GENE FLOW AND GENETIC STRUCTURING OF ILLINOIS CHORUS FROGS (*PSEUDACRIS STRECKERI ILLINOENSIS*) IN CLAY COUNTY, ARKANSAS

FINAL REPORT

SUBMITTED BY

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INTRODUCTION

This final report represents the third of three methods of analyses of the Illinois chorus frog (ICF; *Pseudacris streckeri illinoensis*) in Arkansas. The first phase involved a recent analysis of the numerical distribution and was published in the *Wildlife Society Bulletin* (Appendix A). Recent low rainfall amounts during the spring months along with currently established best management practices of farmland (precision land leveling) in northeast Arkansas have reduced the number of temporary pools available for breeding groups. Both numbers of ICFs and numbers of breeding sites are much lower than previously identified (Trauth 1992; McCallum and Trauth 2002). The second phase of the study was to perform a narrow-scale morphometric analysis of the ICF and Strecker's chorus frog (SCF; *P. s. streckeri*) to help clarify the taxonomic status of the ICF. This study has been submitted to the journal *Zootaxa* (Appendix B). Historically, morphological analyses have been an integral component in studying anuran systematics (e.g., see Gaudin 1974; Trueb 1977; Duellman and Trueb 1986). Morphological differences generally provide overwhelming supportive evidence of underlying genetic differentiation in reproductively-isolated populations. Genetic differences are, thus, inferred from the more obvious phenetic differences, which they cause (Scott 2005). This study provided morphological evidence of geographic (clinal) variation within a species, but did not provide support for the taxonomic elevation of the ICF to species status.

Genetic Basis of Population Analysis

The third phase of the study was designed to examine the genetic diversity and genetic structure of Illinois chorus frog populations. To date, no population-level analyses have been performed on the Illinois chorus frog. Molecular analyses have been used on this species, yet they have been inconsistent in providing taxonomic insight. For example, Hedges (1986) used

allozyme analysis of 33 presumed loci to examine phylogenetic relationships between 30 taxa of Holarctic hylid frogs, including *Pseudacris*. He found genetic distance between the ICF and SCF (D = 0.13) to be similar to distances between some other recognized species (range of 0.02 to 0.16 for 6 other species comparisons), yet much lower than for most species comparisons. Hedges (1986) suggested that *P. s. illinoensis* probably should be recognized as a full species, but that more detailed studies were needed. Conversely, Moriarty and Cannatella (2004) examined the phylogenetic relationships among North American chorus frogs by sequencing 2.4 kb of the 12S and 16S mtDNA genes. They reported the ICF (n = 1 each from AR and MO) sequences to be "nested" within SCF (n = 1 each from KS and TX) sequences. They also recommended further taxonomic study.

Bottlenecking, a sudden and dramatic decline in numbers, has been associated with genetic drift and rapid species change (Nei 1987). Non-lethal techniques such as we performed with microsatellites can provide high resolution as to the genetic partitioning of populations and breeding groups (Paetkau et al. 1997). Best management plans for *P. s. illinoensis* can be enhanced with a better understanding of the genetic diversity of and gene flow between populations. Conservation measures must maximize the genetic diversity of remaining breeding groups. The above findings and questions were the basis of this study which addressed the objectives below.

Objectives of the study

1. Better characterize the genetic structure of breeding groups of ICFs in Clay County.

2. Estimate the gene flow among breeding groups.

METHODS AND MATERIALS

Collection Techniques and DNA Isolation

Sampling sites for the ICF were determined by driving the farm roads of Clay County, Arkansas, and listening for calling males during the February through March breeding season. Illinois chorus frogs were sampled from 10 small temporary pools and water holes within Clay County where calling males were heard (Figure 1; Table 1). Hereafter all breeding sites will be referred to as populations strictly for communication rather than for accuracy purposes. We included breeding sites in the complete range of the approximately 23 km^2 currently inhabited by ICFs in eastern Clay County (Trauth et al. 2005), which is reduced from the range of 59 km^2 previously estimated by McCallum and Trauth (2002). Geographic distances in km were determined between all possible pairs of breeding sites using topographic maps (Table 2).

Adult frogs, mostly males, were collected and transported to the ASU genetics lab. Toe clips were collected using sterile technique from adult frogs from each breeding pool. Frogs were also massed with an electronic balance, and their tibias were measured using electronic calipers for later analysis. Toe clips were transferred to microcentrifuge tubes and stored on ice until they were placed in the -80° C freezer to await DNA extraction. Following toe clipping, the adult ICFs had their toe stumps treated with antibiotic ointment, and they were returned to their respective capture sites.

DNA isolation was performed for individuals utilizing the Qiagen® DNeasy ™ extraction kit. Briefly, the toes were rinsed with PBS (phosphate-buffered saline), minced, and placed in lysis buffer and Proteinase K at 55° C overnight. The homogenate was purified with several washes, ethanol precipitated and eluted in buffer. The elutant was stored at -70° C until the DNA was further processed. DNA concentration within the elutant was determined by

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Figure 1: Illinois Chorus Frog breeding sites 2003 – 2005, Clay County, Arkansas, Township 20 N, Range 9 E, Sections 8 – 10, 15 – 17, 20 – 22, 27 – 29 (adapted from DeLorme 3 – D TopoQuads, 1999).
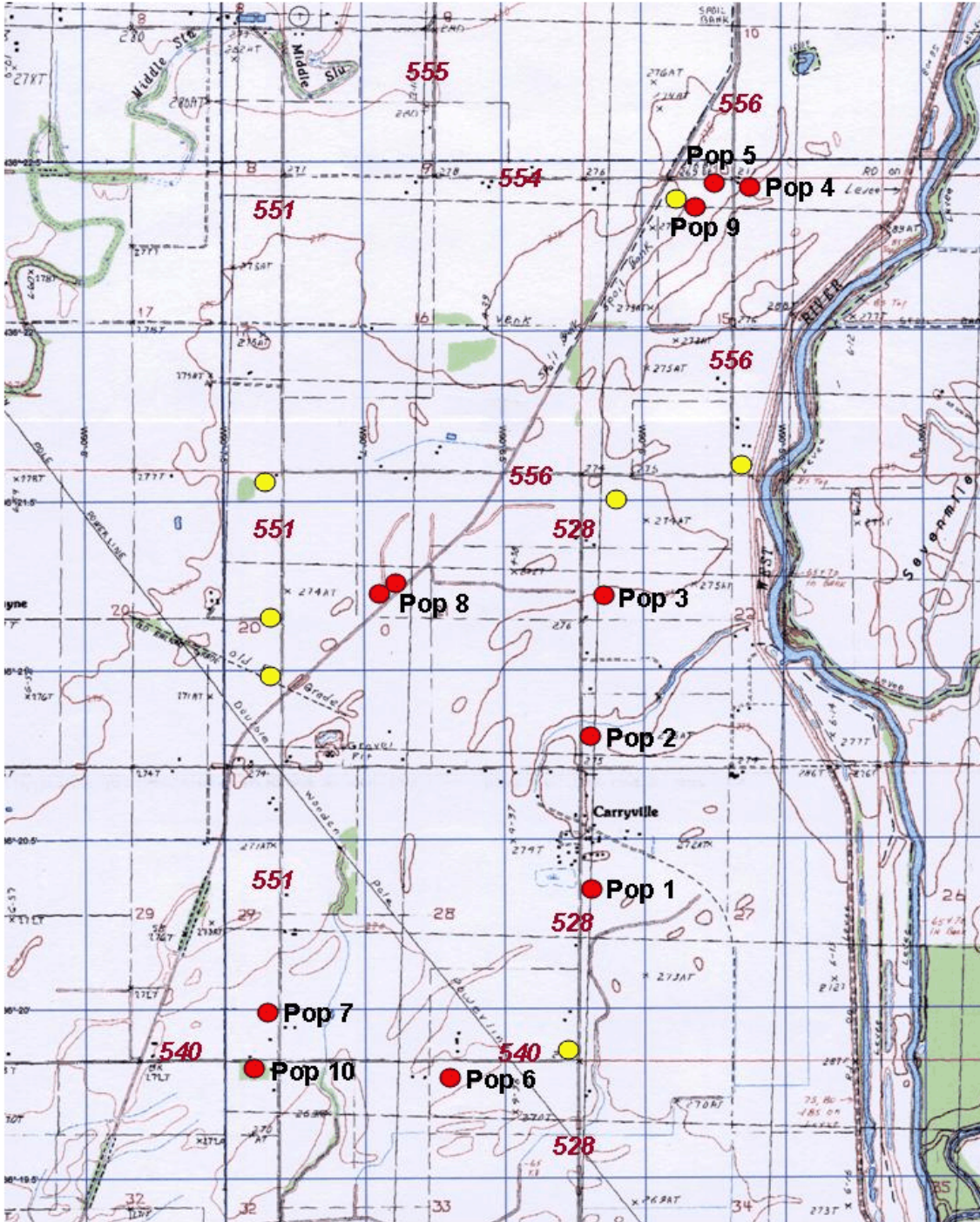
Table 1: Illinois chorus frog population locations, number of individuals sampled per population, and collection dates for Clay County, Arkansas.

