

# The genetic structure of a relict population of wood frogs

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**Abstract** Habitat fragmentation and the associated reduction in connectivity between habitat patches are commonly cited causes of genetic differentiation and reduced genetic variation in animal populations. We used eight microsatellite markers to investigate genetic structure and levels of genetic diversity in a relict population of wood frogs (*Lithobates sylvatica*) in Rocky Mountain National Park, Colorado, where recent disturbances have altered hydrologic processes and fragmented amphibian habitat. We also estimated migration rates among subpopulations, tested for a pattern of isolation-by-distance, and looked for evidence of a recent population bottleneck. The results from the clustering algorithm in Program STRUCTURE indicated the population is partitioned into two genetic clusters (subpopulations), and this result was further supported by factorial component analysis. In addition, an estimate of  $F_{ST}$  ( $F_{ST} = 0.0675$ ,  $P$  value  $< 0.0001$ ) supported the genetic differentiation of the two clusters. Estimates of migration rates among the two subpopulations were low, as were estimates of genetic variability. Conservation of the population of wood frogs may be improved by increasing the spatial distribution of the population and improving gene flow between the subpopulations. Construction or restoration of wetlands in the landscape between the clusters has the potential to address each of these objectives.

**Keywords** Habitat fragmentation · Genetic differentiation · Amphibians · *Lithobates sylvatica*

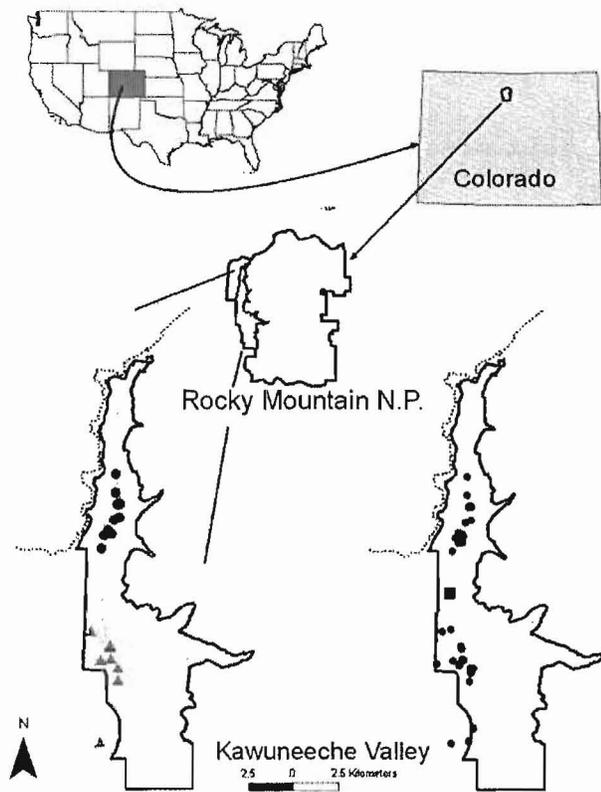
## Introduction

Anthropogenic alterations to landscapes and the subsequent fragmentation of habitat are frequently cited as causes of reduced connectivity among local populations of animals (Shepard et al. 2008; Ford and Fahrig 2008; Pruett et al. 2009). A reduction in connectivity can lead to genetic differentiation among local populations and lower genetic variation within them (Frankham 2006; Allentoft et al. 2009). Relative to other terrestrial vertebrates, amphibians may be particularly vulnerable to landscape changes that fragment habitat due to their small body size and physiological requirement for moist conditions (Gibbs 1998; but see Green 2003). Change in the permeability of the landscape between habitat patches can have important deleterious impacts on amphibian populations (Funk et al. 2005). Several studies have examined the effects of habitat fragmentation on amphibian populations and detected reduced genetic variability in local populations, as well as significant genetic differentiation between them (Andersen et al. 2004; Arens et al. 2007; Noel et al. 2007; Spear and Storfer 2008).

Wood frogs (*Lithobates sylvatica*) are one of the most widespread amphibian species in North America, ranging from southern portions of the eastern seaboard in the U.S. to beyond the Arctic Circle in Canada (Stebbins 2003). In northern Colorado and southern Wyoming, however, wood frog populations occur in a few, relatively small areas (Hammerson 1999) and are relicts from a population that may have once been distributed across much of the southwestern U.S. (Lee-Yaw et al. 2008). Because of their restricted range, they are listed as a species of concern in

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**Fig. 1** Map of the Kawuneeche Valley (KV) in Rocky Mountain National Park, Colorado. The Grand Ditch is represented by the dotted line. The map on the left shows the wetlands from which genetic samples were collected. The results from Program STRUCTURE suggest two genetic clusters in the KV: a northern (black circles) and southern (gray triangles) cluster. The map of the KV on the right shows the locations of wetlands (solid, black circles) where wood frogs were detected during surveys from 2004 to 2006 (the black square in the center represents the only wetland at which breeding by wood frogs was detected in the area between genetic clusters)

Colorado and a species of “greatest conservation need” in Wyoming (Colorado Division of Wildlife 2005; Wyoming Game and Fish Department 2005). The wood frog is one of only 4 extant amphibian species in Rocky Mountain National Park, Colorado (Corn et al. 1997) and is restricted to the Kawuneeche Valley (KV) on the western side of the park (Fig. 1). Therefore, natural and anthropogenic disturbances in the KV may threaten the long-term persistence of this species in the park.

