

AN ICON OF CONSERVATION BIOLOGY: MOLECULAR ECOLOGY OF GREATER
PRAIRIE CHICKEN (*TYMPANUCHUS CUPIDO PINNATUS*) IN ILLINOIS

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Biology
in the Graduate College of the
University of Illinois at Urbana, Champaign, 2011

Urbana, Illinois

Master's Committee:

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ABSTRACT

The Greater Prairie Chicken (GPCH), a once abundant bird in Illinois, declined to fewer than 50 individuals prior to genetic rescue in 1992-1998 manifested by annual translocations of birds from out-of-state populations. These efforts partially restored genetic variability lost through historic inbreeding and low population sizes, yet retention of long-term diversity remains questionable, particularly in light of continued population declines, geographic isolation, and a lek mating system. This study re-evaluated genetic variability of the GPCH populations at the Prairie Ridge State Natural Area (PRSNA) in Illinois by amplifying 24 microsatellite loci using genomic DNA extracted from shed feathers. Genetic profiles accrued from 1,229 feathers identified 74 individuals, and were used to derive population genetic parameters including relatedness, population structure, and gene flow. GPCH showed remarkable lek fidelity. Some individuals (3%) moved among leks within counties but none moved between counties. Bayesian cluster analyses reinforced these data by identifying population substructure at two levels: county and family group. To facilitate comparisons with earlier studies, all analyses were conducted using the full suite of 24 loci, and a reduced panel of 6 (from earlier studies). These comparisons reveal that post-translocation genetic diversity was largely retained in Jasper County, but corresponded in Marion County to characteristics of bottlenecked populations. Both microsatellite panels provided similar answers in the majority of analyses, although the reduced panel failed to elucidate population substructure. This project highlights the necessity of continued genetic monitoring of small populations that elicit conservation concern. These data will promote an ongoing recovery effort with this iconic Illinois species by gauging the success of past conservation actions while guiding future management and restoration efforts.

ACKNOWLEDGEMENTS

The success of this project is attributable to many people who assisted in various capacities. Most importantly, I would like to thank my advisers, Drs. Michael and Marlis Douglas, for their endless help in providing ideas, giving feedback on molecular analyses, reviewing thesis drafts, and always being available to answer questions. I also thank my lab-mates Aubrey and Jared Reynolds for dosing DNA samples and running PCRs whenever asked, and Mark Davis, who was instrumental in conducting primer and multiplex tests. Without their help, this project would never have been completed in a timely fashion.

Many people at the Illinois Department of Natural Resources (IDNR) played vital roles as well. Wade Louis was extremely supportive in providing background literature on Greater Prairie Chickens in Illinois, much of which would have been otherwise difficult, if not impossible, to obtain. Also, special thanks go to everyone at Prairie Ridge State Natural Area (IL), especially Scott Simpson (Supervisor) who graciously provided literature, translocation history, and lek survey data. Both Leroy Harrison and Robert Shelby collected feathers from leks in 2010 and 2011. Obviously these efforts were key to the success of the project.

Institutional support was received from the Illinois Natural History Survey (Dr. Brian D. Anderson, Director), IDNR (John Buhnerkempe, Wildlife Resources Division Chief), and U.S. Fish & Wildlife Service Federal Aid in Wildlife Restoration (Project W-155-R administered through IDNR). Teaching assistantships were provided by the School of Molecular and Cellular Biology at University of Illinois Urbana-Champaign.

Last but not least, I acknowledge the emotional support of my parents, and their understanding of my educational choices. Without them, none of this would have been possible.

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CHAPTER 1

INTRODUCTION

Since its inception, conservation biology has been a crisis discipline bridging the gap between biological research and its practical applications for management of biodiversity (Soulé 1985). Out of necessity this field has been quick in adapting innovative non-invasive sampling methods (Horváth *et al.* 2005) and adopting modern genetic and genomic techniques (Allendorf *et al.* 2010; Schwartz *et al.* 2007). Despite these advances, the trajectory of the discipline remains uphill as biodiversity will continue to decrease over the next century (Pimm *et al.* 1995; Sala *et al.* 2000). These predictions largely reflect anthropogenic impacts, and a chronic failure by society to adequately compensate for their negative effects. These have most often closely followed the spread of civilization (McKee *et al.* 2003).