Pop.	Location	N	Collection Dates
1	E side of CR 528, 0.8 km S of CR 546	13	21 Feb 03
2	0.16 km N of CR 546 on E side of CR 528	13	21 Feb 03; 7 Mar 03
3	1.0 km N of CR 546 on E side of CR 528	6	21 Feb 03; 07 Feb 05
4	E side of intersection of CR 554 and CR 556	2	21 Feb 03
5	0.16 km W of CR 556, S side of CR 554	13	21 Feb 03
6	S side of CR 540, 0.6 km W of CR 528	25	19 Mar 03; 07 Feb 05
7	0.32 km N of CR 551/540 junction on CR 551	8	01 Mar 04
8	0.64 km S of CR 556, 0.8 km E of CR 551	5	01 Mar 04
9	1.3 km E of CR 555 on S side of CR 554	6	06 Mar 04
10	0.16 km W CR 551 on S side of CR 540 at radio tower	8	07 Feb 05

Table 2: Geographic distances in km between populations of Illinois chorus frogs measured from center of population to center of population.

	1	2	3	4	5	6	7	8	9	10
1	0.0	0.8	1.6	4.0	3.9	1.3	1.9	2.0	3.8	2.1
2		0.0	7.7	3.1	3.1	2.0	2.3	1.4	3.0	2.6
3			0.0	2.4	2.4	2.8	2.9	1.2	2.2	3.2
4				0.0	0.2	5.2	5.2	2.9	0.3	5.5
5					0.0	5.1	5.1	2.8	0.2	5.4
6						0.0	0.9	2.7	4.9	0.9
7							0.0	2.4	5.0	0.3
8								0.0	2.7	2.4
9									0.0	5.3
10										0.0

uv visible spectrophotometry. DNA concentrations were then standardized for all samples (20 ng/ul).

Microsatellite Methods

The development of microsatellites was achieved using two different approaches. First, we obtained microsatellite primers initially developed by Call and Hallett (1998) for other related taxa (*Pseudacris* (*Hyla*) *regilla* and *Pseudacris* (*Acris*) *crepitans*). Microsatellite primers developed for one species will often cross-amplify for another related species (Curtis and Taylor 2000; Krupa et al. 2002; Primmer and Merila 2002). The second approach was to develop our own primer sets specific to *P. s. illinoensis*. This method is labor-intensive and is discussed below.

A simple tandem repeat (STR) enriched genomic library was created using a protocol similar to that used in Crowshaw and Glenn (2003). Genomic DNA was extracted from ICF liver tissue using a standard phenol/chloroform extraction method and digested using *Sau*3AI restriction enzyme (New England Biolabs). Restriction fragments with lengths ranging from 400 – 1500 bp were isolated using a CHROMA SPIN® DNA size-fractioning column (Clonetech Laboratories). Polymerase chain reaction (PCR) linkers were ligated to the sticky-ends of ICF restriction fragments and amplified using PCR. The enrichment was carried out by hybridizing biotinylated custom DNA oligonucleotides to the ICF DNA. Biotinylated DNA oligonucleotides were designed with base sequences complimentary to the desired STR motifs that we wanted to locate in the ICF DNA. These oligonucleotides anneal to their complement in the DNA, and these hybridized fragments were captured using Dynabeads MyOne® Straptavidin C1 (Invitrogen). The magnetic beads bound the hybridized DNA and were isolated using a

magnetic particle concentrator (Dynal MPC®). All other DNA was washed away. The STR-enriched ICF DNA was then eluted and amplified using PCR. We used a TOPO-TA® cloning kit (Invitrogen) to create the genomic library, and screened the library for STR's using the Phototope® Star Detection Kit (New England Biolabs). Bacterial colonies were lifted from plates using a nylon membrane, lysed on the membrane, and the DNA was crosslinked to the membrane using a UV crosslinking chamber.   Bacterial colonies on the plates were allowed to continue to grow in the incubator for later harvest. In a series of steps, biotinylated probes similar to the ones used above were hybridized to the DNA, streptavidin was bound to the probes, and biotinylated alkaline phosphatase was bound to the streptavidin. The CDP-star reagent was added to activate the chemiluminescence, and the glowing membrane was exposed to x-ray film. Plates were placed on the developed x-ray film, and colonies were chosen based on the intensity of the light emitted from the previous step. The intensity of the colony indicated the presence of the targeted STRs. These colonies were mini-prepped to isolate their DNAs, and each plasmid insert was sequenced. Sequencing chromatograms were used to detect the actual STR present in each plasmid, and PCR primers were developed from the flanking regions of STR's that appeared to be potential microsatellites. The computer program Primerquest® (Integrated DNA Technologies) was then used to generate the flanking PCR primers.

We screened the suitability of primers purchased by using two or three primer combinations per PCR reaction (multiplex PCR). Each PCR reaction was performed with different $MgCl_2$ concentrations (1.5 mM, 2.0 mM, and 2.5 mM) and a range of differing annealing temperatures (40.8℃ to 57.9℃) using a temperature gradient thermal cycler.

Multiplexed PCR products were electrophoresed on 6.5% LongRanger® polyacrylamide gels (Cambrex) and stained with SyBr Green. Initial optimization PCR reactions resulting in

amplification of microsatellite DNAs were identified. Single-plex PCR reactions were then

conducted using each forward and reverse primer combination alone at those annealing

temperatures and MgCl$_2$ concentrations found to amplify within the multiplex framework. The

optimal annealing temperatures and MgCl$_2$ concentrations were thus identified for each primer

combination. The four primer combinations yielding the best amplification and polymorphisms

were used for population analysis (Table 3).

PCR reactions were performed for each ICF and each primer set. PCR conditions were

as follows: 100 ng of DNA, 5 pm of each primer, 0.4 ul of NTPs, 1.5 - 2.5 mM MgCl$_2$ (see Table

2) and 1 unit of Taq polymerase (Sigma) for a total volume of 20 ul. Cycling parameters

included an initial denaturation at 94º C for 1 min, followed by 30 cycles of denaturation at 94º C

for 30 s, annealing temperature at 42.0 or 48.8º C (see Table 2) for 30 s, and extension at 72º C

for 1 min. The amplified products were denatured with formamide and heat (90º C for 3 min).

