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# COMPLEX SPATIAL DYNAMICS MAINTAIN NORTHERN LEOPARD FROG (*LITHOBATES PIPIENS*) GENETIC DIVERSITY IN A TEMPORALLY VARYING LANDSCAPE

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***Abstract.*—In contrast to most local amphibian populations, northeastern populations of the Northern Leopard Frog (*Lithobates pipiens*) have displayed uncharacteristically high levels of genetic diversity that have been attributed to large, stable populations. However, this widely distributed species also occurs in areas known for great climatic fluctuations that should be reflected in corresponding fluctuations in population sizes and reduced genetic diversity. To test our hypothesis that Northern Leopard Frog genetic diversity would be reduced in areas subjected to significant climate variability, we examined the genetic diversity of *L. pipiens* collected from 12 sites within the Prairie Pothole Region of North Dakota. Despite the region's fluctuating climate that includes periods of recurring drought and deluge, we found unexpectedly high levels of genetic diversity approaching that of northeastern populations. Further, genetic structure at a landscape scale was strikingly homogeneous; genetic differentiation estimates ($D_{est}$) averaged 0.10 (SD = 0.036) across the six microsatellite loci we studied, and two Bayesian assignment tests (STRUCTURE and BAPS) failed to reveal the development of significant population structure across the 68 km breadth of our study area. These results suggest that *L. pipiens* in the Prairie Pothole Region consists of a large, panmictic population capable of maintaining high genetic diversity in the face of marked climate variability.**

*Key Words*.—genetic diversity; microsatellites; Northern Leopard Frog; Prairie Pothole Region

## INTRODUCTION

In general, local amphibian populations harbor low levels of genetic diversity (Neff and Gross 2001) with limited gene flow (Shaffer et al. 2000). This low genetic diversity is associated with high variance in reproductive success (Merrell 1968; Scribner et al. 1997; Wilson et al. 2008), skewed sex ratios (Vieites et al. 2004; Lode et al. 2005), low vagility (Blaustein et al. 1994), and naturally fluctuating population sizes (Berven 1990; Seppä and Laurila 1999). Climate cycles that alter the quantity and quality of suitable habitat across the landscape can also affect levels of genetic diversity by contributing to population size fluctuations (Newman and Squire 2001). In contrast to this general trend of low genetic diversity in amphibian populations, Hoffman et al. (2004) reported remarkably high levels of diversity (mean heterozygosity [$H_e$] averaging 0.86 to 0.92 per population) for populations of the Northern Leopard Frog, *Lithobates pipiens* (Fig. 1), occurring in the northeastern United States. They hypothesized that the high genetic diversity of these local populations was high due to larger, more stable, effective population sizes ($N_e$) as compared to other amphibians that had been studied (e.g., Seppä and Laurila 1999; Beebee and Rowe 2000; Newman and Squire 2001; Monsen and Blouin 2003).

**FIGURE 1.** The Northern Leopard Frog (*Lithobates pipiens*). (Photographed by David Mushet).

Hoffman and Blouin (2004a) identified two distinct haplotypes of the Northern Leopard Frog divided by the Mississippi River and Great Lakes region. While eastern populations appear to be large and stable, the conservation status of this species within western portions of its range is a topic of concern. However, a petition to list the species as threatened under the

Endangered Species Act in all areas of its range west of the Mississippi River was recently determined to be unwarranted by the U.S. Fish and Wildlife Service (USFWS 2011).

One prediction emanating from the work of Hoffman et al. (2004) is that, compared to northeastern U.S. populations, lower levels of genetic diversity would occur in populations occupying areas with more highly dynamic inter-annual climates where great fluctuations in population sizes are expected. The Prairie Pothole Region (PPR) of North Dakota is an area subjected to such seasonal and inter-annual variability in air temperature and precipitation including recurring drought/deluge climate cycles that can persist for 10–20 years (Karl and Riebsame 1984). Northern Leopard Frogs in the PPR frequently undergo large fluctuations in the number of individuals at breeding wetlands associated with the cyclical drying and wetting of these wetlands in response to drought/deluge cycles (Larson et al. 1998; Mushet 2010). Given observed fluctuations at breeding sites and therefore inferred fluctuations in overall population sizes, Northern Leopard Frogs of the PPR should have less genetic diversity compared to northeastern populations where more stable climate conditions prevail (e.g., the populations sampled by Hoffman et al. 2004). We test this prediction using the same microsatellite markers used by Hoffman et al. (2004).

## MATERIALS AND METHODS

***Samples.***—During August 2007, we sampled Northern Leopard Frogs from the following 12 sites within Stutsman County in east-central North Dakota: Pipestem Reservoir (PR), Jamestown Reservoir (JR), Spiritwood Lake (SWL), Stink Lake (SL), Barnes Lake (BL), Fischer Lake (FL), Refugium #1 (R1), Refugium #2 (R2), Cottonwood Lake Study Area (CL), the Woodworth Field Station (WW), an unnamed waterfowl production area (NE), and Hawk's Nest Wildlife Management Area (HN; Fig. 2). The greatest distance between any two sites sampled was 68 km (SL to SWL) and the shortest was 3 km (R1 to R2). At each sample site, we collected tissue samples from 40 recently metamorphosed individuals captured with nylon nets. We avoided capturing multiple individuals from a single breeding effort (i.e., siblings) by distributing our collection locations around the periphery of each site. We also targeted recent metamorphs instead of tadpoles to further reduce the likelihood that captured individuals would be siblings (i.e., recently hatched from a single egg mass).

We restrained and handled all captured individuals following procedures described in the Amphibian Research and Monitoring Initiative (ARMI) Restraint and Handling of Live Amphibians standard operating procedure (ARMI SOP #100, http://www.nwhc.usgs.gov/publications/amphibian_research_procedures/handling_and_restraint.jsp [Accessed 10 April 2007]). We clipped the number IV digit from the right hind foot of captured leopard frogs where the webbing began following procedures described in the ARMI Toe Clipping of Frogs and Toads standard operating procedure (ARMI SOP #110, http://www.nwhc.usgs.gov/publications/amphibian_research_procedures/toe_clipping.jsp [Accessed 10 April 2007]). Tissue samples consisted of toe clips preserved and stored individually in 95% ethanol in 3-dram glass vials.

We extracted and purified total genomic DNA from the tissue samples using DNeasy® Blood and Tissue kits from Qiagen® following their bench protocol for animal tissues. After extraction and purification, we stored all DNA at -20 °C until needed. We amplified six microsatellite loci using five primer sets (Rpi100, Rpi101, Rpi103, Rpi107, and Rpi108) developed for the Northern Leopard Frog (Hoffman et al. 2003) and one primer set (RP197) developed for the Oregon Spotted Frog (*L. pretiosa*; Hoffman and Blouin 2004b). We conducted amplifications using polymerase chain reactions (PCR). Each 10 µL PCR consisted of 2.0 µL template, 1X PCR buffer, 1.5 mmol $MgCl_2$, 0.1 mmol deoxyribonucleotide triphosphates (dNTPs), 0.2 U *Taq* DNA polymerase, 0.25 µM fluorescently labeled forward primer, and 0.5 µmol unlabeled reverse primer. We carried out reactions in Eppendorf Mastercyclers® using the following temperature profiles: initial denaturing at 95 °C for 9 min; 34 cycles of denaturing at 95 °C for 30 s, annealing at 52 °C for 60 s, and extension at 72 °C for 90 s; and a final extension at 72 °C for 10 min.