By most measures, the collective footprint of humankind in North America is best reflected in the Midwest (Ramankutty & Foley 1999). This region has been continuously fragmented for 300 years with forests felled, prairies plowed, and streams sequestered for agricultural and urban purposes (Turner & Rabalais 2003). Natural heritage impacts are indeed evident. Furthermore, global climate change has seriously compounded these effects (Ewers & Didham 2006; McCarty 2001). At issue is the rate at which climate change occurs (i.e., potentially too rapid for organisms to track genetically) and how this impacts natural communities (Carroll *et al.* 2007; Gienapp *et al.* 2008). Those species most severely impacted will exhibit superficial cohesiveness, and unidirectional migrations from high to low quality habitats (i.e., from source-to-sinks) will not counterbalance losses due to habitat constriction (Purvis *et al.* 2000). This will be particularly severe for those with limited dispersal capabilities,

or with specific habitat requirements (Thomas *et al.* 2004). Consensus argues for proactive regional management strategies, yet natural resource agencies continue to operate under traditional fish and wildlife paradigms, even as these prove inadequate to address new challenges (Buysee & Verbeke 2002). Management must instead be entrepreneurial, with synthetic policies developed progressively for entire ecosystems (Simberloff 1998).

These issues serve as a bookmark for the management of an upland game bird in the Midwest [i.e., the Greater Prairie Chicken (GPCH: *Tympanuchus cupido pinnatus*; Galliformes: Phasianidae) (Appendix A)]. This species has served as an icon of conservation biology (Campbell & Reece 2005; Freeman & Herron 2003; Hedrick & Kalinowski 2000). The issues impacting GPCH are not necessarily local or even regional, but instead reflect a broader trend (Foley *et al.* 2005; Pertoldi *et al.* 2007) that has reduced its numbers and established it as a species with range-wide conservation concerns (Appendix B). Although several healthy GPCH populations persist on the Great Plains, smaller populations remain scattered across the Central Lowlands of the Midwest (Svedarsky *et al.* 2000). Recent years have seen an emphasis on the intensive study and management of these smaller populations, particularly in Illinois where deteriorating conditions necessitated a translocation of birds into the state from more stable populations elsewhere in the range (Westemeier *et al.* 1998a).

Illinois is one of four states to successfully reintroduce or augment an existing population through translocation (Snyder *et al.* 1999), a practice that may have mixed success or unpredictable outcomes (Appendix C). This drastic action was prompted by a precipitous decline in GPCH numbers from millions in the mid-19th century to 2000 in 1962, and finally to an estimated 46 individuals in 1994 (Westemeier *et al.* 1998a). The first translocation was made in 1979, when 57 GPCH from Kansas were released in Illinois (Sparling 1979). Ultimately, 35

of these birds were relocated onto reclaimed strip-mine land in Perry County, IL and none could be relocated five weeks post-release despite being fitted with radio transmitters. Nine mortalities (26%) were known to have occurred, and no lek was ever established. In 1991, a successful intrastate translocation was conducted by swapping two egg clutches between Marion and Jasper counties, IL (Westemeier *et al.* 1991). Twelve translocated eggs hatched in Jasper County, while eight were successful in Marion County. Movement of birds over the intervening 60km is possible, but not commonplace (Westemeier & Jansen 1995).

Following failed attempts to save the Illinois population through habitat protection and intensive exotic species management, GPCH were again translocated from Kansas, Minnesota, and Nebraska (Westemeier *et al.* 1998a). Between 1992 and 1998, a total of 506 GPCH were released into Jasper and Marion counties in seven annually occurring translocations [(Scott Simpson – Personal Communication) (Table 1)]. The translocations were deemed successful in rescuing the populations from the brink of extinction (Svedarsky *et al.* 2000). Censuses indicated 25-67% of transplanted birds integrated into the breeding population, with hatching success rising from 76% to 94% post-translocation, and mean fertility rates increasing from 91% to 99% (Westemeier *et al.* 1998a).

The loss of neutral genetic diversity in the Illinois GPCH was documented through a comparison of pre-bottleneck museum specimens with larger, contemporary populations (Bouzat 2000). Initial studies evaluated IL GPCH across six microsatellite (msat) loci originally developed for Chicken (*Gallus gallus*), and discovered that post-bottleneck Illinois populations had fewer alleles per locus (3.67) and reduced heterozygosity (0.571) when contrasted with Kansas (5.83/ 0.597), Minnesota (5.33/ 0.654), and Nebraska (5.83/ 0.626) (Bouzat *et al.* 1998a). Genetic analysis of Illinois museum specimens discovered several alleles found in both historical

Illinois specimens and large modern populations were now lacking in the post-bottleneck Illinois population (Bouzat *et al.* 1998b). Furthermore, the historical specimens also possessed alleles that were absent in the modern populations.