Each sample was electrophoresed in a vertical 6.5% polyacrilamide gel (LongRanger™) and

stained in SyBr® green. Stained fragments were documented and number of base pairs per

fragment estimated by way of the UVP Bioimaging System®. Most individual samples were

duplicated and electrophoresed in different gel combinations to verify repeatability.

Statistical Analysis

Standard diversity measures (expected and observed heterozygosities (H) and number of

alleles/locus) were determined for all loci and populations. Population #4 was not used in

further statistical analyses due to a sample size of 2. Tests for Hardy-Weinberg equilibrium at

each locus and each sample were performed using exact tests with a modified Markov chain

algorithm in *Arlequin* version 3.0 (Excoffier et al. 2007), under the following parameters: 10,000

Table 3: Microsatellite primers used for PCR amplification of Illinois chorus frogs in Clay County, Arkansas.

Locus	Primer Sequences (5' –> 3')	Annealing Temperature	MgCl$_2$ Concentration
D	F-AGG AGC TGC AGC AAT CTG TT	42.0° C	2.5 mM
	R- TCA CTT GGT TAC ACT CAT GCA CT		
I	F- TAC CTG GCT ACC TGT CTG GC	48.8° C	2.5 mM
	R- CTG ATT AGC AGT GAG CGG C		
J	F- GAG TTT ATG CCG TTC TTG GG	48.8° C	2.5 mM
	R- ACA AGG GGG AGA CAC AGG C		
K	F- CCC TTC CCT TCA GAC TCC C	48.8° C	2.5 mM
	R- TGC AAG TGT GAA CTC ATC CC		

dememorization and 100,000 Monte Carlo steps (Levene 1949; Guo and Thompson 1992). The software *Arlequin* was also used to estimate linkage disequilibrium among locus pairs using

Fisher's exact probablility test (Slatkin 1994; Slatkin and Excoffier 1996). Alpha levels for all significance tests were established at 0.05. However, the sequential-comparison Bonferroni technique was used to adjust significance levels for determining H.W. equilibrium to reduce Type I error of multiple tests (Sokal and Rohlf, 1995; $\alpha = 0.05/n$). Pairwise F_{ST} values were calculated based upon an infinite alleles model (Kimura and Crow 1964; Weir and Cockerham 1984) among samples using *Arlequin* to determine population structure (Wright 1951). F_{ST} values were then used to construct a phenogram using the unweighted pair-group method using arithmetic mean (UPGMA; Sokal and Sneath 1963) using the Phylogeny Inference Package (PHYLIP; Felsenstein 1995). Numbers of migrants per generation were calculated by

using $Nm = 1/(4 \, F_{ST} + 1)$ [Wright 1951].

To test an isolation-by-distance model, F_{ST} values were compared to geographic distance in km using a Mantel test with 1000 randomizations (Mantel 1967). We predicted a significant correlation to support isolation-by-distance. The assignment of individuals to specific populations was achieved in the Arlequin package using the log-likelihood method (Paetkau et al. 1995; Paetkau et al. 1997; Waser and Strobeck 1998). Due to the close proximity of populations 4, 5, and 9, and populations 7 and 10, these populations were combined, and all analyses above were duplicated for these combined samples.

RESULTS AND DISCUSSION

<u>Genetic Diversity</u>

Alleles per locus for the ICF ranged from 2 (Locus K) to 9 (Locus J) for the four loci studied ($\bar{x} = 5$; Table 4). Mean heterozygosity for all populations ranged from a low of 0.48000 for Locus I to a high of 0.74000 for Locus K ($\bar{x} = 0.61149$). Locus K was consistent in the number of alleles expressed (n = 2 for all populations). Conversely, Locus J was highly variable among populations, both in number of alleles expressed (Table 4) and in the distribution of those alleles (Table 5). The I locus was monomorphic for populations 9 and 10.

Two of the four loci (D and I) had a single allele in high frequency with some strong variation among populations. For example, allele 73 represented almost 2/3 (0.64) of all D locus alleles, yet frequencies were far lower in populations 2, 3 and 9. Additionally, allele 59 represented almost 3/4 (0.72) of all I locus alleles, yet was also far lower in populations 3, 4, and 7. Conversely, allele 59 was fixed in populations 9 and 10. Locus J had a greater diversity of alleles (n = 9) than the other loci with a broader distribution of those alleles. No allele represented more than half of the Locus J alleles (allele 123 = 0.48). Lastly, Locus K had two

Table 4: Genetic diversity measures of alleles per locus (A) and observed heterozygosity (H_o) for Illinois chorus frog populations (and combined adjacent populations 4,5 and 9 in addition to 7 and 10) as measured by number of alleles and observed heterozygosity. Number of individuals sampled per population are in parentheses. Loci not in Hardy-Weinberg equilibrium using Bonferroni correction are in bold.

| Population | Locus | | | | | | | | | |
| | D | | I | | J | | K | | Mean | |
	A	H_o	A	H_o	A	H_o	A	H_o	A	H_o
1 (13)	3	0.92308	2	0.15385	6	0.53846	2	0.53846	3.25	0.53846
2 (13)	**4**	**1.00000**	2	0.38462	4	0.61538	2	0.38462	3.00	0.59615
3 (6)	3	1.00000	2	0.85714	2	0.57143	2	0.57143	2.25	0.75000
4 (2)	2	0.50000	2	1.00000	2	0.50000	2	0.50000	2.00	0.62500
5 (13)	3	0.38462	3	0.69231	3	0.61538	**2**	**1.00000**	2.75	0.67308
6 (25)	2	0.48000	4	0.56000	5	0.64000	**2**	**0.88000**	3.25	0.64000
7 (8)	2	0.00000	2	1.00000	2	0.42857	2	0.75000	2.00	0.54464
8 (5)	2	0.40000	2	0.40000	3	0.60000	2	1.00000	2.25	0.60000
9 (6)	2	1.00000	1	0.00000	4	0.50000	2	1.00000	2.25	0.62500
10 (8)	2	0.62500	1	0.00000	2	0.75000	2	0.62500	1.75	0.50000
4,5,9 (21)	3	0.57143	3	0.52381	5	0.57143	2	**0.95238**	3.25	0.65476
7,10 (16)	3	0.31250	2	0.50000	2	0.60000	2	0.67850	2.25	0.52500
Total	5	0.63000	4	0.48000	9	0.59596	2	0.74000	5.00	0.61149

Table 5: Microsatellite allele frequencies for Illinois chorus frog populations from Clay County, Arkansas

Locus						Population						
Allele	1	2	3	4	5	6	7	8	9	10	Mean	
D												
59	0.35	0.04	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	
65	0.00	0.00	0.00	0.25	0.12	0.24	0.00	0.20	0.50	0.31	0.15	
67	0.00	0.12	0.50	0.75	0.15	0.00	0.00	0.00	0.00	0.00	009	
73	0.54	0.42	0.36	0.00	0.73	0.76	0.88	0.80	0.50	0.69	0.64	
81	0.12	0.42	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.08	
I												
47	0.00	0.00	0.00	0.00	0.23	0.26	0.50	0.20	0.00	0.00	0.15	
59	0.92	0.81	0.43	0.50	0.50	0.70	0.50	0.80	1.00	1.00	0.72	
65	0.08	0.20	0.57	0.50	0.27	0.02	0.00	0.00	0.00	0.00	0.13	
69	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.01	
J												
123	0.23	0.50	0.57	0.75	0.62	0.56	0.36	0.50	0.42	0.38	0.48	
134	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.17	0.00	0.02	
146	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	
171	0.15	0.00	0.00	0.00	0.04	0.02	0.00	0.00	0.00	0.00	0.04	
184	0.35	0.38	0.43	0.25	0.35	0.38	0.64	0.30	0.25	0.63	0.40	
194	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	
201	0.12	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	
214	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.01	
230	0.04	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.17	0.00	0.02	
K												
172	0.65	0.58	0.43	0.25	0.50	0.52	0.38	0.50	0.50	0.31	0.50	
189	0.35	0.42	0.57	0.75	0.50	0.48	0.63	0.50	0.50	0.69	0.50	

alleles, 172 and 189, equally distributed (0.50 each), yet with high variation among populations.