We visualized lengths of PCR products (i.e., microsatellite fragments) using a capillary electrophoresis system (Beckman Coulter, Inc., Brea, California, USA, Model CEQ 8800). To minimize scoring errors, we included a known sample in each eight-sample row run through the electrophoresis system. When a known sample did not score properly, all samples within that row were reanalyzed. If an individual sample did not provide a clear peak or pair of peaks (heterozygotes), we re-amplified the extracted DNA as described above and ran the new PCR product through the capillary electrophoresis system. When a clear score was still not possible, we re-extracted total genomic DNA from the tissue sample before again repeating the amplification and visualization procedures. Following these procedures, we were able to successfully amplify, visualize, and score PCR microsatellite products for all individuals and loci sampled while minimizing the potential for scoring errors.

***Statistical analyses.***—We used MICRO-CHECKER version 2.2.3 (van Oosterhout et al. 2004) to identify microsatellite genotyping errors of the dataset and to

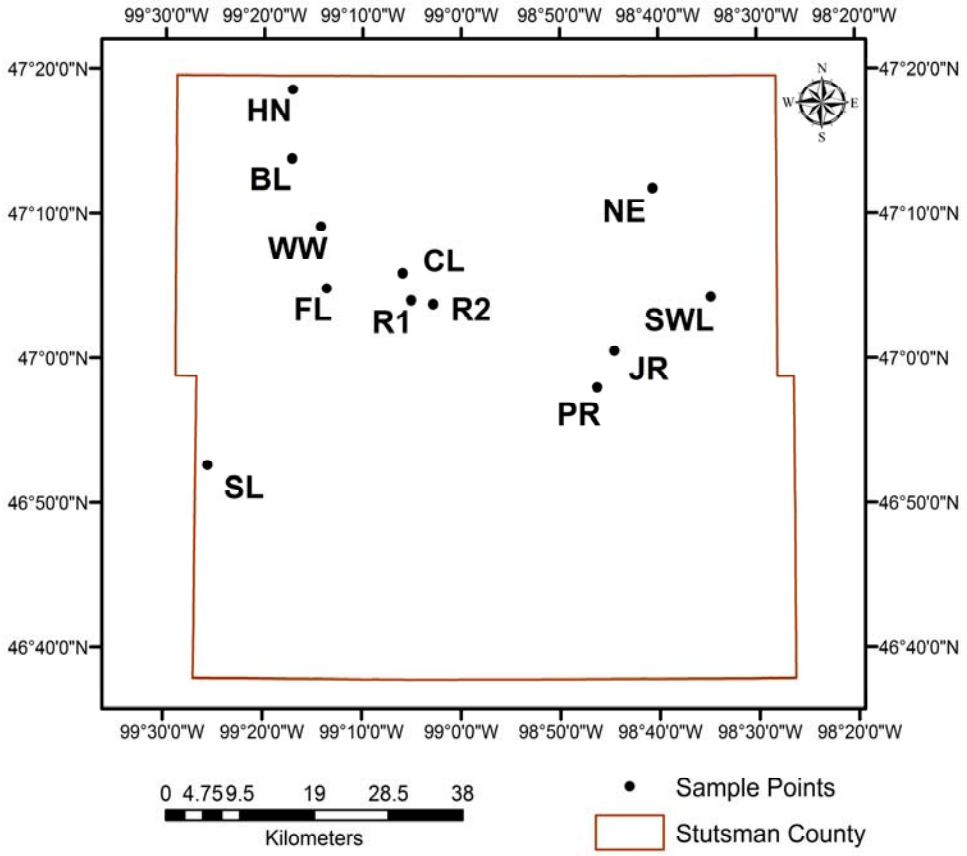


**FIGURE 2.** Locations of 12 sites in Stutsman County, North Dakota where Northern Leopard Frogs (*Lithobates pipiens*) were sampled in August 2007. BL = Barnes Lake, CL = Cottonwood Lake Study Area, FL = Fischer Lake, HN = Hawk's Nest Wildlife Management Area, JR = Jamestown Reservoir, NE = unnamed Waterfowl Production Area, PR = Pipestem Reservoir, R1 = Refugium #1, R2 = Refugium #2, SL = Stink Lake, SWL = Spiritwood Lake, and WW = Woodworth Field Station).

check for null alleles. We used GENEPOP (Raymond and Rousset 1995) to test for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium. GENEPOP implements a Markov chain method to test for Hardy-Weinberg and linkage equilibriums. We ran chains using 1,000 batches and 10,000 iterations per batch. We used a modified false discovery rate correction to maintain an overall significance level of 0.05 (Narum 2006). We also used GENEPOP version 4.0 to calculate allelic richness, allele frequencies, observed heterozygosities ($H_o$), and expected heterozygosities ($H_e$).

To explore population differentiation among sites, we calculated $D_{est}$ (Jost 2008) using SMOGD version 1.2.5 (Crawford 2010) and $F_{ST}$ using GENEPOP. We computed the regression of $D_{est}$ and $F_{ST}$ on linear (Euclidean) geographic distance to test for isolation by distance. We also used two Bayesian clustering programs including STRUCTURE version 2.1 (Pritchard et al. 2000) and BAPS version 4.0 (Corander and Marttinen 2006; Corander et al. 2008) to quantify genetic structure. In STRUCTURE, testing was conducted to estimate the most likely population from which a sample of individuals was derived. For this analysis, we used 100,000 iterations after an initial burn-in of 10,000. Five independent replicate chains, both with and without *a priori* population information, were performed for K = 1 to 15. When *a priori* population information was used, the LOCPRIOR model in STRUCTURE provided priors to the Bayesian assignment process. These priors were based on the site from which an individual was sampled, not the geographic location of the site. The LOCPRIOR model can be useful for identifying cryptic structure while not being biased towards detecting structure when none is present (Hubisz et al. 2009). We used an admixture ancestry model in all STRUCTURE runs and allowed for correlated allele frequencies. We used Evanno's delta K method (Evanno et al. 2005)

**TABLE 1.** Summary statistics for six microsatellite loci from 12 Northern Leopard Frog (*Lithobates pipiens*) populations sampled in Stutsman County, North Dakota. Significant ($P < 0.05$) departure from Hardy-Weinberg equilibrium after a modified false discovery rate correction (Narum 2006) is designated by an asterisk. n = number of individuals sampled per population, A = number of alleles per locus, $H_o$ = observed heterozygosity, $H_e$ = expected heterozygosity.