With respect to wetlands and amphibians, there have been 2 important disturbances to the landscape and biota of the KV over the last 50–100 years: construction of the Grand Ditch and significant reduction in the abundance of beaver (*Castor canadensis*). The Grand Ditch is a water diversion ditch that is over 22 km long and runs along the western slope of the KV at an elevation of approximately 3,100 m (Fig. 1). Construction of the ditch and diversion of

water started in the 1890s, and diversion over its entire length began in 1936 (Perry 2008). Woods (2000) estimates the ditch intercepts about 29 % of the total runoff from the watershed each year. Consequently, operation of the ditch has reduced the surface flow of water into the KV, reduced the amount and frequency of flooding, and lowered water levels in streams and rivers (Woods 2000, 2001). Each of these processes is an important mechanism for maintaining water table levels in the KV, particularly given its location in the relatively arid interior of the western U.S.

In addition to the disturbance caused by the ditch, the beaver population in the KV has declined over 90 % in the last 60 years (Westbrook et al. 2006). Whether the decline in beaver populations in the KV is related to the operation of the Grand Ditch is not known but the lack of newly constructed beaver dams and maintenance on existing dams has also reduced the frequency, extent, and duration of flooding on the floor of the valley (Woods 2000; Westbrook et al. 2006).

Though these disturbances appear to have fragmented wetland habitat, effects on the population of wood frogs are not known. Our objectives were to document the distribution of genetic variation across the KV to determine if population structure exists, and document levels of genetic diversity within the KV. Additionally, we were interested in investigating whether any genetic structure found was consistent with the changing hydrology of the KV, thereby providing indirect evidence for the potential impacts of changes in hydrology on the genetic structure and levels of genetic variation in this relict population of wood frogs.

## Methods

### Study area

The west side of Rocky Mountain National Park is dominated by the KV. This valley and the adjacent mountainsides up to 3,048 m elevation is the study area (Fig. 1). The KV contains the headwaters of the Colorado River and some of the largest remaining sub-alpine wetland complexes in Colorado (Woods 2000). Over 80 % of the study area is upland and is dominated by forests of lodgepole pine (*Pinus contorta*) and mixed coniferous species (*Abies lasiocarpa*, *Picea engelmannii*; Salas et al. 2005). The valley bottom is a mix of wetlands dominated by herbaceous vegetation (e.g., *Carex* spp.) and/or shrubs (*Salix* spp.) and riparian areas.

### Field methods

Wood frogs deposit their eggs in clumps (i.e., egg masses) that are approximately 8 cm in diameter (Hammerson 1999), and individual egg masses can be distinguished

easily from one another. In the southern Rocky Mountains, reproducing females deposit a single egg mass in the spring (Corn and Livo 1989). Therefore, collecting eggs from different egg masses minimizes the probability of sampling known siblings.

We developed a list of over 40 wetlands to search for egg masses based on 2 years of extensive surveys. Wetlands were included in the list if wood frog reproduction (i.e., egg masses or tadpoles) or wood frogs were observed in either of the 2 years. In the spring of 2006, each of these wetlands was surveyed for egg masses by at least two field workers. Multiple eggs or hatchlings were collected from each egg mass but only one egg or hatchling was used in the subsequent genetic analyses. Oviposition in populations of wood frogs occurs over a short period of time in the spring (5–10 days; Petranka et al. 2004). To maximize the probability that oviposition at each wetland was complete at the time of the survey, surveys were conducted a minimum of 6 days after observing the first egg mass in the KV. The fact that egg masses are highly detectable (Grant et al. 2005; Scherer 2008) and no new egg masses were observed on subsequent visits to a subset of the wetlands suggests a high percentage of egg masses were present at the time of the surveys.