Subsequent application of these same six msat loci to Wisconsin populations prompted the discovery of problems with three loci (Bellinger *et al.* 2003) that were consequently replaced with three loci developed for Red Grouse (*Lagopus lagopus scoticus*). This updated suite of loci was later applied to the pre- and post-bottleneck Illinois specimens analyzed for previous studies, in addition to post-translocation samples collected in 2003 (Bouzat *et al.* 2009). Translocation successfully increased allelic richness (5.5) and heterozygosity (0.676) relative to the pre-translocation situation (4.7/ 0.654). However, these values remained low relative to larger populations such as those in Kansas (8.4/ 0.763) and Nebraska (8.0/ 0.731).

Although translocation buoyed the Illinois population in the short-term and increased neutral genetic diversity, it remains unclear if this diversity has been retained to date. Annual census of GPCH leks has been conducted since 1963 (Figure 1). The number of lekking males has steadily declined since 2007, with 73 observed on 11 leks in 2011 (Scott Simpson – Personal Communication). This study re-evaluates genetic diversity in the Illinois GPCH population by genotyping contemporary samples at the six msat loci used previously (Bellinger *et al.* 2003), and at 18 additional loci. Post-translocation genetic diversity was assessed using these two marker systems and genetic structure/ gene flow were quantified among the remaining six leks distributed in fragmented habitats across two IL counties. Microsatellites were also used as genetic tags (Palsbøll 1999) to genotype individuals within the population, estimate population size, and contrast the latter against estimates based on counts of GPCH on leks.

CHAPTER 2

METHODS

2.1 Sample Collection and DNA Extraction

All available feathers were collected from the ground at six GPCH leks in Prairie Ridge State Natural Area (PRSNA: Marion and Jasper Counties, Illinois) during March and April, 2010 and 2011 (Figure 2). Each lek was sampled six times per season, at alternating intervals of 2 and 5 days between collections. Feathers were stored at -20°C. DNA was extracted from 1,726 (75%) of approximately 2,300 feathers (Table 2). Additionally, DNA was extracted from 3 muscle samples harvested from incidental mortalities found at the Copple (2005), Donsbach and Guymon leks (2010). Down feathers were not extracted because of expected genotypic redundancy with other feather types. To test this hypothesis, down feathers from the Bainbridge and Donsbach leks (2010) were extracted to determine if they contain unique genotypes.

DNA extraction protocols utilized the QIAamp DNA Micro Kit (Qiagen-CAT# 56304) following modifications based on Qiagen user-submitted protocol DY04 Aug-06 (Appendix D). This kit compensated for low DNA yields from molted feathers, and has proven successful in previous non-invasive (NIS) bird sampling (Bush *et al.* 2005). All DNA extractions were performed inside a dead air box (AirClean Systems 600 PCR Workstation). Lab coats, latex gloves, and surgical masks were worn while handling samples. Sterilized scissors and forceps were used to cut each feather, with a sterile glass Petri dish as a cutting surface. All scissors, forceps, and Petri dishes were first autoclaved then subjected to 20 minutes of UV exposure prior to use. Samples were extracted in sets of 24 (i.e., 20 GPCH samples + 4 negative controls).

Determination of feather type and condition following Hogan *et al.* (2008). DNA was quantified for a subset of 390 samples of varying feather type and quality using a Hoefer DyNA Quant 200 Fluorometer. These data were utilized to identify additional samples with potential for high DNA yield, which were subsequently dosed. All DNA concentrations were diluted to 5 ng/μl.

2.2 Molecular Methods

Eighty-three microsatellite DNA loci originally developed for eight Galliform species were tested on GPCH (Appendix E). Tests were conducted in 10μl volume polymerase chain reaction (PCR) using 2μl Go-taq 5x buffer (PROMEGA), 2.0mM MgCl₂, 0.25mM dNTPs, 0.2μg BSA, 1 unit Go-taq DNA polymerase (PROMEGA), 0.1μM each of forward and reverse primer, and approximately 20ng template DNA. All PCR conditions were identical with the exception of annealing temperature (variable by locus: Appendix E). These consisted of an initial denaturation step at 94°C for 3 minutes, followed by 15 cycles of 45 seconds at 94°C, 45 seconds annealing, 30 seconds at 72°C, 25 cycles of 30 seconds at 94°C, 30 seconds annealing, and 45 seconds at 72°C, followed by a final extension of 3 minutes at 72°C. Amplification was confirmed using 2.5% agarose gels stained with a 3X solution of GelGreen nucleic acid stain (BIOTIUM). Fifty-nine of the loci (71%) amplified successfully in GPCH.