The number of loci studied and the genetic diversity of those loci was not sufficient to "fingerprint" individual chorus frogs. There were 20 genotypes shared among individuals. This lack of discriminating power is not surprising based upon the small effective population sizes, the close geographic proximity, and the high potential for inbreeding for these frogs. Many more loci would be needed to effectively fingerprint these frogs. For example, Lutz-Carrillo et al. (2006) were not able to fingerprint individual largemouth bass *Micropterus salmoides* and *M. floridanus* from much larger populations and larger geographic ranges using 11 microsatellite loci.

Heterozygosity for individual populations was also highly variable (Table 4). All individuals within the same populations were heterozygous for specific loci (populations 3 and 9, Locus D; population 7, Locus I; and populations 5, 8, and 9, Locus K). Conversely, a couple of populations had no heterozygosity for specific loci (populations 7 and 8, Locus D).

Diversity values for the ICF are somewhat lower than for other related taxa. For example, alleles per locus ranged from 2 - 19 for 50 northern water snakes *Nerodia sipedon* (Prosser et al. 1999), 7-32 for 80 spotted salamanders *Ambystoma maculatum* (Julian et al. 2003), and 9 - 34 for wood frogs *Rana sylvatica* (Julian and King 2003). We studied a higher number of individuals than these other studies, which should have resulted in a greater number of microsatellite alleles being identified (Nei 1987). For example, in our study we found a significantly positive correlation between sample size for populations and number of alleles per locus ($r_s = 0.70$; $p = 0.026$). Conversely, ranges of other species studied were far greater than in the present study, possibly contributing to the greater number of alleles for several of the loci studied in other species. Heterozygosity values were also low relative to those studies mentioned above, where H ranged up to 0.96. Frankham et al. (2002) in an extensive review of

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the literature of microsatellite analyses of populations, identified a reduction in the genetic diversity of threatened versus non-threatened species. For example, number of alleles per locus identified by Frankham et al. (2002) typically ranged from 5 - 10 per locus for non-threatened species, whereas threatened species had 2 - 4 alleles per locus. Likewise, H values ranged from 0.60 - 0.80 for non-threatened species, and from 0.30 - 0.56 for threatened species. Diversity values for the ICF are at the low end for non-threatened species, yet only slightly greater than for threatened species.

Three loci were not in Hardy-Weinberg equilibrium among the 10 populations, representing 0.075 of the locus combinations tested (Table 4). Each of the loci not in equilibrium was due to homozygote deficiency. Populations exhibiting disequilibrium are in a state of genetic flux with the exception of those exhibiting null alleles. Many explanations have been offered for disequilibrium within populations, including null alleles, bottleneck effects, Wahlund effect, linkage disequilibrium, inbreeding, and selection (Singh and Green 1984). Null alleles represent the loss of expression of STRs due to mutations within the primer annealing site. If primers cannot anneal to the microsatellite flanking regions, then amplification cannot occur. Null alleles are common among microsatellite loci, with the result being heterozygote deficiency, or even a lack of alleles expressed altogether (Callen et al. 1993; Paetkau and Strobeck 1995; Pemberton et al. 1995). Although there was one individual without expression of the J locus in population 7, that particular population/locus combination was not in Hardy-Weinberg disequilibrium. Additionally, disequilibrium in this study was due to homozygote deficiency rather than excess.

The combined effects of drought and habitat modification (precision land leveling) could be producing a bottleneck effect, in which genetic drift is serving as a dominant force in allele

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frequency changes of these populations (Nei et al. 1975). The sharp declines in breeding choruses over the past couple of decades (Trauth et al. 2005) may be a causative factor of this disequilibrium. Bottleneck effects have been demonstrated to reduce heterozygosity for hundreds of thousands of years following population recovery (Nei et al. 1975).

Independently reproducing subgroups (Wahlund effect) would not be a factor in this study, as small breeding sites were treated as discrete populations. Linkage disequilibrium may also result in Hardy-Weinberg disequilibrium (Hornbach et al. 1980). However, linkage disequilibrium among loci was identified in only two of the 10 populations tested. For population 6, locus D was linked with locus I, and in population 9 locus D was linked with locus K. This inconsistency in linkage disequilibrium among populations probably represents a Type I error, with multiple linkage tests performed and no Bonferroni correction applied.

Inbreeding has also been proposed as an explanation for homozygote excess (Hornbach et al. 1980). Koehn et al. (1971) stated that if inbreeding is indeed occurring, heterozygote deficiency should be consistent for all polymorphic loci. Homozygote deficiency rather than excess was the norm for these frogs, not supporting inbreeding as a mechanism for disequilibrium. Nei et al. (1975) have previously identified significant negative correlations of population size with heterozygosity values, with inbreeding enhanced in smaller populations. We used our sample size for each population as a crude estimation of effective population size. The basis of this estimation is that only breeding individuals would be out during our collection period, and the primary limiter of sampling size was availability of chorus frogs. No significant correlation was observed between effective population size and heterozygosity (r_S = -0.078; p = 0.83), thus not supporting population size-related inbreeding. Surprisingly, we see no genetic evidence for inbreeding for ICF, although we anticipate inbreeding will become problematic based upon current population numbers. There is a lag period between population declines and

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the genetic effects resulting from those declines. Selection has been associated with Hardy-Weinberg disequilibrium in many other studies (e.g., Hornbach et al. 1980; Singh and Green 1984; Diamond et al. 1991), yet microsatellite loci typically are not subject to selection (however, see Goldstein and Schlotterer 1999; Moxon and Wills 1999).

Population Structure and Gene Flow

F-statistics were used to investigate the genetic interactions of these populations (Table 6). Genetic structuring was evident among subpopulations. Despite the low geographic distances between populations, F_{ST} values demonstrated moderate to high differentiation among populations as defined by Hartl (1980), who described F_{ST} values of 0.05 - 0.15 as representative of moderately differentiated populations, F_{ST} values of 0.15 - 0.25 for highly differentiated populations, and very great differentiation for F_{ST} values greater than 0.25. The overall mean F_{ST} value was 0.11608 (+/- 0.01194 SE), indicative of overall moderate genetic differentiation. Thirty-one of the 45 (69 %) F_{ST} pairwise combinations were either moderately (18/45), highly (10/45) or greatly (3/45) differentiated. Significance tests demonstrated that a majority of populations were significantly differentiated from one another even with Bonferroni correction (corrected $\alpha = 0.0011$; Table 6). Significance of F_{ST} values slightly differed when adjacent populations were combined (Table 7). Fewer populations were different using this approach, with 13 of the 21 (62 %) pairwise comparisons having moderate to high differentiation. It must be noted that sample size for the statistics performed is low, necessitated by the effective population size of Illinois chorus frogs in Arkansas. Further confounding these analyses are the usage of individuals from differing years and therefore differing generations for populations 3 and 6 (see Table 1) in an effort to obtain an adequate sample size, introducing pseudoreplication issues for those two populations (Waples 1989). Interestingly, these two populations showed the greatest divergence from geographic predictions (Table 6, see also Figures 2 and 3, discussed

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Table 6: Population pairwise genetic differentiation (F_{ST}) below diagonal and corresponding p values above diagonal for Illinois chorus frogs of Clay County, Arkansas, using the infinite alleles model. Bold p values indicate significant differentiation among populations with (underlined) and without Bonferroni correction for significance ($\alpha = 0.0011$ and 0.05, respectively).