#### Genetic processing of samples

DNA was extracted using the Wizard Genomic DNA Purification System (Promega) following manufacturer's specifications. All 160 wood frog samples were screened using eight nuclear microsatellite loci. We used eight microsatellite markers (*RsyC11*, *RsyC63*, *RsyD20*, *RsyD32*, *RsyD40*, *RsyD55*, *RsyD70*, and *RsyD88*) that were isolated and characterized for *L. sylvatica* (Julian and King 2003). Amplifications were performed using a M13-tailed forward primer as described by Boutin-Ganache et al. (2001). Each 12.5  $\mu$ l reaction contained 125  $\mu$ M each dNTP, 1 $\times$  *Taq* buffer (Kahn et al. 1998), 0.034  $\mu$ M M13-tailed forward primer, 0.5  $\mu$ M non-tailed reverse primer, 0.5  $\mu$ M M13 dye-labeled primer with Beckman Coulter dyes D2, D3 or D4 (Proligo), and 0.31U *Taq* polymerase (Promega). The thermal profile for the M13 dye-labeled reactions was as follows with the annealing temperature varying: preheat at 94  $^{\circ}$ C for 2 min, denature at 94  $^{\circ}$ C for 40 s, anneal for 1 min, and extend at 72  $^{\circ}$ C for 1 min. Each PCR had 35 amplification cycles (MJ Research PTC-200, Bio-Rad) and concluded with a 10 min post heat at 72  $^{\circ}$ C for 5 min. The annealing temperature for *RsyD20* was 53  $^{\circ}$ C, for *RsyC11* and *RsyD40* was 58  $^{\circ}$ C, and for all other loci was 55  $^{\circ}$ C. The PCR products were diluted and run on the CEQ8000 XL DNA Analysis System (Beckman Coulter). All loci were run with the S400 size standard (Beckman Coulter) and analyzed using the Frag 3 default method.

#### Genetic analyses

Initially, we pooled all individuals into one "population" and tested for departures from HWE using GENEPOP on the web (Raymond and Rousset 1995; Markov chain parameters: 10,000 dememorization steps, 1,000 batches, and 10,000 iterations per batch). To determine if *P* values indicated a significant departure from H–W equilibrium, we used a Bonferroni-corrected *P* value of  $\alpha = 0.006$ . We also tested for linkage disequilibrium for each pair of loci GENEPOP (Raymond and Rousset 1995; Markov chain parameters: 10,000 dememorization steps, 1,000 batches, and 10,000 iterations per batch).

We used Program STRUCTURE 2.0 to evaluate the genetic structure of the population of wood frogs. Specifically, we estimated the number of genetically-distinct population clusters (Pritchard et al. 2000). Program STRUCTURE uses a model-based clustering algorithm to estimate the number of clusters, *K*, given the data (Pritchard et al. 2000). We set the range of *K* from 1 to 12. Final runs were performed using the admixture model with correlated allele frequencies at burn-in length of 100,000 and MCMC of 250,000 (20 runs per *K*). We used the estimated average log likelihood of the data given *K*,  $\text{Ln}(\bar{X}IK)$ , and  $\Delta K$  (Evanno et al. 2005) as the metrics for selecting the number of genetic clusters given the data. We visualized these metrics in STRUCTURE HARVESTER (Earl and vonHolt 2011). Higher values of the metrics indicate greater support for a given number of genetic clusters (Evanno et al. 2005; Allentoft et al. 2009). The use of multiple runs to evaluate *K* in STRUCTURE can produce several distinct solutions due to label switching across replicates. Therefore, we used CLUMPP (Jakobsson and Rosenberg 2007) to produce the final output. We visualized the results from CLUMPP analysis using DISTRUCT (Rosenberg 2002). We used a factorial component analysis (FCA) in GENETIX (Belkhir et al. 2004) to conduct a second test of population genetic structure. FCA uses microsatellite genotypes to determine structural relationships among individuals with no a priori assumptions about those relationships.

After identifying genetic clusters in Program STRUCTURE, we treated each cluster as a subpopulation and used GENEPOP to test for departures from HWE (using a Bonferroni-corrected *P* value of 0.003) and ARLEQUIN 2.0 (Schneider et al. 2000) to estimate genetic variability within each subpopulation and  $F_{ST}$  between them. We also tested each subpopulation for evidence of a bottleneck using two separate approaches. In ARLEQUIN, we calculated Garza and Williamson's *M*-ratio (Garza and Williamson 2001). Additionally, we used the software BOTTLENECK (Cornuet and Luikart 1996) using all three models of mutation (infinite alleles model [IAM], stepwise mutation model [SMM] and two-phased mutation model

[TPM]). Both methods investigate population bottlenecks by measuring post-decline reductions in heterozygosity and number of alleles. We estimated migration rates between the subpopulations using BayesAss ver. 1.3 (Wilson and Rannala 2003) and used GeneClass2 (Piry et al. 2004) to determine if putative migrants were first generation migrants. In the analysis using BayesAss, we ran the MCMC for 3,000,000 iterations with a burn-in period of 999,999 and sampled every 2,000 iterations. Delta values were varied to optimize maximum likelihood values. We used the approach of Paetkau et al. (1995) in GeneClass2 and 10,000 simulations [following Paetkau et al. (2004)] with a Type I error rate of 0.01.