The forward primers of 54 loci were assigned one of four fluorescent Applied Biosystems (ABI) dyes (6-FAM, NED, PET, or VIC). To test for diversity, these were amplified in 8 individuals and run on an ABI 3730 automated sequencer using either LIZ-500 or LIZ-600 internal size standard. Fourteen (26%) were monomorphic, while an additional six (11%) amplified spurious peaks within the expected allele size range. Two others (4%) produced

fragments too large to be accurately sized using LIZ-600. Of the 32 remaining loci, 24 (44%) were successfully combined into six multiplex panels of four loci each. Modifications to the PCR conditions necessary to test each locus are provided for each multiplex in Appendix F.

2.3 Identification of Genotypes and Mark-Recapture

The 1,729 samples were genotyped using the eight loci in multiplexes 3 and 6 (Table 3). The binning method in GeneMapper 4.0 (ABI) was used to call peaks. Loci were only scored when fragments greater than 100 units of intensity were amplified. Six loci were used to condense the samples into 98 unique genotypes using GENECAAP (Wilberg and Dreher 2004). Two other loci (LLSD7 and MCW146) were excluded from this analysis due to the suspected presence of null alleles. Only samples yielding genotypes for all six loci (N=1229, 71%) were used to identify unique individuals. GENECAAP calculated the probability of two individuals (P_{ID}) sharing the same genotype (Paetkau & Strobeck 1994). It also enhanced detection of scoring errors by identifying genotypes that matched as well as those that differed at one or two alleles. The latter were re-scored in GeneMapper to verify differences. Scoring errors were corrected, after which GENECAAP was utilized to generate capture histories of all unique genotypes. To verify capture histories, a sample representing each capture of a genotype (N=311) was amplified at the 16 additional loci.

The mark-recapture data generated from GENECAAP were input into RCAPTURE (Baillargeon & Rivest 2007); a package for R 2.14 (R Development Core Team 2011) designed to estimate population size. RCAPTURE applied the Jolly-Seber model to mark-recapture histories for the purpose of estimating the total number of birds each year visiting the six leks in this

study. An open-world model was selected to compensate for variation in the number of birds visiting leks between capture events.

Counties were sampled on different days (i.e. feathers were collected in Jasper County on Mondays and Wednesdays, while in Marion County on Tuesdays and Thursdays). As such, a capture event was defined as one complete sampling cycle of all leks (i.e., Monday/ Tuesday equaled one capture event). Sampling was conducted for three weeks each year, resulting in six capture events per year. Bainbridge was excluded from 2011 estimates since it yielded no genotypes for that year. The individual collected in 2005 from Copple was also excluded. Population estimates were compared to visual counts of male GPCH observed on the six leks in 2010 and 2011.

2.4 Linkage Disequilibrium and Hardy-Weinberg Equilibrium

Noninvasive sampling techniques present unique challenges for the quantification of genotyping error (Paetkau 2003). To minimize these, MICROCHECKER v2.2.3 (Van Oosterhout *et al.* 2004) was utilized to detect null alleles and scoring errors. GENEPOP v4.1 (Raymond & Rousset 1995; Rousset 2008) was utilized to test for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD). Default settings were used for HWE and LD tests with the exception of batch number (N=200). These values were determined by running exploratory analyses until the standard error for most tests fell below 0.01. GENEPOP uses a Markov chain method to assess deviation from HWE for all loci with greater than four alleles in a population. The program defaulted to the exact test for all other loci. The probability test in GENEPOP was used to evaluate LD by assessing nonrandom associations among alleles. Significance was assessed using a Bonferroni adjusted P-value for 24 loci ($\alpha = 0.00208$).

2.5 Genetic Diversity and Divergence

A multifaceted approach was used to evaluate genetic similarities and differences between leks. MICROSATELLITE ANALYZER v4.05 (Dieringer & Schlötterer 2003) calculated expected (H_E) and observed (H_O) heterozygosities for each locus. Mean H_E and H_O , were then calculated for each lek and county. Rarefaction (HP-RARE: Kalinowski 2004a) was used to compensate for variation in sample size when calculating allelic richness (A_R). Lek estimates were calculated for 6 individuals (the smallest lek sample size), while county A_R estimates were made for 18 individuals to facilitate comparisons with values from past studies. GENALEX v6.1 (Peakall & Smouse 2006) provided the mean number of alleles (A_M) and private allele counts (A_{PR}) for each county and lek.

Pairwise F_{ST} values were calculated to assess genetic differences among leks and counties, while isolation by distance (IBD) was tested using a Mantel test in ARLEQUIN v3.5 (Excoffier & Lischer 2010). The Copple lek was excluded from the Mantel test due to its representation by a single individual. Calculations were performed using both the full panel of 24 msats (MSP24) and a reduced panel of six (MSP6) (Bouzat *et al.* 2009).