					Population					
	1	2	3	4	5	6	7	8	9	10
1	---------	**0.01802**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00901**	**0.02703**	**0.00000**	**0.00000**
2	0.04868	----------	**0.00000**	**0.01802**	**0.00000**	**0.00000**	**0.00000**	**0.02703**	**0.00901**	**0.00000**
3	0.17233	0.09534	----------	**0.00901**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**	**0.00000**
4	0.26547	0.14369	-0.07314	---------	**0.03604**	**0.00000**	**0.01802**	**0.03604**	**0.02703**	**0.00000**
5	0.11734	0.06947	0.04933	0.11411	---------	0.11712	**0.00901**	0.20721	**0.00000**	**0.00000**
6	0.10195	0.08814	0.16575	0.23468	0.00868	---------	**0.00901**	0.77477	**0.02703**	**0.00901**
7	0.16126	0.14130	0.19947	0.32572	0.04344	0.04101	----------	0.11712	**0.00000**	**0.00000**
8	0.05611	0.05360	0.14522	0.21683	-0.00799	-0.04345	0.03166	----------	0.17117	**0.04505**
9	0.07956	0.08489	0.19454	0.20976	0.10006	0.04591	0.19350	0.00412	---------	**0.00901**
10	0.11498	0.11294	0.21688	0.30394	0.10955	0.05437	0.13007	0.02230	0.03147	----------

Table 7: Population pairwise genetic differentiation (F_{ST}) below diagonal and corresponding p values above diagonal for Illinois chorus frogs of Clay County, Arkansas, using the infinite alleles model. Population 4* represents the combining of adjacent populations 4, 5 and 9, whereas 7* represents the combining of adjacent populations 7 and 10. All other population numbers remain unchanged. Bold p values indicate significant differentiation among populations with (underlined) and without Bonferroni correction for significance (α = 0.0024 and 0.05, respectively).

| | Population | | | | | | |
	1	2	3	4 *	6	7*	8
1	---------	0.07207	0.05405	**<u>0.00000</u>**	0.36937	0.77477	0.30631
2	0.04868	---------	**<u>0.00000</u>**	**<u>0.00000</u>**	**<u>0.00000</u>**	**<u>0.00000</u>**	0.05405
3	0.17233	0.09534	---------	**0.00901**	**<u>0.00000</u>**	**<u>0.00000</u>**	**<u>0.00000</u>**
4*	0.09179	0.04961	0.05389	---------	0.08108	**<u>0.00000</u>**	0.36937
6	0.10195	0.08814	0.16575	0.00772	----------	**0.02703**	0.77477
7*	0.11933	0.10881	0.19360	0.04901	0.02044	----------	0.30631
8	0.05611	0.05360	0.14522	-0.01671	-0.04345	-0.00542	---------

below).

Number of migrants per generation ranged from less than one to more than 60 (Table 8). Nm values > 1 are considered sufficient to offset effects of genetic drift within populations (Wright 1951). Although F statistics are normally used to generate Nm values, this application has been seriously questioned by Gaggiotti et al. (1999). These Nm values appear spuriously high to us, even with the close proximity of these populations, due to the life history characteristics of the Illinois chorus frog being typical of species having low levels of gene flow: small size, burrowing during most of the year, and short 2-3 year lifespan (for review, see Templeton 2006). The fact that there was a gender bias in our sampling (male dominance) probably is one factor impacting these Nm calculations, as males tend to have greater dispersal tendencies than do females (Duellman and Trueb 1985).

Results of the Mantel test (non-parametric correlation of geographic distance versus F_{ST} values) strongly supported isolation by distance ($r = 0.885$; $p < 0.01$), consistent with the high F_{ST} values discussed previously. The generated UPGMA phenogram (Figure 2) also demonstrates some geographic partitioning of these populations, yet there were some inconsistencies among geography and genetic structuring. For example, populations 5 and 6 partitioned out genetically, yet were at extremes geographically. Additionally, population 3 was central geographically yet was distinct from other groups genetically. This may demonstrate the effects of genetic drift and recent bottlenecking (but note the discussion previously on pseudoreplication) [Garza and Williamson 2001]. The UPGMA dendrogram generated by the combining of adjacent populations was similar to that generated using individual populations (Figure 3).

Assignment of individuals to populations using the log-likelihood method indicated high probability of success (74.4%; Table 9). Most individuals not assigned to their appropriate

Table 8: Estimated number of migrants per generation among Illinois chorus frog populations of Clay County,

Arkansas, based upon F statistics. Note that the population 4 sample size was 2 individuals.

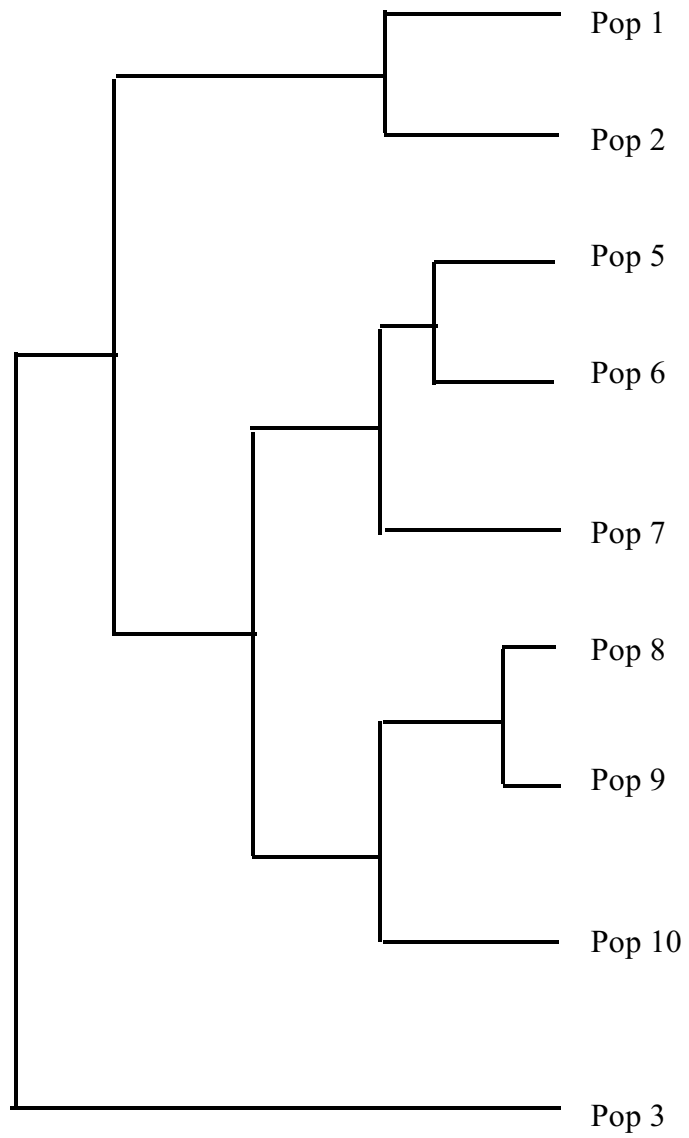|	1	2	3	4	5	6	7	8	9	10
1	*****									
2	4.89	*****								
3	1.20	2.37	*****							
4	0.69	1.49	3.17	*****						
5	1.88	3.35	4.82	1.94	*****					
6	2.20	2.59	1.26	0.82	28.55	*****				
7	1.30	1.52	1.00	0.52	5.51	5.85	*****			
8	4.21	4.41	1.47	0.90	31.04	5.50	7.65	*****		
9	2.89	2.69	1.04	0.94	2.25	5.20	1.04	60.43	*****	
10	1.92	1.96	0.90	0.57	2.03	4.35	1.67	10.96	7.69	*****