Finally, we investigated patterns of spatial genetic structure using the spatial autocorrelation test implemented in the software Alleles in Space (Miller 2005). We conducted the spatial autocorrelation test within the northern and southern subpopulations separately and then pooled all individuals and tested for spatial autocorrelation within the larger population. For all tests, we used ten distance classes constructed using distance classes of equal size but unequal sample sizes and 10,000 permutations.

## Results

We collected eggs or hatchlings from 160 egg masses at 19 wetlands in the study area and from one wetland on private property west of the KV (WRR Pond; Fig. 1; Table 1). All microsatellite loci were polymorphic, and the number of alleles for each locus ranged from 3 to 18 (Table 2). When all individuals were pooled into one population, four loci were out of H–W equilibrium; indicating a possible Wahlund effect. We observed no evidence of linkage disequilibrium across all pairs of loci (smallest  $P$  value = 0.16).

The most appropriate number of unique genetic clusters for the data was 2 using both the method of Pritchard et al. (2000) and the method of Evanno et al. (2005) (Fig. 2). The two unique genetic clusters were spatially disjunct and consisted of a northern and a southern cluster (Figs. 1, 3). Hereafter, we will refer to these clusters as the northern and southern subpopulations. Of the 77 individuals sampled from wetlands in the northern part of the KV, 65 individuals (84 %) had a high probability (>0.80) of assignment to the northern subpopulation (Figs. 1, 3). Sixty-nine of 83 individuals (83 %) sampled from southern wetlands had a high probability of assignment to the southern subpopulation. A few individuals, however, had high probabilities of assignment to subpopulations that were not consistent with the location from which they were sampled (e.g., the last individual in wetland #5050 had a high probability of assignment to the southern subpopulation even though it was sampled from a northern wetland; Fig. 3). Our tests for

departures from HWE within the two subpopulations revealed that no locus was out of HWE in both subpopulations. Two loci in the northern subpopulation remained out of HWE. We then looked for evidence of genetic structure at finer resolution by analyzing the data from the northern and southern subpopulations separately in Program STRUCTURE. The data provided no evidence of finer genetic structure (the model of  $K = 1$  in the northern and southern subpopulations received the most support). The results from the FCA analysis were consistent with the results from STRUCTURE. FCA revealed relationships among the individuals that were consistent with two populations that were summarized by two underlying dimensions (Fig. 4). The first two factors explained 5.45 and 4.37 % of the variation respectively. The average numbers of alleles across loci in each subpopulation were 5.5 (northern) and 7.63 (southern; Table 1), though there was no evidence that observed heterozygosity varied between the subpopulations. Estimates of  $F_{ST}$  indicate high levels of genetic differentiation between the northern and southern subpopulations ( $F_{ST} = 0.0675$ ,  $P$  value <0.0001).

Regarding the tests for bottlenecks in each subpopulation, the mean  $M$ -ratio for the northern subpopulation was 0.83 (SD = 0.20) and for the southern subpopulation was 0.83 (SD = 0.19). These values are well within the range of stable populations described in Garza and Williamson (2001). The BOTTLENECK results varied depending on which mutation model was used. The results from SMM were drastically different than results from the IAM or the TPM. Because many of the loci we used were bimodally distributed with regard to allele sizes, the SMM is likely not a good fit for these data. In the northern subpopulation, neither the IAM ( $P = 0.09$ ) nor TPM ( $P = 0.09$ ) provided statistically significant evidence of a bottleneck using the sign test, whereas the Wilcoxon test showed a significant bottleneck with the IAM ( $P = 0.004$ ) but not with the TPM ( $P = 0.10$ ). The mode shift test did not support a bottleneck in the northern subpopulation. In the southern subpopulation, the sign test did indicate a statistically significant bottleneck using the IAM ( $P = 0.02$ ) but not with the TPM ( $P = 0.57$ ). The Wilcoxon test had a similar pattern of significance with the IAM ( $P = 0.002$ ) but not with the TPM ( $P = 0.13$ ). The mode shift test also did not support a bottleneck in the southern subpopulation.

Recent migration rates between the northern and southern subpopulations were low. The estimated proportion of individuals in the northern subpopulation that were migrants from the southern subpopulation was 0.00611 (95 % CI = 0.00017–0.02200), which is lower than the proportion of individuals in the southern subpopulation that were migrants from the northern subpopulation, 0.03191 (95 % CI = 0.00163–0.09158). GeneClass2 identified four

**Table 1** Number of sampled egg masses and genetic variation across the 20 sampled wetlands in the Kawuneeche Valley (KV)