| | | | Site | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | A | | PR | JR | SWL | SL | BL | FL | R1 | R2 | CL | WW | NE | HN | Mean (SE) |
| RP193 | 16 | n | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | |
| | | A | 12 | 13 | 10 | 12 | 10 | 11 | 10 | 11 | 13 | 12 | 11 | 9 | 11.2 (1.27) |
| | | $H_o$ | 0.98 | 0.90 | 0.88 | 0.88 | 0.90 | 0.88 | 0.88 | 0.88 | 0.85 | 0.90 | 0.88 | 0.85 | |
| | | $H_e$ | 0.87 | 0.88 | 0.88 | 0.85 | 0.85 | 0.88 | 0.86 | 0.86 | 0.88 | 0.90 | 0.87 | 0.85 | |
| Rpi100 | 18 | n | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | |
| | | A | 14 | 14 | 14 | 15 | 13 | 12 | 12 | 13 | 13 | 12 | 13 | 11 | 13.0 (1.13) |
| | | $H_o$ | 0.93 | 0.98 | 0.98 | 0.90 | 0.98 | 0.90 | 0.90 | 0.88 | 0.88 | 0.83 | 0.80 | 0.75 | |
| | | $H_e$ | 0.91 | 0.93 | 0.91 | 0.90 | 0.85 | 0.83 | 0.87 | 0.87 | 0.86 | 0.86 | 0.87 | 0.77 | |
| Rpi101 | 14 | n | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | |
| | | A | 9 | 11 | 10 | 10 | 11 | 10 | 10 | 9 | 8 | 10 | 8 | 9 | 9.6 (1.00) |
| | | $H_o$ | 0.80 | 0.88 | 0.78 | 0.83 | 0.78 | 0.78 | 0.80 | 0.80 | 0.88 | 0.98 | 0.73* | 0.78 | |
| | | $H_e$ | 0.84 | 0.82 | 0.85 | 0.85 | 0.86 | 0.87 | 0.85 | 0.88 | 0.82 | 0.87 | 0.85 | 0.86 | |
| Rpi103 | 41 | n | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | |
| | | A | 29 | 25 | 24 | 22 | 17 | 18 | 20 | 22 | 21 | 19 | 20 | 17 | 21.2 (3.54) |
| | | $H_o$ | 0.93 | 0.93 | 0.90 | 0.98 | 0.90 | 0.93 | 0.95 | 0.98 | 0.90 | 0.93 | 0.88 | 0.93 | |
| | | $H_e$ | 0.95 | 0.94 | 0.95 | 0.94 | 0.90 | 0.92 | 0.93 | 0.92 | 0.90 | 0.92 | 0.93 | 0.92 | |
| Rpi107 | 12 | n | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | |
| | | A | 8 | 8 | 9 | 7 | 9 | 8 | 7 | 8 | 8 | 8 | 6 | 10 | 8.0 (1.04) |
| | | $H_o$ | 0.80 | 0.83 | 0.65* | 0.70 | 0.80 | 0.80 | 0.83 | 0.78 | 0.80 | 0.60 | 0.65 | 0.70* | |
| | | $H_e$ | 0.76 | 0.80 | 0.77 | 0.80 | 0.76 | 0.82 | 0.75 | 0.76 | 0.77 | 0.74 | 0.67 | 0.76 | |
| Rpi108 | 26 | n | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | |
| | | A | 17 | 17 | 19 | 18 | 13 | 14 | 17 | 15 | 13 | 11 | 16 | 12 | 15.2 (2.55) |
| | | $H_o$ | 0.95 | 0.85 | 0.93 | 0.78 | 0.80 | 0.90 | 0.80 | 0.90 | 0.65 | 0.80 | 0.75 | 0.83 | |
| | | $H_e$ | 0.90 | 0.88 | 0.89 | 0.90 | 0.76 | 0.88 | 0.80 | 0.87 | 0.74 | 0.76 | 0.85 | 0.72 | |
| Average | 21.2 | n | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | |
| across all | | A | 14.8 | 14.7 | 14.3 | 14.0 | 12.2 | 12.2 | 12.7 | 13.0 | 12.7 | 12.0 | 12.3 | 11.3 | 13.0 (1.15) |
| Loci | | $H_o$ | 0.90 | 0.85 | 0.89 | 0.84 | 0.86 | 0.86 | 0.86 | 0.87 | 0.83 | 0.84 | 0.80 | 0.80 | |
| | | $H_e$ | 0.87 | 0.88 | 0.88 | 0.87 | 0.83 | 0.86 | 0.84 | 0.86 | 0.84 | 0.84 | 0.84 | 0.81 | |

implemented using Structure Harvester web version 0.6.92 (Earl and vonHoldt 2012) to infer the most likely number of population clusters from our STRUCTURE results.

While each individual was used as a sampling unit in STRUCTURE, BAPS allowed for modeling at the group-level where a group of individuals sampled from a given area were considered as the sampling unit.

Additionally, BAPS incorporated spatial geographic location information into the analysis. For this analysis, individuals from each of the 12 sites sampled were considered as groups. Spatial information for each group consisted of the longitude and latitude of the centroid of the area from within which individuals of that group were captured. We conducted analyses in BAPS both with (using the 'Spatial Clustering by Groups' function) and without (using the 'Clustering of Groups' function) this spatial information. For both analyses, we used maximum Ks from 2 through 15 with three replications of each.

## RESULTS

The six microsatellite loci used in this study were highly polymorphic. Over the 12 sites sampled, the total number of alleles per locus ranged from 14 for Rpi101 to 41 in Rpi103 (Appendix A). MICRO-CHECKER revealed no evidence of scoring error due to stuttering, large allele dropout, or null alleles for the loci. Genotype frequencies met Hardy-Weinberg expectations for all six loci and all 12 sites with three exceptions: Rpi101 for NE, Rpi107 for SWL, and Rpi 107 for HN (Table 1). There was no evidence of linkage disequilibrium between pairs of loci.

All allelic richness and heterozygosity measures showed very high levels of genetic diversity. At the site-specific scale, allelic richness ranged from a low of 6 at locus Rpi107 for NE to a high of 29 at locus Rpi103 for PR. Average allelic richness across all loci ranged from 11.3 for HN to 14.8 for PR (Table 1). High expected heterozygosity values ($H_e$) ranged from 67% at locus Rpi107 for NE to 95% at locus Rpi103 for PR and SWL. Averaged over all loci, $H_e$ ranged from 81% for HN to 88% for JR and SWL (Table 1). Allelic richness and $H_e$ values were only slightly lower than values reported by Hoffman et al. (2004) for stable populations in the northeastern United States (Table 2).

$D_{est}$ across loci averaged 0.10 (SD = 0.036) and ranged from 0.06 at Rpi107 to 0.16 at Rpi103. A plot of $D_{est}$ and linear (Euclidean) geographic distance (Fig. 3) revealed a trend of increased differentiation with increasing distance between sites ($r^2 = 0.45$). Global $F_{ST}$ was estimated to be 0.016 and also showed the same trend of isolation by distance ($r^2$=0.45).

Despite the apparent isolation by distance, measures of population differentiation by STRUCTURE failed to reveal any structure among the sites (i.e., the most likely number of populations, K, equaled one, unless *a priori* geographic information was included). When we included this population information, K = 2 also was identified as a possibility. However, a plot of the log likelihood versus K (Fig. 4) revealed a nearly flat line among all K's when using prior population information, thus indicating little, if any, differentiation among groups. Additionally, even though delta K was largest for K = 2 (Fig. 4), the value was very small relative to the log likelihood values (Fig. 4) further supporting our finding of a single, panmictic population. Likewise, BAPS assigned all individuals sampled to a single population, both with and without the inclusion of spatial information.