2.6 Relatedness

Average relatedness (r) within and among leks was calculated using COANCESTRY (Wang 2011). This program was set to derive allele frequencies from the entire dataset, utilize 100 reference individuals, and perform 1000 bootstraps. First, a traditional approach (Queller & Goodnight 1989) was used for calculating r (results available upon request). Secondly, a more contemporary likelihood approach was used to calculate relatedness by comparing dyads of individuals to a third reference individual (Wang 2007). Mean relatedness within each lek was

then tested for significance using a one-sample *t*-test (following Gibson *et al.* 2005). A value of $r=0$ indicates individuals are unrelated, while $r=0.125$ designates a 1st cousin relationship, $r=0.25$ represents half-siblings, and $r=0.5$ indicates parent/offspring or full sibling relationships (Blouin 2003). Correlation between distance and mean pairwise relatedness among leks was examined with a Mantel test via ARLEQUIN v3.5, with the Copple lek again being excluded from analysis. All analyses were performed using both msat panels.

2.7 Population Structure

STRUCTURE v2.3.3 (Pritchard *et al.* 2000) was applied to assess if genetic structure exists within the Illinois GPCH population. This program, which utilizes a Bayesian Markov Chain Monte Carlo approach, does not make *a priori* assumptions regarding physical sampling location, and was run using two models. First, the admixture model with no prior population information was applied, using correlated allele frequencies (CAF) and K values of 1 through 12 (where K = numbers of aggregates). The program was run for 1,100,000 generations, with the first 100,000 discarded as burn-in. Ten iterations were run for each K -value to calculate ΔK (Evanno *et al.* 2005) and to test for consistency. The program was then run with identical conditions but with the independent allele frequencies model (IAF). STRUCTURE HARVESTER (Earl & vonHoldt 2011) was used to calculate ΔK , which was used alongside $\Pr(K)$ and compared to biological and geographical patterns to determine the appropriate K value. All simulations were performed using both msat panels. DISTRUCT (Rosenberg 2004) was used to visualize population assignments determined by STRUCTURE. Discriminant analysis was conducted in GENALEX to validate the assignments of individuals to aggregates.

CHAPTER 3

RESULTS

3.1 Identification of Genotypes and Mark-Recapture

GENECAP identified 98 genotypes, of which 74 (76%) were unique and thus representative of individual birds, with 67 of these (91%) being detected multiple times (i.e., via two or more feathers). The P_{ID} was low (9.7×10^{-8}), indicating that two unique individuals did not share the same genotype. Five individuals (7%) were identified only once while two (3%) were documented solely from carcasses. The remaining 24 genotypes (24%) had amplification difficulties that prevented their confirmation as unique. Nine of these (38%) were eliminated due to large allele dropout ($N=5$; 21%) or scoring error ($N=4$; 17%) due to a 104 base pair (bp) artifact in the ADL230 locus. Thirteen others (54%) failed to amplify at three or more loci and were thus excluded from downstream analyses. Two additional genotypes (8%) were distinguishable at the TTD1 locus by a single allelic difference and were thus excluded as potential genotyping error.

On a seasonal basis, 2005 yielded one individual (as a carcass). The 2010 season yielded 57 unique individuals, 35% more than were recorded in 2011 ($N=37$). Twenty-one individuals were detected in both years (i.e., 37% of those identified in 2010 reappeared again in 2011, and comprised 57% of the population that year; Table 4). Remarkable lek fidelity was noted. Twenty of 21 individuals detected in both years (95%) were resident each year at the same lek. Only two (3%) were recorded at multiple leks. One was at Survey lek in 2010 but visited Guymon in 2011 before returning again that same year to Survey (both are separated by 2.74 km). The second bird was undetected in 2010 but visited Guymon in 2011 before shifting to Survey later that year.

Seventy-two of 74 individuals (97%) were detected solely at single leks. No movements were recorded between Marion and Jasper counties.

Extraction of down feathers from Bainbridge and Donsbach led to the discovery of only one individual that would have otherwise gone undetected. This bird was identified (twice) from two (of 118) down feathers extracted in 2010. Seventy-eight of the remaining 116 down feathers (67%) yielded genotypes redundant with those derived from other feather types, while the remaining 38 (33%) were deficit in amounts of DNA necessary for analyses. By contrast, other feather types had a combined 71% success rate.

Application of the Jolly-Seber mark-recapture model yielded poor estimates of lek visitation when compared to lek survey counts (Table 5). Closed-world model estimates implemented by RCAPTURE (data not presented) did not improve upon population estimates. Lek surveys only count male GPCH, whereas genotypes studied here are assumed to be a mixture of males and females. Estimated population size for 2010 barely exceeded the observed number of male GPCH (64.1 vs. 63). Population estimates for the five leks studied in 2011 failed to exceed the observed number of male GPCH (38.3 vs. 46).