Wetland	<i>N</i>	Subpopulation	<i>N</i> <sub>mean</sub>	Mean <i>H</i> <sub>O</sub> (SE)	Mean <i>H</i> <sub>E</sub> (SE)
03CR02	3	North	1.63	0.438 (0.151)	0.262 (0.081)
PSTR pond	1	North	1.25	0.250 (0.164)	0.125 (0.082)
FP pond	22	North	4.25	0.382 (0.069)	0.397 (0.082)
5050	4	North	2.63	0.469 (0.153)	0.430 (0.101)
5048	15	North	3.25	0.381 (0.088)	0.452 (0.081)
03BP02	4	North	2.63	0.438 (0.091)	0.426 (0.071)
BD pond	5	North	3.00	0.588 (0.114)	0.500 (0.074)
03TC02	2	North	1.88	0.500 (0.134)	0.344 (0.081)
5284	5	North	2.63	0.450 (0.118)	0.435 (0.089)
LDD pond	1	North	1.00	0.250 (0.164)	0.125 (0.082)
03TC03	4	North	2.63	0.542 (0.130)	0.469 (0.083)
03NS02	11	North	3.88	0.456 (0.097)	0.483 (0.094)
Gaskil pond	1	South	1.38	0.375 (0.183)	0.188 (0.091)
03GT01	8	South	3.63	0.398 (0.113)	0.483 (0.110)
03GT02	13	South	5.38	0.617 (0.077)	0.588 (0.064)
HS pond	32	South	6.00	0.567 (0.069)	0.593 (0.076)
5219	6	South	3.75	0.504 (0.079)	0.520 (0.076)
03OV03	12	South	4.50	0.711 (0.082)	0.621 (0.051)
03GM05	2	South	2.38	0.563 (0.148)	0.469 (0.081)
WRR pond	9	South	3.00	0.424 (0.076)	0.490 (0.081)
Northern population	77		5.50	0.428 (0.069)	0.495 (0.083)
Southern population	83		7.63	0.561 (0.054)	0.614 (0.068)

Results from Program STRUCTURE indicated the population was comprised of two genetic clusters (a northern and southern cluster); therefore, the number of sampled egg masses and genetic variation for the northern (03CR02 to 03NS02) and southern (Gaskil Pond to WRR Pond) clusters are also shown

*N* number of sampled egg masses, mean *N*<sub>mean</sub> mean number of alleles across loci, mean *H*<sub>O</sub> mean observed heterozygosity across loci, mean *H*<sub>E</sub> mean expected heterozygosity across loci

**Table 2** Genetic variation across the microsatellite loci

Locus	<i>N</i> <sub>A</sub>	<i>H</i> <sub>O</sub>	<i>H</i> <sub>E</sub>	<i>F</i> <sub>IS</sub>
<i>RsyC11</i>	14	0.690	0.75	0.75
<i>RsyD20</i>	7	0.52	0.72	0.28
<i>RsyD32</i>	4	0.39	0.39	0.02
<i>RsyD40</i>	3	0.16	0.16	0.08
<i>RsyD55</i>	6	0.56	0.56	0.01
<i>RsyD88</i>	7	0.60	0.60	0.17
<i>RsyC63</i>	18	0.87	0.87	0.28
<i>RsyD70</i>	9	0.58	0.58	0.06

*N*<sub>A</sub> total number of alleles at a locus across wetlands, *N*<sub>min</sub> the minimum number of alleles per locus at a wetland, *N*<sub>max</sub> the maximum number of alleles per locus at a wetland, and *N*<sub>mean</sub> the average number of alleles per locus across wetlands. *RsyD20* and *RsyD32* did not amplify from the single egg mass collected at LDD Pond; therefore, the minimum number of alleles for those microsatellite loci was 0

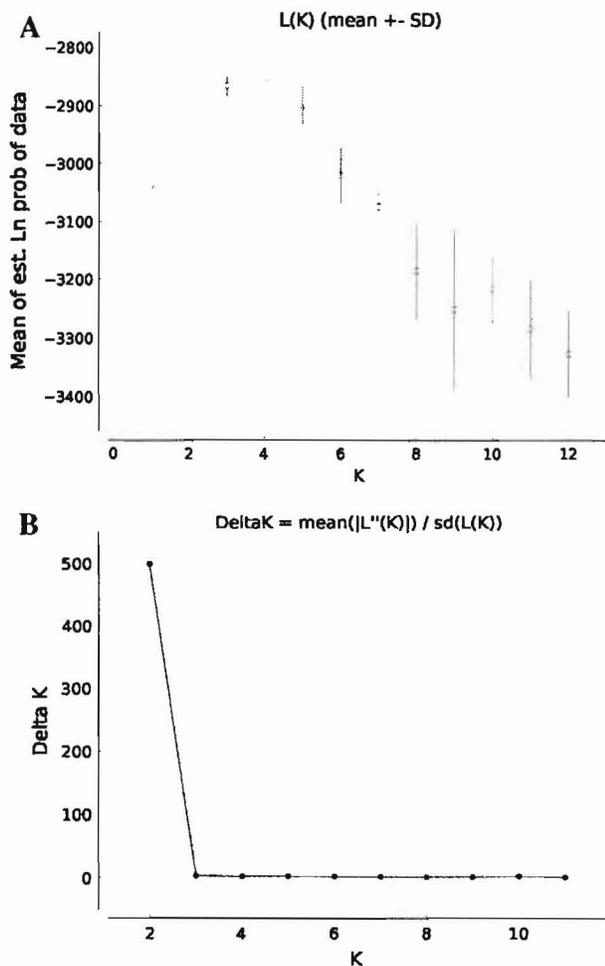
first generation migrants. Three (one from wetland 5050, one from wetland 03NS02, and one from Gaskil pond) were identified as migrants from the southern subpop-

ulation and one (from wetland 03GT01) was identified as a migrant from the northern subpopulation.