**TABLE 2.** Range of allelic richness and expected heterozygosity ($H_e$) for microsatellite loci from Northern Leopard Frog (*Lithobates pipiens)* populations sampled in the northeastern United States (from Hoffman et al. 2004) and North Dakota.

| | Allelic Richness | | Heterozygosity | |
|---|---|---|---|---|
| Locus | Northeastern United States | North Dakota | Northeastern United States | North Dakota |
| RP193 | 8–15 | 9–13 | 0.72–0.91 | 0.85–0.90 |
| RP415 | 9–15 | not used | 0.85–0.92 | not used |
| Rpi100 | 12–23 | 11–15 | 0.88–0.97 | 0.77–0.93 |
| Rpi101 | 9–17 | 8–11 | 0.78–0.92 | 0.82–0.87 |
| Rpi103 | 18–33 | 17–29 | 0.92–0.96 | 0.90–0.95 |
| Rpi106 | 16–35 | not used | 0.95–0.97 | not used |
| Rpi107 | not used | 6–10 | not used | 0.67–0.82 |
| Rpi108 | 8–23 | 11–19 | 0.88–0.91 | 0.72–0.90 |

## DISCUSSION

We predicted that the genetic diversity of Northern Leopard Frogs would be significantly reduced under the dynamic environmental conditions present within our study region. However, we found that this was not the case. Allelic richness and heterozygosity were much greater than expected, and both were only slightly lower than reported by Hoffman et al. (2004) for northeastern US populations of the species (Table 2). This suggests that Northern Leopard Frog populations in our study area have very large effective population sizes ($N_e$) and/or very high levels of gene flow. We hypothesize that genetic diversity is maintained by a combination of these two factors.

We can estimate $N_e$ under the Stepwise Mutation Model (Ohta and Kimura 1973) by using our estimates of heterozygosity as:

$$N_e = (1 / (1-H_e)^2-1) / 8\mu.$$

Using the expected heterozygosities (Table 1) and the range of mutation rates used by Hoffman et al. (2004; $10^{-3}$–$10^{-4}$), our estimates of $N_e$ range from 3,338 ($\mu$ = 0.001) to 33,376 ($\mu$ = 0.0001) for a $H_e$ of 0.81 and 8,556 ($\mu$ = 0.001) to 85,556 ($\mu$ = 0.0001) for a $H_e$ of 0.88. Thus, even at the high mutation rate of 0.001, the maintenance of heterozygosity values in our data set requires an effective population in the thousands.

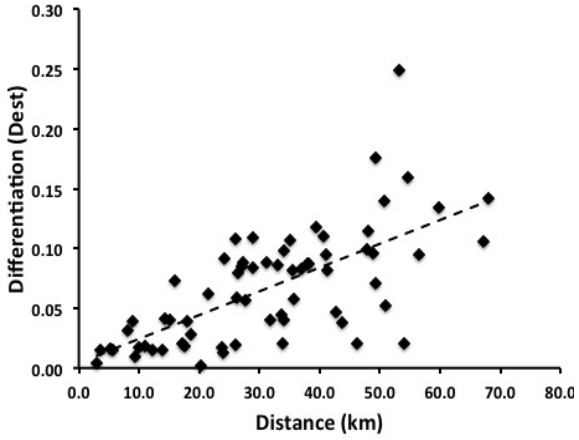


**FIGURE 3.** Plot of pairwise genetic differentiation ($D_{est}$) versus Euclidean distances (km) between sites where Northern Leopard Frogs (*Lithobates pipiens*) were sampled in Stutsman County, North Dakota. Trend-line formula is $y = 0.002x + 0.0045$ ($r^2 = 0.45$).

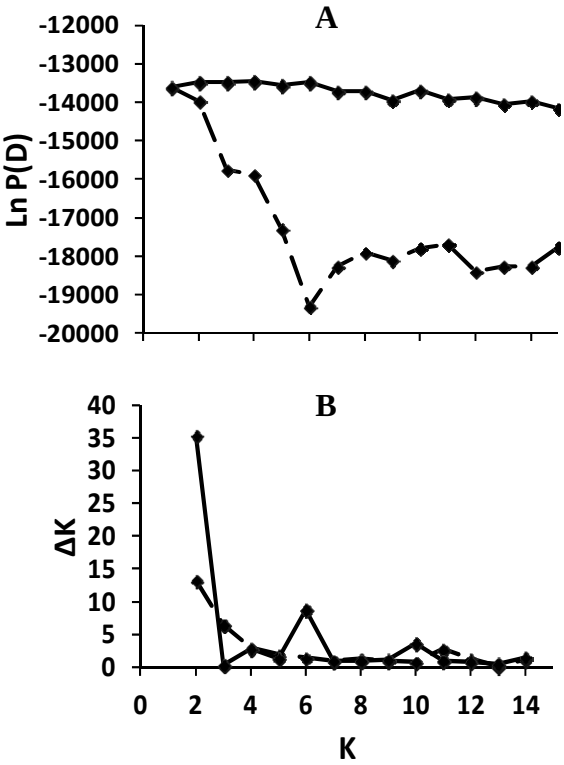


**FIGURE 4. A**) Log likelihood (ln P(D)) and **B**) Evanno's delta K (ΔK) versus number of populations (K) from 480 Northern Leopard Frogs (*Lithobates pipiens*) collected from 12 locations in Stutsman County, North Dakota. Values plotted are mean values from five independent STRUCTURE runs. Solid line indicates runs conducted using *a priori* location information while dashed line indicates runs conducted without including location information.

High genetic diversity could still be maintained even with substantially lower effective population sizes given rapid mixing of populations following drought because, as with Hoffman et al. (2004), our Northern Leopard Frog populations are unlikely to be closed systems. High gene flow is consistent with the work conducted by Merrell (1970) who showed that Northern Leopard Frog populations in Minnesota have high levels of vagility. Merrell (1970) studied migration activities and gene dispersal of Northern Leopard Frogs in Minnesota by examining the spatial distribution of a dominant gene, *burnsi*, coding for the lack of spots. This gene was uniformly maintained at low frequencies across populations, a pattern consistent with relatively high levels of gene flow rather than spatially varying frequencies that would be expected with random genetic drift (Merrell 1970).

Our results using nuclear microsatellite markers from sites in North Dakota confirm Merrell's earlier observations. In fact, relatively low genetic divergence occurs among our sites (low $D_{est}$ and low $F_{ST}$) indicating high levels of gene flow. Further, results from both STRUCTURE and BAPS indicate little differentiation among groups (i.e., K = 1) even when *a priori* sample location information are included. The limited level of differentiation at the landscape scale further supports our conclusions that significant mixing among relatively large populations occurs across our study area.

Our findings correspond to spatial and temporal dynamics of overwintering congregations of leopard frogs in North Dakota. These congregations are likely to vary in size depending on the relative abundance of overwintering sites and their proximity to breeding sites. During wet years, winter refugia are abundant and closely spaced on the landscape (Mushet 2010). However, during droughts, these sites become rare and are critical landscape features for sustaining this species in the PPR. During periods when overwintering sites are rare, large numbers of individuals likely congregate at these sites and their highly mobile nature contributes to rapid mixing following drought and thus the ability to maintain high levels of genetic diversity in the face of uncertain habitat conditions.

Our findings contrast with those of a recent study by Wilson et al. (2008), which revealed much lower genetic diversity ($H_e$ ranging from 0.396 to 0.739) in Canadian populations of *L. pipiens*. Wilson et al. (2008) found that genetic diversity declined in a westward progression across their study region with lowest diversity occurring at sites near the northwestern periphery of the species' range. In general, peripheral populations tend to have lower genetic diversity than populations more centrally located within a species' range (Lesica and Allendorf 1995; Hoffman and Blouin 2004b). However, the lower genetic diversity observed by Wilson et al. (2008) follows an east to west precipitation gradient that also extends across the north-central United States and south-central Canada due to the rain shadowing effects of the

Rocky Mountains. This precipitation gradient likely results in a dearth of overwintering and drought refugia wetlands in their westernmost (drier) study sites. Likewise, we predict lower levels of genetic diversity in other areas of Northern Leopard Frog's range (e.g., western North Dakota) where the climate is generally drier, and deep-water habitats are fewer and separated by much greater distances than in the east-central North Dakota sites we sampled.