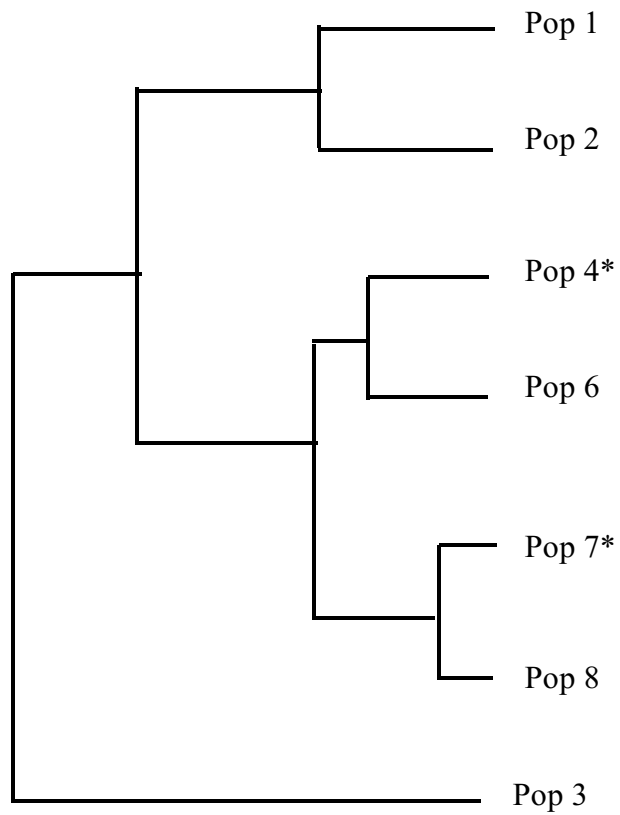Figure 2: UPGMA dendrogram using F statistics of Illinois chorus frog populations in Clay County, AR.

Figure 3. UPGMA dendrogram using F statistics of Illinois chorus frog populations in Clay County, AR. Population 4* represents the combining of adjacent breeding sites of 4, 5, and 9, whereas population 7* represents the combining of 7 and 10.

population were assigned to the most geographically proximal population. This high assignment success is particularly notable considering the close proximity of breeding populations studied (maximum distance of 5.5 km between populations). Likelihood of assignment was slightly lower (68 %) when adjacent populations (4, 5, and 9; 7 and 10) were combined (Table 10).

Conclusions and Management Recommendations

The genetic aspects of small populations must be considered at the onset of management programs in order to maximize the probability of their long-term survival and continued adaptability (Meffe 1986). Loss of genetic diversity is typically associated with populations having sharp population declines (Garza and Williamson 2001). This loss of genetic diversity can not only limit the evolutionary potential of a species (Frankham et al. 1999), but can make that species more vulnerable to newly introduced pathogens and parasites (O'Brien and Evermann 1989). Additionally, genetic drift of these isolated populations can result in the fixation of deleterious alleles (Wright 1977; Madsen et al. 1996; Frankham and Ralls 1998). Efforts should be made to enhance within-site genetic variation while simultaneously maintaining the genetic integrity of differing populations. However, outbreeding depression can result from the introduction of exogenous alleles into existing populations if sound management practices are not followed (Philipp et al. 2003).

Moritz (1994) elaborated on the distinction of evolutionary significant units (ESUs) and management units (MUs) on the basis of the historical framework of a species. Whereas classification of populations as ESUs requires interpretation of the historical relationship of the populations in question, the MU concept focuses on the current genetic and population dynamics.

As more fields have become precision leveled, breeding-pool sizes and locations have varied from year to year with size trending toward smaller. Our genetic data shows the distinct nature of the populations sampled even though some populations only existed at our

recorded location for a single breeding season. Further loss of genetic diversity due to inbreeding and genetic drift are inevitable if population sizes and numbers are allowed to continue to fall. Lack of action to promote reproductive success for the ICF makes its extirpation from Clay County, Arkansas, more likely.

Key to the long-term survival of the Illinois chorus frog in Clay County, Arkansas, is a strategy geared toward creating and maintaining breeding sites of ample size and dimension to allow for some sustained annual recruitment. This is especially crucial for the smaller and more isolated populations that exist today. These small breeding aggregations often utilize temporary pools in marginal habitats and are, thus, at the fringes of most cultivated fields. Fewer than 10 breeding pairs often occur in these breeding pools; the number of these aggregates has dwindled to less than 15. Setting aside small, peripheral tracts of land (marginal pockets specifically at the corners of cultivated lands) and maintaining these sites under a strict conservation plan which increases water retention will greatly benefit reproductive success in this species. This marginal land management approach would require only minimal habitat modifications. The leasing of property easements on this marginal land would demonstrate a major step toward implementing a conservation recovery plan for this frog.

Table 9: Log likelihood of individual genotypes in all populations of Illinois chorus frogs in Clay County, AR. Population number is followed by sample number (eg. 1-1, represents population 1-individual 1).

| | **Population** | | | | | | | | | |
	1	2	3	4	5	6	7	8	9	10
1-1	-5.622	-13.053	-16.201	-20.224	-20.388	-21.558	-19.328	-16.495	-17.065	-18.548
1-2	-4.467	- 7.397	-10.456	-15.456	-14.526	-15.029	-12.955	-11.731	-12.301	-11.792
1-3	-4.467	-11.061	-10.456	-15.456	-14.526	-15.029	-12.955	-11.731	-12.301	-11.792
1-4	-4.524	-11.444	-11.437	-17.248	-15.219	-15.642	-14.159	-12.424	-12.994	-13.273
1-5	-4.061	- 6.480	- 6.385	-13.172	-10.051	-9.807	-8.479	-8.353	-8.923	-7.211
1-6	-5.683	-17.028	-15.913	-19.126	-14.445	-15.696	-18.818	-16.495	-17.065	-17.759
1-7	-6.721	-13.053	-16.201	-20.224	-20.388	-18.587	-19.328	-16.495	-13.399	-18.548
1-8	-5.740	-17.411	-16.894	-20.917	-15.139	-16.309	-20.021	-17.189	-17.758	-19.241
1-9	-5.910	- 7.605	- 6.385	-14.271	-10.670	-13.283	-14.042	-13.405	-14.380	-13.743
1-10	-6.453	- 5.254	- 5.992	-11.263	-4.371	-3.890	-4.920	-3.665	-4.524	-3.460
1-11	-4.872	- 3.127	- 9.070	-11.381	-8.782	-8.726	-4.709	-7.149	-7.719	-7.028
1-12	-5.971	- 3.558	- 9.475	-10.975	-8.900	-9.032	-5.990	-7.331	-7.901	-8.232
1-13	-6.664	- 3.869	- 8.089	-10.687	-8.708	-11.589	-9.067	-11.508	-12.483	-12.080
2-1	-4.929	-3.510	-10.051	-13.172	-9.476	-9.339	-5.913	-7.842	-8.412	-8.510
2-2	-6.201	-4.130	-9.475	-10.975	-9.476	-9.500	-4.891	-7.842	-8.412	-6.933

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Table 9: Continued.