The tests for spatial autocorrelation for the northern and southern subpopulations do not support a pattern of increasing genetic distance with increases in geographic distance (north: *V* = 0.033, *P* = 0.37; south: *V* = 0.044, *P* = 0.22). However, the analysis for which all individuals were pooled across subpopulations supported a pattern of spatial autocorrelation (*V* = 0.068, *P* < 0.00001).

**Discussion**

The genetic clustering algorithm in Program STRUCTURE indicated that the wood frogs in the KV are structured as two subpopulations. This differentiation is supported by a multilocus Bayesian Analysis (STRUCTURE), FCA analysis (GENETIX), and more traditional metrics (*F*<sub>ST</sub>). However, a few individuals had high probabilities of assignment to a subpopulation that differed from the location from which they were sampled (STRUCTURE), and four individuals were identified as first generation



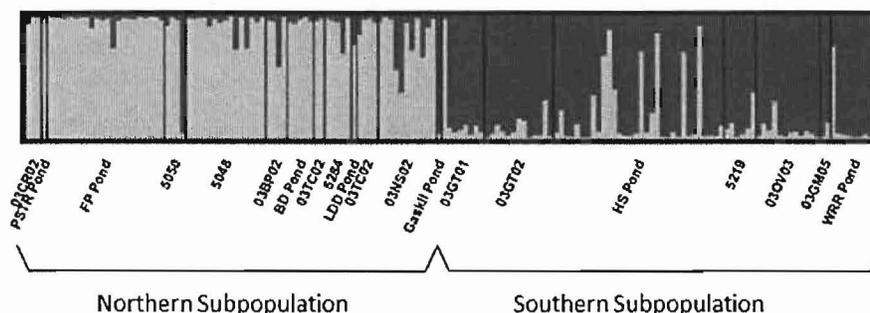
**Fig. 2** Estimation of the most appropriate number of unique genetic clusters using **a** the method described by Pritchard et al. (2000) and **b** the method described by Evanno et al. (2005)

migrants between the subpopulations (GeneClass2). These results suggest that, though rare, individuals are capable of moving between the subpopulations, yet at a much lower

rate than movement between wetlands within a subpopulation. The migration rates estimated in BayesAss confirmed that migration between the two subpopulations was very low (less than 1 % of individuals in the northern subpopulation are migrants from the south and approximately 3 % of individuals in the southern subpopulation are from the north) and showed that migration rates were slightly higher moving from the northern subpopulation into the southern one.

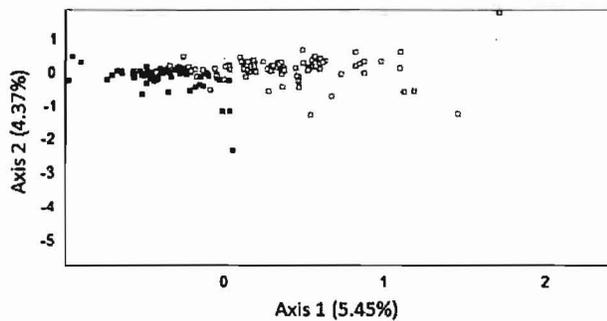
One possible cause of the relatively low rates of movement between the northern and southern subpopulations is the geographic distance between them (approximately 4 km). Despite extensive surveys from 2004 to 2006, evidence of breeding by wood frogs was observed only at one wetland in 1 year in the area between the subpopulations (Scherer et al. 2012; Fig. 1). Corn et al. (1997) conducted amphibian surveys in the KV from 1988 to 1994 and did not detect breeding by wood frogs at any wetlands in the area between the subpopulations. Within subpopulations, on the other hand, the density of occupied wetlands is much higher (Fig. 1). The fact that we found no evidence of finer scale genetic variation when we ran STRUCTURE within each subpopulation suggests that there is significant gene flow among wetlands within each subpopulation. This idea is further supported by the spatial autocorrelation analysis that found no significant autocorrelation within subpopulations, yet significant autocorrelation across the range.