Although Hoffman et al (2004) observed significant differentiation among all sampled sites, their spatial scale (82 to 387 km between site pairs) was much greater than ours and this range does not overlap with the 3 to 68 km distances that separate our sites. Likewise, Wilson et al. (2008) also sampled at a larger spatial scale (up to 1606.3 km between sites) but they included two site pairs separated by a distance of 29.5 and 45.6 km. They did not detect genetic differentiation among these closest sites, yet all remaining sites (distances > 45.6 km) exhibited differentiation.

Climate change is likely to impact genetic diversity in populations of the Northern Leopard Frog. Maintenance of genetic diversity requires a better understanding of all habitat components. The International Panel on Climate Change (IPCC) predicted an increase in frequency and severity of drought events in the upper Midwest (Schneider et al. 2007). Further, wetland simulation models specific to the PPR recently projected that climate change will result in shallower, shorter hydroperiod wetlands (Johnson et al. 2005, 2010). Thus, key overwintering and drought refugium wetlands that are critical to maintaining diverse populations of Northern Leopard Frogs may become increasingly limited. These changes could be even more significant in areas likely to support lower genetic diversity (e.g., Canada, western North Dakota). A better understanding of this species' dispersal capabilities and population dynamics is necessary to understand fully the potential responses of populations to anthropogenic and natural disturbances, including climate change.

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**APPENDIX A**. Allele frequencies of six Northern Leopard Frog (*Lithobates pipiens*) genetic microsatellite loci (Rpi100, Rpi101, Rpi103, Rpi107, Rpi108, and RP193) from 12 populations (40 individuals each) sampled in Stutsman County, North Dakota, August 2007. BL = Barnes Lake, CL = Cottonwood Lake Study Area, FL = Fischer Lake, HN = Hawk's Nest Wildlife Management Area, JR = Jamestown Reservoir, NE = unnamed Waterfowl Production Area (WPA), PR = Pipestem Reservoir, R1 = Refugium #1, R2 = Refugium #2, SL = Stink Lake, SWL = Spiritwood Lake, and WW = Woodworth Field Station.

**Locus Rpi100**

| Population | Alleles | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **172** | **176** | **180** | **184** | **188** | **192** | **196** | **200** | **204** | **208** | **212** | **216** | **220** | **224** |
| CL | 0.000 | 0.013 | 0.088 | 0.025 | 0.000 | 0.038 | 0.300 | 0.000 | 0.125 | 0.113 | 0.038 | 0.000 | 0.113 | 0.050 |
| R1 | 0.000 | 0.000 | 0.050 | 0.025 | 0.000 | 0.100 | 0.238 | 0.000 | 0.200 | 0.088 | 0.013 | 0.013 | 0.050 | 0.125 |
| WW | 0.000 | 0.013 | 0.125 | 0.000 | 0.000 | 0.138 | 0.288 | 0.000 | 0.063 | 0.100 | 0.025 | 0.038 | 0.038 | 0.075 |
| BL | 0.000 | 0.038 | 0.100 | 0.013 | 0.000 | 0.075 | 0.313 | 0.013 | 0.025 | 0.138 | 0.125 | 0.000 | 0.038 | 0.075 |
| HN | 0.000 | 0.000 | 0.100 | 0.038 | 0.000 | 0.063 | 0.450 | 0.025 | 0.025 | 0.063 | 0.050 | 0.075 | 0.075 | 0.038 |
| PR | 0.000 | 0.050 | 0.038 | 0.125 | 0.013 | 0.175 | 0.163 | 0.050 | 0.050 | 0.000 | 0.050 | 0.038 | 0.050 | 0.088 |
| NE | 0.013 | 0.000 | 0.013 | 0.075 | 0.000 | 0.138 | 0.250 | 0.038 | 0.125 | 0.163 | 0.063 | 0.000 | 0.050 | 0.038 |
| SWL | 0.000 | 0.063 | 0.038 | 0.125 | 0.063 | 0.188 | 0.063 | 0.075 | 0.138 | 0.113 | 0.025 | 0.025 | 0.025 | 0.038 |
| JR | 0.000 | 0.075 | 0.075 | 0.075 | 0.050 | 0.100 | 0.138 | 0.038 | 0.088 | 0.088 | 0.038 | 0.050 | 0.013 | 0.063 |
| SL | 0.000 | 0.038 | 0.075 | 0.063 | 0.013 | 0.063 | 0.213 | 0.013 | 0.100 | 0.100 | 0.050 | 0.050 | 0.013 | 0.125 |
| FL | 0.000 | 0.038 | 0.050 | 0.000 | 0.000 | 0.113 | 0.350 | 0.013 | 0.150 | 0.088 | 0.013 | 0.013 | 0.063 | 0.025 |
| R2 | 0.000 | 0.013 | 0.063 | 0.013 | 0.075 | 0.125 | 0.275 | 0.000 | 0.063 | 0.050 | 0.025 | 0.000 | 0.025 | 0.088 |
| All | 0.001 | 0.028 | 0.068 | 0.048 | 0.018 | 0.109 | 0.253 | 0.022 | 0.096 | 0.092 | 0.043 | 0.025 | 0.046 | 0.069 |

| | **228** | **232** | **236** | **240** |
|---|---|---|---|---|
| CL | 0.013 | 0.075 | 0.013 | 0.000 |
| HN | 0.000 | 0.000 | 0.000 | 0.000 |
| PR | 0.025 | 0.088 | 0.000 | 0.000 |
| NE | 0.000 | 0.013 | 0.000 | 0.025 |
| SWL | 0.000 | 0.025 | 0.000 | 0.000 |
| JR | 0.000 | 0.113 | 0.000 | 0.000 |
| SL | 0.000 | 0.075 | 0.000 | 0.013 |
| FL | 0.000 | 0.088 | 0.000 | 0.000 |
| R2 | 0.050 | 0.138 | 0.000 | 0.000 |
| All | 0.013 | 0.067 | 0.001 | 0.003 |