	Population									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
2-3	-7.820	-4.683	-9.475	-12.074	-9.519	-12.507	-11.552	-12.383	-13.359	-14.765
2-4	-7.763	-4.300	-8.494	-10.282	-8.826	-11.894	-10.348	-11.690	-12.665	-13.284
2-5	-12.756	-7.437	-13.546	-14.357	-15.145	-18.665	-13.831	-16.783	-17.758	-16.748
2-6	-7.357	-7.301	-19.578	-19.126	-20.388	-21.638	-15.153	-16.495	-17.065	-17.759
2-7	-6.664	-3.869	-8.089	-10.687	-8.708	-11.589	-9.067	-11.508	-12.483	-12.080
2-8	-6.952	-4.824	-9.070	-12.479	-9.977	-12.669	-9.173	-12.712	-13.687	-12.262
2-9	-7.300	-4.561	-9.881	-10.570	-9.593	-9.805	-6.172	-8.024	-8.594	-8.137
2-10	-4.929	-3.510	-10.051	-13.172	-9.476	-9.339	-5.913	-7.842	-8.412	-8.510
2-11	-8.942	-4.426	-4.152	-7.305	-4.425	-8.726	-8.374	-7.149	-7.719	-7.028
2-12	-10.540	-4.809	-9.714	-9.097	-11.034	-15.948	-11.524	-12.894	-13.176	-13.880
2-13	-9.441	-7.207	-6.049	-9.097	-11.034	-15.948	-15.189	-12.894	-13.176	-13.880
3-1	-12.505	-8.569	-4.493	-6.207	-10.960	-18.970	-18.525	-17.253	-17.940	-17.354
3-2	-11.520	-8.905	-6.049	-10.196	-12.228	-19.891	-19.652	-18.457	-19.144	-19.113
3-3	-11.832	-5.599	-3.576	-6.207	-4.469	-11.894	-14.013	-11.690	-12.665	-13.284
3-4	-10.734	-5.168	-3.171	-6.612	-4.351	-11.589	-12.732	-11.508	-12.483	-12.080
3-6	-13.912	-7.296	-3.576	-7.305	-5.663	-15.837	-18.477	-17.253	-18.633	-18.517
3-7	-10.734	-5.168	-3.171	-6.612	-4.351	-11.589	-12.732	-11.508	-12.483	-12.080
3-8	-13.161	-6.602	-3.982	-5.801	-5.162	-12.667	-14.195	-12.383	-13.359	-13.188

	Population									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
4-1	-18.772	-11.971	-8.563	-2.825	-7.008	-13.820	-19.806	-13.770	-13.359	-13.977
4-2	-17.037	-7.161	-3.528	-3.230	-6.602	-18.890	-19.036	-17.253	-17.940	-18.142
5-1	-17.037	-7.161	-3.528	-3.230	-6.602	-18.890	-19.036	-17.253	-17.940	-18.142
5-2	-11.832	-5.599	-3.576	-6.207	-4.469	-11.894	-14.013	-11.690	-12.665	-13.284
5-3	-13.912	-7.296	-3.576	-7.305	-5.663	-15.837	-18.477	-17.253	-18.633	-18.517
5-4	-8.995	-6.690	-4.606	-12.074	-4.798	-9.922	-13.559	-12.894	-14.563	-13.841
5-5	-10.734	-9.238	-8.089	-7.711	-4.639	-6.133	-12.732	-7.842	-7.719	-7.498
5-6	-10.580	-9.574	-9.657	-14.645	-3.758	-3.414	-4.044	-3.665	-8.594	-8.607
5-7	-9.769	-10.098	-10.233	-16.842	-4.909	-4.189	-2.869	-4.687	-9.616	-7.585
5-8	-9.481	-9.143	-9.252	-15.050	-3.640	-3.108	-2.763	-3.483	-8.412	-7.403
5-9	-10.041	-8.927	-9.475	-7.999	-4.830	-3.576	-9.655	-3.665	-3.137	-3.651
5-10	-10.580	-9.574	-9.657	-14.645	-3.758	-3.414	-4.044	-3.665	-8.594	-8.607
5-11	-9.481	-9.143	-9.252	-15.050	-3.640	-3.108	-2.763	-3.483	-8.412	-7.403
5-12	-14.398	-13.819	-13.140	-11.381	-4.793	-3.568	-7.681	-4.176	-7.719	-7.498
5-13	-4.836	-9.094	-9.945	-15.050	-5.757	-5.756	-8.625	-6.861	-7.719	-7.626
6-1	-9.538	-9.526	-10.233	-16.842	-4.333	-3.722	-3.967	-4.176	-9.105	-8.885
6-2	-10.580	-9.574	-9.657	-14.645	-3.758	-3.414	-4.044	-3.665	-8.594	-8.607
6-3	-9.481	-9.143	-9.252	-15.050	-3.640	-3.108	-2.763	-3.483	-8.412	-7.403

Table 9: Continued.

					Population					
	1	2	3	4	5	6	7	8	9	10
6-4	-9.481	-9.143	-9.252	-15.050	-3.640	-3.108	-2.763	-3.483	-8.412	-7.403
6-5	-10.580	-9.574	-9.657	-14.645	-3.758	-3.414	-4.044	-3.665	-8.594	-8.607
6-6	-10.580	-9.574	-9.657	-14.645	-3.758	-3.414	-4.044	-3.665	-8.594	-8.607
6-7	-9.481	-9.143	-9.252	-15.050	-3.640	-3.108	-2.763	-3.483	-8.412	-7.403
6-8	-15.902	-19.306	-18.885	-15.456	-15.625	-9.547	-18.124	-12.136	-4.277	-12.485
6-9	-10.580	-9.574	-9.657	-14.645	-3.758	-3.414	-4.044	-3.665	-8.594	-8.607
6-10	-9.481	-9.143	-9.252	-15.050	-3.640	-3.108	-2.763	-3.483	-8.412	-7.403
6-11	-9.538	-9.526	-10.233	-16.842	-4.333	-3.722	-3.967	-4.176	-9.105	-8.885
6-12	-10.580	-9.574	-9.657	-14.645	-3.758	-3.414	-4.044	-3.665	-8.594	-8.607
6-13	-9.769	-10.098	-10.233	-16.842	-4.909	-4.189	-2.869	-4.687	-9.616	-7.585
6-14	-10.810	-10.146	-9.657	-14.645	-4.333	-3.882	-2.946	-4.176	-9.105	-7.308
6-133	-8.942	-8.496	-9.070	-8.404	-4.713	-3.271	-8.374	-3.483	-2.955	-2.447
6-134	-8.942	-8.496	-9.070	-8.404	-4.713	-3.271	-8.374	-3.483	-2.955	-2.447
6-135	-8.942	-8.496	-9.070	-8.404	-4.713	-3.271	-8.374	-3.483	-2.955	-2.447
6-136	-10.041	-8.927	-9.475	-7.999	-4.830	-3.576	-9.655	-3.665	-3.137	-3.651
6-137	-10.041	-8.927	-9.475	-7.999	-4.830	-3.576	-9.655	-3.665	-3.137	-3.651
6-138	-16.883	-15.254	-12.853	-11.381	-10.175	-9.688	-17.783	-12.894	-13.176	-13.243
6-139	-9.230	-9.452	-10.051	-10.196	-5.981	-4.352	-8.479	-4.687	-4.159	-2.629