Fragmentation of suitable habitat and reduction in gene flow between fragments can threaten the long-term persistence of populations (Frankham 2006). Water diversion and storage projects and the loss of beaver have heavily modified many riparian systems in the western U.S. (Pepin et al. 2002) and the alteration of hydrologic conditions in the KV has been shown or hypothesized to have negative impacts on peatlands, populations of willow (*Salix* spp.) and benthic invertebrates (Woods 2000, 2001; Chimner



**Fig. 3** The results of the genetic clustering algorithm in Program STRUCTURE. Wetlands are listed along the x axis from the northernmost (03CR02) to the southernmost (WRR Pond). The narrow black lines partition individuals into the wetlands from which they were sampled. Each colored bar across the figure represents an

individual. The proportion of each bar colored in light gray indicates the probability of assignment to the northern population, and the proportion colored in dark gray indicates the probability of assignment to the southern population



**Fig. 4** Two dimensional graph illustrating the relationship between wood frog individuals in the KV of Rocky Mountain National Park based on FCA analysis. The first two dimensions are shown with the percentage of variation described noted in parentheses. The solid colored squares are individuals from the northern subpopulation and the open squares represent individuals from the southern subpopulation

2000; Westbrook et al. 2006; Clayton and Westbrook 2008). The absence of persistent occupancy by wood frogs in the area between the northern and southern subpopulations reflects the fragmentation of wetlands in the KV (Baker 2003; Westbrook et al. 2006) and suggests that the intervening landscape is unsuitable for wood frog occupancy. This lack of suitability may limit dispersal and thus facilitate genetic differentiation. This contention is supported by studies that show lower levels of genetic variation and gene flow in amphibian populations where human activities have fragmented landscapes (Andersen et al. 2004; Arens et al. 2007; Noel et al. 2007; Spear and Storfer 2008).

Several previous studies have reported little evidence of genetic differentiation in populations of wood frogs, despite distances between subpopulations as great as 20 km (Newman and Squire 2001; Squire and Newman 2002; Julian and King 2003). The results of these studies suggest that a geographic distance of 4 km is insufficient to produce genetic differentiation in populations of wood frogs. In contrast, and in support of our result, Crosby et al. (2009) examined genetic structure in populations of wood frogs separated by 0.95–50 km in an area highly fragmented by agriculture and urban development and detected significant genetic differentiation in approximately half of these comparisons. Though they reported significant genetic differentiation, estimates of  $F_{ST}$  in Crosby et al. (2009) were small compared to the present study (estimates of  $F_{ST}$  from significant pairwise comparisons ranged from 0.001 to 0.011 compared to 0.0675 from the present study). Given these results, the significant genetic differentiation between populations separated by only 4 km in the KV suggest that the permeability of the landscape between the northern and southern populations is lower than a landscape dominated by high levels of human development and

infrastructure, although this could also be due to differences in the current and historical context of the populations. Based on visual inspection of a map of land cover types in the KV (Salas et al. 2005), the majority of the intervening landscape is herbaceous wetland that lacks canopy cover from shrubs or trees. The landscape between wetlands within each subpopulation, on the other hand, has higher amounts of canopy cover. Several studies have shown that movement of adult and juvenile wood frogs is preferentially directed toward areas with canopy cover and moist substrates (deMaynadier and Hunter 1999; Vasconcelos and Calhoun 2004; Regosin et al. 2005; Baldwin et al. 2006). The lack of canopy cover in the landscape between the northern and southern subpopulations may constrain movement and, consequently, gene flow.

While landscape change may be responsible for creating the conditions under which the subpopulations have differentiated, genetic drift is the ultimate cause and can act rapidly when populations are small (Newman and Squire 2001; Squire and Newman 2002). Counts of egg masses indicate that the number of wood frogs breeding at individual wetlands in the KV is small. At wetlands where wood frog egg masses were detected, counts of egg masses ranged from 1 to 32, and most counts were less than 10 (Table 2). Wood frog egg masses are highly conspicuous (Grant et al. 2005; Scherer 2008); therefore, it is unlikely that low detectability is the cause of the small counts. Population bottlenecks in the past or a pattern of periodic extinction followed by recolonization could also create the conditions for genetic differentiation to occur (Newman and Squire 2001). Drought is common in the recent climatic history of Colorado (McKee et al. 1999), and large declines in amphibian populations are often associated with years of low precipitation (Pechmann et al. 1991; Berven 1995). While the results from the tests for bottlenecks did not indicate a historic bottleneck, there was evidence for contemporary bottlenecks from both the IAM and the TPM. When combined with the low counts of wood frog egg masses from recent surveys, these results are consistent with the hypothesis that reduced population size is a contributing factor in the genetic differentiation between subpopulations.