3.2 Linkage Disequilibrium and Hardy-Weinberg Equilibrium

Potential null alleles (as homozygote excess) were identified in two loci: CUR06 (Jasper County) and LLSD07 (Marion County). A potential scoring error or presence of a null allele was also noted for LLSD09 (Jasper County). Three statistically significant detections of homozygote excess were also detected by GENEPOP in three of 144 tests (2.1%). These occurred at Donsbach (LLSD09) and YFM (CUR06) leks (Jasper County) and Guymon lek (LLSD7) (Marion County).

These six HWE deviations fall below the 7.2 significant results expected by chance alone at the 5% level.

GENEPOP detected significant LD in 28 tests involving 24 pairs of loci across six leks (Table 6). The preponderance of these ($N=18$; 64%) occurred in Donsbach lek. Four were significant at Guymon, and 6 at YFM. No LD was identified at the remaining three leks. As context, only 13.8 significant results are expected by chance alone when 276 pairwise comparisons are evaluated at the $P=0.05$ confidence level. This is 42% lower than the 24 recorded in this analysis. However, little consistency was detected among the LD results. For example, only two pairs of loci (LLSD7/ MNT12; TTT2/ BG16) reflected LD at more than one lek, and accounted for most failed tests overall. TTT2 failed six tests, MNT12, BG16, and LLSD4 each failed five, while LLSD7, LLST4, and TTT1 each failed four. Yet despite the lack of a clear pattern, the potential violation of LD assumptions at the Donsbach lek must still be interpreted carefully.

3.3 Genetic Diversity and Divergence

Estimates of A_R were similar for most leks, particularly for MSP24 (Table 7A). Survey was the sole exception ($A_R=2.8$), with its value falling well below estimates for all other leks using MSP24 ($A_R=3.8-4.0$). Results using MSP6 were similar (Table 7B), although the difference between Survey ($A_R=3.2$) and the remaining leks ($A_R=3.7-4.2$) was not as great. The A_R values calculated for Jasper County, along with average number of alleles per locus (A_M) and H_E estimates closely matched their corresponding values for the 2003 Jasper County post-translocation estimates (Table 7D). A_R and A_M values for Marion County exceeded those of the

pre-translocation population, however H_E fell short. Estimates for MSP24 (Table 7C) varied slightly from those for MSP6, but reflected the same overall trends.

F_{ST} values were significant when both msat panels were used to compare counties. This trend continued when counties were separated into leks, however F_{ST} values and within-county significance varied by msat panel (Table 8). F_{ST} values were non-significant within either county using MSP6. In contrast, MSP24 yielded small but significant F_{ST} values within Marion County, except when comparing Guymon and Bainbridge. F_{ST} values within Jasper County were low for both panels and non-significant. Both msat panels found the greatest difference between the two leks with the smallest sample sizes (Survey and Hunt). High levels of genetic differentiation were evident for all intercounty lek comparisons as evidenced by the positive correlation between pairwise F_{ST} and geographic distance among leks (Figure 3). Mantel tests found significant isolation by distance (IBD) when comparing F_{ST} values (both panels) vs linear distances among leks.

3.4 Relatedness Among Counties and Leks

Relatedness was estimated using different msat panels and approaches, but overall trends were similar. Relatedness values were first calculated following Queller and Goodnight (1989) (=QG89) and were significant for both msat panels within all leks save Hunt and YFM (data not shown). Mean relatedness in Survey was between half- and full-sib, while Guymon had values equating to first cousins. Estimates for the remaining three leks were also significant for the MSP24 panel, and more variable using MSP6 though overall trends remained similar.

Using the Wang (2007) (=W07) approach, relatedness was significant for all leks across both panels (Table 9), and consistently greater than QG89 estimates. Relationships using the

MSP24 panel corresponded to first-cousin, occasionally higher, especially with MSP6. The Survey lek again had a mean relatedness value between half- and full-sib. The MSP6 panel once again displayed greater variance in its estimates, the exception being Survey and Bainbridge leks.

Mean relatedness among leks offered fewer clear patterns (Table 10). Trends were similar for MSP24 and MSP6 under QG89, with low relatedness among counties and within Jasper County (data not shown). Pairwise estimates for Marion County fell between first cousin/half-sibling. A greater disparity was observed between msat panels using W07, with MSP6 often providing greater relatedness estimates among counties. Both panels found higher relatedness values within Jasper County. Mantel tests comparing relatedness vs. linear distances among leks were non-significant for any combination of panels and relatedness estimators (data not shown).