	Population									
	1	2	3	4	5	6	7	8	9	10
6-140	-8.942	-8.496	-9.070	-8.404	-4.713	-3.271	-8.374	-3.483	-2.955	-2.447
6-141	-8.942	-8.496	-9.070	-8.404	-4.713	-3.271	-8.374	-3.483	-2.955	-2.447
6-142	-9.347	-14.032	-14.121	-12.479	-7.485	-6.603	-12.955	-8.065	-7.537	-7.211
6-143	-8.942	-8.496	-9.070	-8.404	-4.713	-3.271	-8.374	-3.483	-2.955	-2.447
7-1	-9.481	-9.143	-9.252	-15.050	-3.640	-3.108	-2.763	-3.483	-8.412	-7.403
7-2	-10.580	-9.574	-9.657	-14.645	-3.758	-3.414	-4.044	-3.665	-8.594	-8.607
7-3	-9.769	-10.098	-10.233	-16.842	-4.909	-4.189	-2.869	-4.687	-9.616	-7.585
7-4	-12.562	-9.143	-18.415	-15.050	-15.471	-16.325	-6.655	-13.587	-17.940	-18.142
7-5	-20.105	-20.646	-19.760	-22.795	-15.245	-16.020	-5.877	-12.830	-17.758	-18.134
7-6	-9.481	-9.143	-9.252	-15.050	-3.640	-3.108	-2.763	-3.483	-8.412	-7.403
7-7	-11.098	-11.102	-10.638	-16.437	-5.602	-4.963	-3.051	-5.380	-10.309	-7.490
7-8	-11.098	-11.102	-10.638	-16.437	-5.602	-4.963	-3.051	-5.380	-10.309	-7.490
8-1	-10.580	-9.574	-9.657	-14.645	-3.758	-3.414	-4.044	-3.665	-8.594	-8.607
8-2	-14.686	-14.774	-14.121	-13.172	-6.061	-4.649	-7.786	-5.380	-8.923	-7.681
8-3	-4.025	-3.820	-5.181	-12.074	-3.560	-2.811	-3.456	-2.790	-3.648	-2.352
8-4	-9.193	-9.094	-9.945	-15.050	-8.728	-8.726	-8.625	-3.195	-7.719	-7.626
8-5	-14.110	-13.770	-13.833	-11.381	-9.881	-9.186	-13.543	-3.888	-7.026	-7.721
9-111	-10.041	-8.927	-9.475	-7.999	-4.830	-3.576	-9.655	-3.665	-3.137	-3.651

	Population									
	1	2	3	4	5	6	7	8	9	10
9-112	-15.902	-19.306	-18.885	-15.456	-15.625	-9.547	-18.124	-12.136	-4.277	-12.485
9-113	-15.902	-19.306	-18.885	-15.456	-15.625	-9.547	-18.124	-12.136	-4.277	-12.485
9-114	-9.230	-9.452	-10.051	-10.196	-5.981	-4.352	-8.479	-4.687	-4.159	-2.629
9-115	-8.942	-8.496	-9.070	-8.404	-4.713	-3.271	-8.374	-3.483	-2.955	-2.447
9-116	-10.041	-8.927	-9.475	-7.999	-4.830	-3.576	-9.655	-3.665	-3.137	-3.651
10-144	-9.230	-9.452	-10.051	-10.196	-5.981	-4.352	-8.479	-4.687	-4.159	-2.629
10-145	-4.025	-3.820	-5.181	-12.074	-3.560	-2.811	-3.456	-2.790	-3.648	-2.352
10-146	-4.025	-3.820	-5.181	-12.074	-3.560	-2.811	-3.456	-2.790	-3.648	-2.352
10-147	-5.642	-5.779	-6.568	-13.460	-5.522	-4.665	-3.744	-4.687	-5.545	-2.439
10-148	-10.271	-9.499	-9.475	-7.999	-5.406	-4.044	-8.556	-4.176	-3.648	-2.352
10-149	-10.271	-9.499	-9.475	-7.999	-5.406	-4.044	-8.556	-4.176	-3.648	-2.352
10-150	-8.942	-8.496	-9.070	-8.404	-4.713	-3.271	-8.374	-3.483	-2.955	-2.447
10-151	-8.942	-8.496	-9.070	-8.404	-4.713	-3.271	-8.374	-3.483	-2.955	-2.447

Table 10:   Log likelihood of individual genotypes in combined Illinois chorus frog populations of 4,5 and 9 plus populations 7 and 10 from Clay County, AR. Population number is followed by sample number (eg. 4-1, represents population 4-individual 1).

Population 4* (4, 5 and 9)

	1	2	3	4	5	6	7
4-1	-18.772	-11.971	-8.563	-6.235	-13.820	-16.609	-13.770
4-2	-17.037	-7.161	-3.528	-6.607	-18.890	-20.597	-17.253
5-1	-17.037	-7.161	-3.528	-6.607	-18.890	-20.597	-17.253
5-2	-11.832	-5.599	-3.576	-4.721	-11.894	-14.953	-11.690
5-3	-13.912	-7.296	-3.576	-6.433	-15.837	-20.556	-17.253
5-4	-8.995	-6.690	-4.606	-5.853	-9.922	-15.059	-12.894
5-5	-10.734	-9.238	-8.089	-4.284	-6.133	-9.133	-7.842
5-6	-10.580	-9.574	-9.657	-4.547	-3.414	-4.406	-3.665
5-7	-9.769	-10.098	-10.233	-5.773	-4.189	-3.313	-4.687
5-8	-9.481	-9.143	-9.252	-4.467	-3.108	-3.166	-3.483
5-9	-10.041	-8.927	-9.475	-3.959	-3.576	-4.917	-3.665
5-10	-10.580	-9.574	-9.657	-4.547	-3.414	-4.406	-3.665
5-11	-9.481	-9.143	-9.252	-4.467	-3.108	-3.166	-3.483
5-12	-14.398	-13.819	-13.140	-4.690	-3.568	-4.083	-4.176
5-13	-4.836	-9.094	-9.945	-6.221	-5.756	-8.676	-6.861
9-111	-10.041	-8.927	-9.475	-3.959	-3.576	-4.917	-3.665
9-112	-15.902	-19.306	-18.885	-8.236	-9.547	-14.962	-12.136
9-113	-15.902	-19.306	-18.885	-8.236	-9.547	-14.962	-12.136
9-114	-9.230	-9.452	-10.051	-5.185	-4.352	-3.824	-4.687
9-115	-8.942	-8.496	-9.070	-3.879	-3.271	-3.677	-3.483
9-116	-10.041	-8.927	-9.475	-3.959	-3.576	-4.917	-3.665

Table 10: Continued.

Population 7* (7 and 10)

	1	2	3	4	5	6	7
7-1	-9.481	-9.143	-9.252	-4.467	-3.108	-3.166	-3.483
7-2	-10.580	-9.574	-9.657	-4.547	-3.414	-4.406	-3.665
7-3	-9.769	-10.098	-10.233	-5.773	-4.189	-3.313	-4.687
7-4	-12.562	-9.143	-18.415	-16.846	-16.325	-8.218	-13.587
7-5	-20.105	-20.646	-19.760	-16.845	-16.020	-7.816	-12.830
7-6	-9.481	-9.143	-9.252	-4.467	-3.108	-3.166	-3.483
7-7	-11.098	-11.102	-10.638	-6.371	-4.963	-3.360	-5.380
7-8	-11.098	-11.102	-10.638	-6.371	-4.963	-3.360	-5.380
10-144	-9.230	-9.452	-10.051	-5.185	-4.352	-3.824	-4.687
10-145	-4.025	-3.820	-5.181	-3.656	-2.811	-2.761	-2.790
10-146	-4.025	-3.820	-5.181	-3.656	-2.811	-2.761	-2.790
10-147	-5.642	-5.779	-6.568	-5.560	-4.665	-2.954	-4.687
10-148	-10.271	-9.499	-9.475	-4.477	-4.044	-3.724	-4.176
10-149	-10.271	-9.499	-9.475	-4.477	-4.044	-3.724	-4.176
10-150	-8.942	-8.496	-9.070	-3.879	-3.271	-3.677	-3.483
10-151	-8.942	-8.496	-9.070	-3.879	-3.271	-3.677	-3.483

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Appendix A. Article published in *Wildlife Society Bulletin* pertaining to populations

declines and current agriculture practices in northeast Arkansas.

Appendix B. Article submitted to *Zootaxa* pertaining to morphometric analyses of

Illinoisand Strecker's chorus frogs.