**Locus Rpi101**

| Population | Alleles | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **163** | **167** | **171** | **175** | **179** | **183** | **187** | **191** | **195** | **199** | **203** | **207** | **211** | **219** |
| CL | 0.000 | 0.000 | 0.038 | 0.000 | 0.000 | 0.075 | 0.000 | 0.188 | 0.238 | 0.088 | 0.275 | 0.075 | 0.025 | 0.000 |
| R1 | 0.000 | 0.000 | 0.125 | 0.038 | 0.013 | 0.063 | 0.000 | 0.238 | 0.225 | 0.100 | 0.125 | 0.050 | 0.025 | 0.000 |
| WW | 0.000 | 0.000 | 0.113 | 0.075 | 0.025 | 0.075 | 0.013 | 0.163 | 0.063 | 0.138 | 0.225 | 0.113 | 0.000 | 0.000 |
| BL | 0.000 | 0.000 | 0.050 | 0.038 | 0.025 | 0.075 | 0.013 | 0.225 | 0.200 | 0.075 | 0.213 | 0.050 | 0.038 | 0.000 |
| HN | 0.000 | 0.000 | 0.100 | 0.100 | 0.013 | 0.138 | 0.000 | 0.150 | 0.088 | 0.263 | 0.113 | 0.038 | 0.000 | 0.000 |
| PR | 0.000 | 0.000 | 0.038 | 0.000 | 0.100 | 0.250 | 0.025 | 0.063 | 0.250 | 0.113 | 0.100 | 0.063 | 0.000 | 0.000 |
| NE | 0.000 | 0.000 | 0.038 | 0.075 | 0.000 | 0.088 | 0.000 | 0.138 | 0.225 | 0.200 | 0.200 | 0.038 | 0.000 | 0.000 |
| SWL | 0.000 | 0.000 | 0.063 | 0.050 | 0.063 | 0.125 | 0.025 | 0.075 | 0.275 | 0.175 | 0.013 | 0.138 | 0.000 | 0.000 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JR | 0.000 | 0.013 | 0.025 | 0.025 | 0.038 | 0.075 | 0.000 | 0.138 | 0.363 | 0.125 | 0.113 | 0.050 | 0.038 | 0.000 |
| SL | 0.013 | 0.000 | 0.013 | 0.000 | 0.038 | 0.250 | 0.000 | 0.138 | 0.188 | 0.125 | 0.125 | 0.088 | 0.000 | 0.025 |
| FL | 0.000 | 0.000 | 0.113 | 0.038 | 0.063 | 0.050 | 0.013 | 0.188 | 0.225 | 0.150 | 0.125 | 0.038 | 0.000 | 0.000 |
| R2 | 0.000 | 0.000 | 0.163 | 0.013 | 0.038 | 0.113 | 0.000 | 0.138 | 0.163 | 0.113 | 0.163 | 0.100 | 0.000 | 0.000 |
| All | 0.001 | 0.001 | 0.073 | 0.038 | 0.034 | 0.115 | 0.007 | 0.153 | 0.208 | 0.139 | 0.149 | 0.070 | 0.010 | 0.002 |

**Locus Rpi103**

| **Population** | **Alleles** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **143** | **145** | **147** | **149** | **151** | **153** | **155** | **157** | **159** | **161** | **163** | **165** | **167** | **169** |
| CL | 0.050 | 0.013 | 0.000 | 0.000 | 0.000 | 0.013 | 0.075 | 0.038 | 0.000 | 0.025 | 0.000 | 0.013 | 0.000 | 0.000 |
| R1 | 0.025 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.075 | 0.038 | 0.000 | 0.025 | 0.000 | 0.075 | 0.000 | 0.000 |
| WW | 0.088 | 0.113 | 0.000 | 0.013 | 0.013 | 0.000 | 0.000 | 0.013 | 0.013 | 0.013 | 0.000 | 0.063 | 0.000 | 0.025 |
| BL | 0.075 | 0.063 | 0.000 | 0.000 | 0.025 | 0.013 | 0.038 | 0.063 | 0.013 | 0.000 | 0.000 | 0.088 | 0.000 | 0.000 |
| HN | 0.125 | 0.075 | 0.000 | 0.000 | 0.000 | 0.000 | 0.088 | 0.025 | 0.063 | 0.000 | 0.013 | 0.025 | 0.000 | 0.000 |
| PR | 0.013 | 0.025 | 0.013 | 0.000 | 0.013 | 0.000 | 0.013 | 0.075 | 0.025 | 0.013 | 0.000 | 0.025 | 0.000 | 0.000 |
| NE | 0.038 | 0.100 | 0.000 | 0.038 | 0.013 | 0.000 | 0.013 | 0.038 | 0.000 | 0.050 | 0.000 | 0.038 | 0.000 | 0.000 |
| SWL | 0.013 | 0.063 | 0.000 | 0.013 | 0.025 | 0.000 | 0.025 | 0.063 | 0.000 | 0.013 | 0.000 | 0.088 | 0.000 | 0.000 |
| JR | 0.013 | 0.088 | 0.000 | 0.000 | 0.013 | 0.000 | 0.050 | 0.050 | 0.000 | 0.013 | 0.000 | 0.138 | 0.000 | 0.000 |
| SL | 0.100 | 0.088 | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 | 0.038 | 0.025 | 0.000 | 0.000 | 0.100 | 0.013 | 0.000 |
| FL | 0.013 | 0.063 | 0.000 | 0.000 | 0.000 | 0.000 | 0.063 | 0.050 | 0.000 | 0.063 | 0.000 | 0.038 | 0.000 | 0.000 |
| R2 | 0.025 | 0.025 | 0.000 | 0.000 | 0.000 | 0.000 | 0.050 | 0.050 | 0.000 | 0.013 | 0.000 | 0.013 | 0.013 | 0.000 |
| All | 0.048 | 0.059 | 0.001 | 0.005 | 0.008 | 0.002 | 0.043 | 0.045 | 0.011 | 0.019 | 0.001 | 0.058 | 0.002 | 0.002 |
| | **171** | **173** | **175** | **177** | **179** | **181** | **183** | **185** | **187** | **189** | **191** | **193** | **195** | **197** |
| CL | 0.000 | 0.000 | 0.000 | 0.113 | 0.000 | 0.013 | 0.225 | 0.125 | 0.000 | 0.138 | 0.013 | 0.013 | 0.013 | 0.000 |
| R1 | 0.000 | 0.100 | 0.000 | 0.138 | 0.000 | 0.000 | 0.088 | 0.113 | 0.013 | 0.100 | 0.050 | 0.000 | 0.000 | 0.025 |
| WW | 0.000 | 0.000 | 0.000 | 0.163 | 0.013 | 0.000 | 0.113 | 0.075 | 0.000 | 0.000 | 0.113 | 0.000 | 0.013 | 0.000 |
| BL | 0.000 | 0.025 | 0.000 | 0.063 | 0.000 | 0.000 | 0.250 | 0.063 | 0.000 | 0.000 | 0.075 | 0.000 | 0.025 | 0.000 |
| HN | 0.000 | 0.088 | 0.013 | 0.063 | 0.013 | 0.000 | 0.163 | 0.113 | 0.000 | 0.000 | 0.063 | 0.000 | 0.000 | 0.000 |
| PR | 0.000 | 0.075 | 0.025 | 0.125 | 0.013 | 0.000 | 0.075 | 0.138 | 0.000 | 0.063 | 0.050 | 0.013 | 0.025 | 0.013 |
| NE | 0.000 | 0.038 | 0.000 | 0.075 | 0.000 | 0.000 | 0.200 | 0.100 | 0.000 | 0.000 | 0.063 | 0.013 | 0.000 | 0.050 |
| SWL | 0.000 | 0.100 | 0.000 | 0.038 | 0.000 | 0.000 | 0.050 | 0.125 | 0.000 | 0.013 | 0.025 | 0.013 | 0.025 | 0.025 |
| JR | 0.000 | 0.038 | 0.000 | 0.113 | 0.025 | 0.000 | 0.100 | 0.038 | 0.013 | 0.063 | 0.038 | 0.013 | 0.000 | 0.000 |
| SL | 0.013 | 0.075 | 0.000 | 0.125 | 0.000 | 0.000 | 0.075 | 0.013 | 0.013 | 0.113 | 0.038 | 0.013 | 0.000 | 0.000 |
| FL | 0.013 | 0.025 | 0.000 | 0.113 | 0.000 | 0.000 | 0.200 | 0.038 | 0.013 | 0.063 | 0.075 | 0.000 | 0.000 | 0.025 |
| R2 | 0.000 | 0.050 | 0.038 | 0.100 | 0.013 | 0.000 | 0.150 | 0.188 | 0.000 | 0.050 | 0.063 | 0.000 | 0.000 | 0.025 |
| All | 0.002 | 0.051 | 0.006 | 0.102 | 0.006 | 0.001 | 0.141 | 0.094 | 0.004 | 0.050 | 0.055 | 0.006 | 0.008 | 0.014 |
| | **201** | **203** | **205** | **207** | **209** | **211** | **213** | **215** | **217** | **219** | **223** | **227** | **235** | |
| CL | 0.000 | 0.000 | 0.000 | 0.000 | 0.038 | 0.000 | 0.038 | 0.000 | 0.000 | 0.013 | 0.000 | 0.013 | 0.025 | |
| R1 | 0.000 | 0.000 | 0.025 | 0.025 | 0.038 | 0.013 | 0.013 | 0.000 | 0.000 | 0.013 | 0.000 | 0.000 | 0.013 | |
| WW | 0.000 | 0.000 | 0.000 | 0.000 | 0.050 | 0.013 | 0.088 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.013 | |
| BL | 0.013 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.088 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 | |
| HN | 0.000 | 0.000 | 0.013 | 0.000 | 0.000 | 0.000 | 0.038 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 | |
| PR | 0.025 | 0.000 | 0.025 | 0.000 | 0.013 | 0.013 | 0.025 | 0.000 | 0.000 | 0.013 | 0.013 | 0.013 | 0.038 | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NE | 0.050 | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 | 0.038 | 0.000 | 0.000 | 0.000 | 0.013 | 0.000 | 0.013 |
| SWL | 0.038 | 0.000 | 0.075 | 0.000 | 0.013 | 0.063 | 0.063 | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | 0.013 |
| JR | 0.038 | 0.013 | 0.025 | 0.000 | 0.000 | 0.063 | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 | 0.000 | 0.000 |
| SL | 0.038 | 0.000 | 0.000 | 0.013 | 0.000 | 0.050 | 0.013 | 0.000 | 0.000 | 0.013 | 0.000 | 0.000 | 0.013 |
| FL | 0.000 | 0.000 | 0.000 | 0.000 | 0.075 | 0.000 | 0.050 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 |
| R2 | 0.013 | 0.000 | 0.013 | 0.013 | 0.050 | 0.000 | 0.025 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 |
| All | 0.018 | 0.001 | 0.015 | 0.004 | 0.023 | 0.020 | 0.041 | 0.001 | 0.001 | 0.007 | 0.003 | 0.002 | 0.019 |

