Ontario (west of James Bay), Manitoba, Saskatchewan, and British Columbia (no Alberta samples were used in this study) and an eastern group that also occurred in eastern Ontario, as well as Quebec and New Brunswick. Molecular clock estimates suggested that these groups had been separated for two million years (Hoffman and Blouin 2004b) and are evolutionarily distinct. Translocations into an area inhabited by individuals of a different evolutionary history can result in the loss of any unique genetic characteristics of this region through hybridization or replacement (Dobzhansky 1970; Rhymer and
Simberloff 1996). Therefore, we recommend that only populations with a western haplotype be considered for reintroduction efforts. While some of the haplotypes observed in this study had not been previously described by Hoffman and Blouin (2004b), phylogenetic analysis supported (78% maximum parsimony bootstrap) their inclusion in the western haplotype group. Consequently, none of the populations from this study should be eliminated as potential sources for translocation based on evolutionary history.

Of the three genetic criteria outlined here, only genetic variability had an effect on the desirability of the populations used in our study as translocation sources. Genetic differentiation is expected to be large except at fine scales, and all populations share a common evolutionary history. The genetically diverse populations from Manitoba and Ontario may then be good sources for translocation. However, Allendorf and Luikart (2007) listed a fourth consideration for source populations: environmental similarity. Using source populations from environments similar to the location for reintroduction may limit the possibility of maladaptation to the new environment (Allendorf and Luikart 2007). This criterion could support the use of source populations from Alberta, as their proximity may suggest higher levels of environmental similarity. However, comparisons would have to be performed on a site by site basis, given the range of habitats and microhabitats within the province. For instance, northern leopard frogs occur in the parklands, grasslands, and foothills natural regions in southern Alberta, and the Canadian Shield in northern Alberta (Alberta Sustainable Resource Development 2003). Allendorf and Luikart (2007) also stated that if no environmentally similar populations exist, founders from multiple sources could be mixed to maximize genetic diversity, suggesting that a mixture of sources from Alberta may be a method for maximizing diversity while presumably taking environmental similarity into account. While this scenario would likely increase diversity over using a single Alberta source, this province still has lower levels of diversity than Manitoba and Ontario when analyzed as a single unit (AR = 4.59, $H_1 = 0.616$). Other considerations exist, such as the potential for the introduction of disease and effects on the source population of losing individuals during a reintroduction, but taking into account the genetic backgrounds of potential source populations will increase the likelihood of restoring viable populations within the original range of northern leopard frogs in Alberta while preserving existing, currently isolated populations.
5.4 Management recommendations

The results of this study can be used to establish recommendations for the reintroduction of northern leopard frogs to parts of their Alberta range. It must be noted, however, that these recommendations are based on a genetic perspective, and do not take into account other possible factors that should be weighed when choosing source populations for reintroduction, such as disease presence or source population size.

1) Where possible, choose source populations geographically close to the reintroduction site. Source populations close to a reintroduction site are more likely to have adapted to similar environments. A reintroduction is more likely to succeed if its founders have evolved under similar selection pressures as their introduction location. However, due to the low vagility of amphibians in general, populations tend to only be genetically similar over fine spatial scales. In our study, populations separated by ~50 km had high levels of genetic differentiation, suggesting that genetic drift is a powerful force controlling relationships among northern leopard frog populations. Genetic drift may dilute the effect of selection for similar environments over large spatial scales, and increase the possibility of fine-scale local adaptation.

2) It is not necessary for the source population to occur within the same drainage. In some instances, frogs sampled within the same Alberta drainage on a fine spatial scale (~1 km) were genetically similar enough to be classified as members of a single population. However, over larger spatial scales watershed-based distances among populations were not better predictors of genetic relationships than straight line distances, so there is no reason to think that frogs found farther than a few km apart within a watershed are any more closely related than those found in different watersheds separated by the same geographic distance. This could be due in part to the ability of northern leopard frogs to migrate over land under appropriate weather conditions rather than strictly following riparian corridors. Therefore, populations from different drainages would be suitable sources for reintroduction efforts.
3) **The choice of source populations within Alberta may depend on environment and availability.** If nearby source populations are unavailable, there is no obvious area within Alberta that would be better (or worse) than any other from a genetic standpoint. Populations with high numbers of alleles are more likely to contain genetic information that will be selected for in their new environment, and those with high levels of heterozygosity are least likely to suffer from inbreeding effects. However, mean number of alleles and heterozygosity are similar throughout the sampled Alberta populations. Due to the high levels of genetic differentiation found among northern leopard frog populations in Alberta, it is possible that these populations have become locally adapted to their current environments. Consequently, ecological similarity between the source and reintroduction locations for factors such as latitude, elevation, habitat, and presence of predators and diseases should increase the likelihood of reintroduction success. However, no studies have been done that would allow the relative importance of these factors to be estimated.

4) **Diversity will be maximized if eggs from multiple sources are used.** All else being equal, genetic diversity in a recently founded population will increase with the number of founders used. Most eggs from a single mass are likely related at the full sib level. As such, using a number of eggs from a single mass will result in a resampling of the genetic material present in the parents, and will add little to the diversity of the re-established population. In order to increase the effective number of founders, eggs from a number of masses should be used at a single reintroduction site. As sampled populations in Alberta have similar levels of genetic diversity, obtaining founding individuals from large, healthy, ecologically similar areas is likely more important than obtaining founders from multiple source populations. However, using founders from more than one source population is unlikely to have any negative genetic effects.

5) **Manitoba and Ontario may offer good source populations for northern leopard frogs.** As mentioned, genetic differentiation is high among the sampled northern leopard frog populations. Thus, source populations genetically similar to extant populations close to where reintroductions are to occur are unlikely to
be found. The best source of founders for reintroduction efforts may then be populations that are most genetically diverse. Genetic diversity was similar among Alberta frog populations. However, diversity was higher in populations from northern Ontario and Manitoba. From a genetic standpoint, these provinces would then be suitable sources for reintroduction efforts. But, it may be more difficult to find ecologically exchangeable populations due to the large geographic distance separating these regions from Alberta. Caution must be taken if frogs from Ontario are used as founders, since two clades that have been separated for two million years are present in this province. Only frogs from the western group, which is the only group found within Alberta, were found in our Ontario sample.

6) **Genetic monitoring of recently founded populations would serve as an early warning system for loss of diversity.** Genetic diversity can be rapidly lost from small populations, such as most recently founded ones, due to genetic drift or differential reproductive success. This can result in a decrease in fitness over the short- and long-term. The collection of genetic samples from recently founded populations will allow for the monitoring of diversity levels, and provide valuable lessons about the maintenance of genetic diversity to future reintroduction efforts.
6.0 LITERATURE CITED


luteiventris) is strongly affected by the landscape. Molecular Ecology 14: 483-496.


Lode, T., M.J. Holveck, and D. Lesbarreres. 2005. Asynchronous arrival pattern, operational sex ratio and occurrence of multiple paternities in a territorial


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