Genetic diversity (as measured by the number of alleles per locus) in both subpopulations was low relative to other populations of wood frogs. Julian and King (2003) collected genetic samples from 113 individuals at three wetlands and used the same microsatellite markers as used in this study. Despite a smaller total sample size, they observed considerably higher numbers of alleles at every locus. Crosby et al. (2009) had four markers in common with this study. They sampled from 29 to 96 adults at each of 9 wetlands in areas of high human density. In most cases, the number of alleles at each wetland was higher

than the number of alleles across all individuals sampled from the KV. Though recent fragmentation of wetlands in the KV may have contributed to the reduced genetic variation, other factors have also contributed. For example, genetic variation is generally lower in areas that have been colonized by a species since the last period of wide-spread glaciation (10,000–15,000 years ago; Beebee and Rowe 2000; Newman and Squire 2001; Ficetola et al. 2007; Allentoft et al. 2009). Ficetola et al. (2007) found that genetic variation within populations of the frog, *R. latastei*, was affected by patterns of postglacial colonization, as well as contemporary isolation. Wood frog populations in Colorado occur in a few isolated mountain valleys (Hammerston 1999) and appear to be the result of colonization from populations in present-day Wisconsin following the last glaciation (Lee-Yaw et al. 2008). In addition, the colonization and extinction events associated with metapopulation dynamics can cause reduced genetic variation (Newman and Squire 2001; Frankham et al. 2002). Many authors have argued that amphibian populations are spatially structured as metapopulations (Pechmann and Wilbur 1994; Alford and Richards 1999), though there is disagreement over the generality of this characterization (Marsh et al. 1999; Petranka et al. 2004; Smith and Green 2005). Regardless of the causes, the low genetic variation of wood frogs in the KV is a concern. Weyrauch and Grubb (2006) reported higher mortality rates for wood frog eggs and larvae from populations with lower genetic variability. In addition, they found that larvae from populations with lower genetic variation were more susceptible to the deleterious effects of UV-B radiation. Similarly, Johansson et al. (2007) reported lower larval survival and body size in common frogs (*R. temporaria*) from wetlands with lower levels of genetic variation.

Previous research has generated considerable evidence that operation of the Grand Ditch and loss of beaver have reduced the frequency and magnitude of flooding in the KV and lowered water tables (Woods 2000, 2001; Peinetti et al. 2002; Westbrook et al. 2006). These alterations, although initiated decades ago, are still shaping the landscape and have probably reduced the density of wetlands in the KV and increased the resistance of the intervening landscape to movement by amphibians. While there are no historic data with which to test this hypothesis, the results of this analysis indicate the population of wood frogs in the KV is fragmented and offer several insights relevant to the management and conservation of the population in this landscape. The degree to which habitat fragmentation causes deleterious genetic effects on any population of animals depends largely on the number of subpopulations into which a population is divided, the size of the subpopulation on each

habitat fragment, and the level of gene flow between the fragments (Frankham 2006). The genetic clustering algorithm indicated that wood frogs in the KV are subdivided into two subpopulations, and evidence of significant genetic differentiation suggests gene flow between the subpopulations is highly limited. In addition, compared to other populations of wood frogs, levels of genetic variation in each subpopulation are low. Consequently, conservation of wood frogs in the KV would benefit from increasing the number of subpopulations and the number of individuals in each subpopulation, as well as enhancing gene flow between them. The results of a concurrent study of occupancy patterns of wood frogs in the KV indicates that occupied wetlands tended to be located near streams and other wetlands occupied by wood frogs (Scherer et al. 2012). To increase the number of subpopulations and enhance gene flow between the northern and southern subpopulations, one management option is to construct or restore wetlands between the subpopulations but near streams and other wetlands used by breeding wood frogs. Because of its location in a national park, however, direct manipulation of the landscape in the KV may not be feasible. Other management options include restoring beaver populations and altering the operation of the Grand Ditch. While vegetation conditions are currently unsuitable in most of Rocky Mountain National Park, managers are currently planning for future reintroductions of beaver (M. Watry and T. Johnson, *personal communication*). Clayton and Westbrook (2008) argued that the Grand Ditch could be decommissioned or operated such that its impacts on important hydrologic processes are minimized.

Habitat loss and fragmentation have been identified as key threats to amphibian populations (Cushman 2006), and their negative effects on the degree of connectivity in animal populations from a variety of taxa are becoming more apparent (Shepard et al. 2008; Ford and Fahrig 2008). One approach for characterizing the status of a population, therefore, is to elucidate its current genetic structure. Evidence of genetic differentiation between subpopulations may indicate fragmentation of habitat and facilitate identification of the landscape features that are responsible. Our goal was to assess the current genetic structure of the population of wood frogs in the KV, and based on the results of our study, we suspect that human-caused alterations to the hydrology of the KV have reduced the spatial distribution of wetlands and increased the resistance of the landscape to movement by wood frogs. Future management action may focus on mitigating the effects of the alterations, and subsequent assessments of the genetic structure of the population may provide insights into the effectiveness of the actions (i.e., genetic monitoring; Schwartz et al. 2006).

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