**Locus Rpi107**

| **Population** | **Alleles** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **181** | **185** | **189** | **193** | **197** | **201** | **205** | **209** | **213** | **217** | **221** | **225** |
| CL | 0.000 | 0.063 | 0.038 | 0.113 | 0.000 | 0.025 | 0.025 | 0.300 | 0.088 | 0.350 | 0.000 | 0.000 |
| R1 | 0.000 | 0.050 | 0.000 | 0.150 | 0.000 | 0.013 | 0.050 | 0.275 | 0.075 | 0.388 | 0.000 | 0.000 |
| WW | 0.000 | 0.025 | 0.000 | 0.113 | 0.000 | 0.063 | 0.038 | 0.375 | 0.050 | 0.325 | 0.000 | 0.013 |
| BL | 0.000 | 0.025 | 0.013 | 0.138 | 0.000 | 0.013 | 0.038 | 0.300 | 0.100 | 0.375 | 0.000 | 0.000 |
| HN | 0.000 | 0.100 | 0.013 | 0.050 | 0.013 | 0.063 | 0.100 | 0.175 | 0.013 | 0.438 | 0.038 | 0.000 |
| PR | 0.000 | 0.000 | 0.063 | 0.063 | 0.013 | 0.038 | 0.075 | 0.425 | 0.113 | 0.213 | 0.000 | 0.000 |
| NE | 0.000 | 0.038 | 0.000 | 0.113 | 0.000 | 0.013 | 0.000 | 0.413 | 0.038 | 0.388 | 0.000 | 0.000 |
| SWL | 0.000 | 0.038 | 0.050 | 0.050 | 0.000 | 0.063 | 0.038 | 0.213 | 0.063 | 0.413 | 0.000 | 0.075 |
| JR | 0.000 | 0.050 | 0.050 | 0.050 | 0.000 | 0.125 | 0.100 | 0.375 | 0.075 | 0.175 | 0.000 | 0.000 |
| SL | 0.013 | 0.163 | 0.000 | 0.175 | 0.000 | 0.038 | 0.075 | 0.300 | 0.000 | 0.238 | 0.000 | 0.000 |
| FL | 0.000 | 0.050 | 0.000 | 0.188 | 0.000 | 0.075 | 0.050 | 0.288 | 0.113 | 0.225 | 0.013 | 0.000 |
| R2 | 0.000 | 0.025 | 0.050 | 0.150 | 0.000 | 0.013 | 0.025 | 0.250 | 0.100 | 0.388 | 0.000 | 0.000 |
| All | 0.001 | 0.052 | 0.023 | 0.113 | 0.002 | 0.045 | 0.051 | 0.307 | 0.069 | 0.326 | 0.004 | 0.007 |

**Locus Rpi108**

| **Population** | **Alleles** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 265 | 267 | 269 | 271 | 273 | 275 | 277 | 279 | 283 | 285 | 287 | 289 | 291 | 293 |
| CL | 0.000 | 0.013 | 0.000 | 0.000 | 0.175 | 0.050 | 0.013 | 0.000 | 0.013 | 0.475 | 0.000 | 0.000 | 0.013 | 0.000 |
| R1 | 0.000 | 0.013 | 0.025 | 0.025 | 0.100 | 0.038 | 0.075 | 0.000 | 0.013 | 0.425 | 0.025 | 0.000 | 0.013 | 0.000 |
| WW | 0.000 | 0.013 | 0.038 | 0.000 | 0.100 | 0.025 | 0.013 | 0.000 | 0.000 | 0.450 | 0.013 | 0.000 | 0.000 | 0.000 |
| BL | 0.000 | 0.000 | 0.038 | 0.000 | 0.113 | 0.025 | 0.050 | 0.013 | 0.000 | 0.463 | 0.000 | 0.000 | 0.013 | 0.013 |
| HN | 0.000 | 0.000 | 0.063 | 0.000 | 0.025 | 0.013 | 0.088 | 0.000 | 0.000 | 0.513 | 0.000 | 0.000 | 0.013 | 0.000 |
| PR | 0.000 | 0.000 | 0.075 | 0.013 | 0.063 | 0.075 | 0.025 | 0.025 | 0.000 | 0.250 | 0.025 | 0.013 | 0.000 | 0.000 |
| NE | 0.000 | 0.000 | 0.050 | 0.025 | 0.150 | 0.013 | 0.050 | 0.050 | 0.000 | 0.338 | 0.025 | 0.013 | 0.000 | 0.025 |
| SWL | 0.000 | 0.000 | 0.063 | 0.000 | 0.150 | 0.063 | 0.025 | 0.050 | 0.013 | 0.263 | 0.013 | 0.025 | 0.013 | 0.013 |
| JR | 0.000 | 0.000 | 0.088 | 0.000 | 0.163 | 0.088 | 0.025 | 0.038 | 0.000 | 0.238 | 0.013 | 0.025 | 0.000 | 0.000 |
| SL | 0.013 | 0.038 | 0.038 | 0.013 | 0.075 | 0.113 | 0.138 | 0.000 | 0.013 | 0.225 | 0.013 | 0.025 | 0.025 | 0.000 |
| FL | 0.000 | 0.013 | 0.025 | 0.000 | 0.100 | 0.050 | 0.063 | 0.000 | 0.113 | 0.288 | 0.038 | 0.000 | 0.000 | 0.013 |
| R2 | 0.000 | 0.000 | 0.013 | 0.063 | 0.088 | 0.038 | 0.013 | 0.000 | 0.050 | 0.300 | 0.038 | 0.000 | 0.000 | 0.000 |
| All | 0.001 | 0.007 | 0.043 | 0.011 | 0.108 | 0.049 | 0.048 | 0.015 | 0.018 | 0.352 | 0.017 | 0.008 | 0.007 | 0.005 |