3.5 Population Structure and Relatedness

Results from STRUCTURE were variable by model, microsatellite panel, and method of K estimation. Evanno's ΔK was the most consistent delineator, finding $K=2$ as the best estimate for numbers of populations, independent of model choice or msat panel (Figure 4). These populations corresponded to Marion and Jasper counties (Figure 5). Calculating the posterior probability of each K allowed for evaluation of the full spectrum of K values, but led to more variable results (Figure 6). Analyzing MSP6 using both correlated (CAF) and independent allele frequency (IAF) models produced best supported values of $K=3$. For populations above $K=3$, STRUCTURE encountered difficulty in assigning individuals, often determining a near-equal probability of assigning each individual to multiple populations.

The IAF and CAF models converged on different answers for MSP24. The CAF model, for example, found the greatest posterior probability at $K=10$, with each county being divided into multiple subpopulations (Marion = 4, Jasper = 6), none of which aligned with leks (aside from Survey, which had 6 individuals assigned). On the other hand, the IAF model found the best supported estimate to be $K=4$ populations, with Jasper County being subdivided into three subpopulations (none of which corresponded to a lek), while Marion County remained intact.

Mean relatedness for clusters identified by the CAF model in STRUCTURE underscore that the subpopulations within each county correspond to family groups (Table 11). For example, relatedness within 80% of these subpopulations (=family groups) was higher than the minimum value of a half-sib relationship (i.e., $r > 0.25$). The two subpopulations (one in each county) that failed to achieve this standard did so by less than 10 and 12% (i.e., 0.222 and 0.227 vs. 0.25; Table 9). Eleven (of 13) pairwise comparisons among subpopulations within Jasper County ranked higher than second cousin, while the remaining two exceeded first cousin status. Six (of seven) cross-comparisons within Marion County were similarly greater than that for second cousin ($r > 0.03125$), with the seventh exceeding first cousin ($r > 0.125$). However, when subpopulations were compared across counties, only three of 24 (12.5%) scored higher than second cousin. All pairwise F_{ST} values for these subpopulations were significant (Table 12).

The IAF model in STRUCTURE yielded four subpopulations, three in Jasper and one in Marion counties. Relatedness within two (of three) Jasper County subpopulations ranked higher than that for half-sibs, while the third superseded first cousin (Table 13). Relatedness within the sole Marion County subpopulation exceeded that for second cousin. Cross-comparisons ($N=3$) within Jasper County yielded relatedness values greater than second cousins. Cross-comparisons

($N=3$) between Jasper and Marion counties ranked less than second cousin. All pairwise F_{ST} values for the IAF-identified groupings were significant (Table 14).

Results of the discriminant analysis verified $K=4$ and $K=10$ as valid clusters of individuals. Values of $K=4$ (97%) and $K=10$ (96%) saw high percentages of individuals assigned to the STRUCTURE-defined populations. Assignment was highest when GPCH were divided by county (99%). However, this trend breaks down when individuals are partitioned by lek, which found only 49% correctly assigned.

CHAPTER 4

DISCUSSION

4.1 Identification of Genotypes and Mark-Recapture

Extracting and amplifying DNA from molted feathers was quite successful. The 71% genotyping success rate was similar to that observed in other NIS studies. Svobodová *et al.* (2011) reported a 56.3% success rate for shed feathers, while Alda *et al.* (2011) had a 69% success rate. Complete sampling of all individuals visiting a lek was not achieved, but results compared across years do reflect known demographic trends. Sixty-three male GPCH were sighted on the six observed leks in 2010, but only 57 individuals were identified from shed feathers. These data suggest that not all males were sampled, and the 57 individuals likely include several females that were not included in lek counts.

Fewer individuals were detected in 2011, but this was consistent with population fluctuations and sampling difficulties. For example, three leks (Donsbach, Guymon, and YFM) each reflected declines of 3-5 males. The other three leks saw increases of 1-2 individuals each. Excluding Bainbridge, for which seven males were observed but no genotypes could be produced, 46 male GPC were observed while 37 genotypes (80%) were detected.

Mark-recapture models failed to estimate populations significantly larger than the number of observed males. These discrepancies may have several causes. Individual detection probability varies with behavior and sex (Boulanger *et al.* 2004), and can be troublesome for producing accurate population estimates (Link 2003). Heterogeneity was observed in exploratory analyses, particularly the 2011 dataset (data not shown) which produced marginally poorer estimates than in 2010. Feathers for genotyping were lost during combat or mating

attempts between birds. Fighting also decreases later in the season, once territories are established (Hamerstrom 1939). Logically, it follows that less aggressive GPCH will lose fewer feathers through combat or copulation and thus will be more difficult to detect as a result. Lek visitations also vary by sex. Robel (1970) found the number of males attending a lek to decrease as the breeding season progressed, and repeat visitations by females were relatively fewer in number.