| | **295** | **297** | **299** | **301** | **303** | **305** | **307** | **309** | **311** | **313** | **315** | **317** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CL | 0.000 | 0.000 | 0.013 | 0.013 | 0.088 | 0.038 | 0.050 | 0.050 | 0.000 | 0.000 | 0.000 | 0.000 |
| R1 | 0.013 | 0.013 | 0.000 | 0.000 | 0.100 | 0.038 | 0.025 | 0.050 | 0.013 | 0.000 | 0.000 | 0.000 |
| WW | 0.000 | 0.000 | 0.000 | 0.000 | 0.038 | 0.075 | 0.125 | 0.113 | 0.000 | 0.000 | 0.000 | 0.000 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BL | 0.000 | 0.013 | 0.000 | 0.000 | 0.088 | 0.025 | 0.038 | 0.100 | 0.013 | 0.000 | 0.000 | 0.000 |
| HN | 0.000 | 0.013 | 0.000 | 0.000 | 0.038 | 0.038 | 0.063 | 0.100 | 0.038 | 0.000 | 0.000 | 0.000 |
| PR | 0.038 | 0.013 | 0.063 | 0.013 | 0.138 | 0.038 | 0.063 | 0.075 | 0.000 | 0.000 | 0.000 | 0.000 |
| NE | 0.088 | 0.013 | 0.000 | 0.050 | 0.063 | 0.000 | 0.038 | 0.013 | 0.000 | 0.000 | 0.000 | 0.000 |
| SWL | 0.000 | 0.025 | 0.038 | 0.013 | 0.100 | 0.000 | 0.038 | 0.050 | 0.025 | 0.000 | 0.000 | 0.025 |
| JR | 0.038 | 0.000 | 0.000 | 0.013 | 0.150 | 0.013 | 0.038 | 0.025 | 0.025 | 0.013 | 0.013 | 0.000 |
| SL | 0.013 | 0.000 | 0.000 | 0.025 | 0.100 | 0.050 | 0.013 | 0.075 | 0.000 | 0.000 | 0.000 | 0.000 |
| FL | 0.013 | 0.000 | 0.000 | 0.000 | 0.063 | 0.075 | 0.100 | 0.050 | 0.000 | 0.000 | 0.000 | 0.000 |
| R2 | 0.025 | 0.038 | 0.000 | 0.038 | 0.113 | 0.013 | 0.100 | 0.075 | 0.000 | 0.000 | 0.000 | 0.000 |
| All | 0.019 | 0.010 | 0.009 | 0.014 | 0.090 | 0.033 | 0.057 | 0.065 | 0.009 | 0.001 | 0.001 | 0.002 |

**Locus RP193**

| **Population** | **Alleles** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **134** | **146** | **150** | **154** | **158** | **162** | **166** | **170** | **174** | **178** | **182** | **186** | **190** | **194** |
| CL | 0.000 | 0.075 | 0.038 | 0.050 | 0.238 | 0.150 | 0.150 | 0.113 | 0.063 | 0.013 | 0.063 | 0.025 | 0.013 | 0.013 |
| R1 | 0.000 | 0.050 | 0.075 | 0.150 | 0.075 | 0.275 | 0.100 | 0.113 | 0.113 | 0.000 | 0.013 | 0.000 | 0.000 | 0.038 |
| WW | 0.000 | 0.138 | 0.038 | 0.113 | 0.125 | 0.150 | 0.125 | 0.100 | 0.038 | 0.013 | 0.088 | 0.050 | 0.025 | 0.000 |
| BL | 0.000 | 0.063 | 0.025 | 0.125 | 0.150 | 0.250 | 0.100 | 0.188 | 0.013 | 0.000 | 0.075 | 0.013 | 0.000 | 0.000 |
| HN | 0.000 | 0.150 | 0.000 | 0.100 | 0.175 | 0.175 | 0.225 | 0.100 | 0.000 | 0.000 | 0.025 | 0.038 | 0.000 | 0.013 |
| PR | 0.000 | 0.050 | 0.050 | 0.050 | 0.175 | 0.225 | 0.163 | 0.125 | 0.013 | 0.025 | 0.100 | 0.013 | 0.000 | 0.000 |
| NE | 0.013 | 0.063 | 0.038 | 0.025 | 0.200 | 0.200 | 0.063 | 0.125 | 0.138 | 0.000 | 0.125 | 0.013 | 0.000 | 0.000 |
| SWL | 0.000 | 0.025 | 0.050 | 0.113 | 0.150 | 0.138 | 0.100 | 0.225 | 0.050 | 0.050 | 0.100 | 0.000 | 0.000 | 0.000 |
| JR | 0.000 | 0.075 | 0.050 | 0.050 | 0.138 | 0.263 | 0.050 | 0.050 | 0.063 | 0.025 | 0.100 | 0.088 | 0.013 | 0.038 |
| SL | 0.000 | 0.013 | 0.063 | 0.100 | 0.038 | 0.275 | 0.225 | 0.025 | 0.125 | 0.000 | 0.063 | 0.013 | 0.000 | 0.050 |
| FL | 0.000 | 0.063 | 0.075 | 0.138 | 0.163 | 0.125 | 0.113 | 0.213 | 0.038 | 0.013 | 0.050 | 0.013 | 0.000 | 0.000 |
| R2 | 0.000 | 0.025 | 0.038 | 0.150 | 0.213 | 0.225 | 0.100 | 0.100 | 0.075 | 0.000 | 0.050 | 0.013 | 0.000 | 0.013 |
| All | 0.001 | 0.066 | 0.045 | 0.097 | 0.153 | 0.204 | 0.126 | 0.123 | 0.060 | 0.011 | 0.071 | 0.023 | 0.004 | 0.014 |

| | **198** | **214** |
|---|---|---|
| CL | 0.000 | 0.000 |
| R1 | 0.000 | 0.000 |
| WW | 0.000 | 0.000 |
| BL | 0.000 | 0.000 |
| HN | 0.000 | 0.000 |
| PR | 0.000 | 0.013 |
| NE | 0.000 | 0.000 |
| SWL | 0.000 | 0.000 |
| JR | 0.000 | 0.000 |
| SL | 0.013 | 0.000 |
| FL | 0.000 | 0.000 |
| R2 | 0.000 | 0.000 |
| All | 0.001 | 0.001 |