These differential detection probabilities are evidenced by variation in the number of feathers representing each individual in this study. Some individuals were detected only once, while one individual was identified from 145 feathers, accounting for 8.4% of all samples. The probability of two individuals sharing the same genotype was low [$P_{ID}=9.7 \times 10^{-8}$], translating to a 1 in 10,353,475 chance that two unique individuals will share the same genotype. This indicates that the six selected markers were suitable for identifying individuals, and all individuals were accounted for in samples from which complete genotypes could be produced. Unique genotypes could, however, remain undetected among the 500 feathers that failed to amplify at all loci.

A low P_{ID} also increases certainty in the observed lek fidelity, as it increases confidence that genotypes detected multiple times from the same lek reflect the same individual. The high levels of observed lek fidelity within and among years match trends observed among closely related North American grouse species. Sharp-Tailed Grouse exhibited a 95% rate of lek fidelity for males and 81% for females (Drummer *et al.* 2011). Schroeder and Robb (2003) found that 82% of male Greater Sage-Grouse (*Centrocercus urophasianus*) visited one lek, while the same was true for only 59% of females. Female GPCH (85%) have been observed visiting more than one lek in a season (Schroeder 1991). In contrast to Illinois, this population inhabited a less

fragmented landscape and had many more leks from which to choose, with an average of 43 active leks within a 301 km² area each year. If these trends hold true across species (i.e., female GPCH are more likely to visit multiple leks than male GPCH), then the high lek fidelity observed in this study may indicate more males were sampled than females. This hypothesis will be testable as sex-determining markers (Griffiths *et al.* 1998) are applied to GPCH samples.

4.2 Linkage Disequilibrium and Hardy-Weinberg Equilibrium

Statistically significant levels of LD are present within leks, although this may not result exclusively from physical linkage. Only two pairs of loci exhibited consistent patterns of linkage at multiple leks. The high levels of observed LD may instead be the product of bottleneck and founder events stemming from translocation. Wang *et al.* (1998) noted that changes in gene frequencies resulting from these events can have lasting effects on perceived LD among loci after multiple generations, even when they are in HWE. Also, a Wahlund effect can result when multiple subpopulations are grouped and analyzed for LD (Law *et al.* 2003). Even when loci are physically unlinked, a pooling of multiple subpopulations can discern statistically significant linkage (Durand *et al.* 2003). This is manifested in the Donsbach lek, where subpopulation structure was found using the CAF model (which is less susceptible to overestimation of *K* when populations include closely related individuals). The second greatest number of linked loci was found at YFM, which contained similar subpopulation structure. Thus, the pooling of multiple subpopulations at this lek could artificially inflate the actual number of linked loci.

As the three significant tests of deviation from HWE indicate, null alleles are likely present at these loci. This is unsurprising for LLSD7, given its high rate of failure at the Bainbridge lek (i.e., only 49% of feathers successfully amplified at this locus). In contrast, the

remaining three loci had a combined 22% failure rate. Null alleles for LLSD9 were also anticipated, as this locus had null alleles in the pre-translocation population (Bouzat *et al.* 2009). It also displayed a complicated and highly variable stutter pattern which makes scoring difficult. To address this issue, tailed primers will be utilized to reduce stutters and these samples will be re-amplified and re-scored (Brownstein *et al.* 1996).

4.3 Genetic Diversity and Divergence

To understand the current status of the Illinois GPCH, the data presented here must be contrasted against previous studies. This is accomplished by comparing estimates calculated from MSP6 to genetic diversity estimates presented by Bouzat *et al.* (2009) for the 2003 Jasper County population. Those estimates are very similar (2003: $H_E=0.676$; $A_R=5.5$ vs. 2010/ 2011: $H_E=0.688$; $A_R=5.6$), indicating that heterozygosity levels have held steady in the intervening 8 years.

Unfortunately, no post-translocation reference point exists for Marion County. STRUCTURE results combined with significant F_{ST} and IBD values demonstrate the two counties represent unique populations. This isolation event occurred approximately 30 years ago (Westemeier *et al.* 1998a). Therefore, the 2003 Jasper County estimates would be an inadequate baseline. However, it is worth noting that 2010/ 2011 heterozygosity values for Marion County ($H_E=0.611$; $A_R=5.1$) are lower than pre-translocation values ($H_E=0.654$; $A_R=5.2$; Bouzat *et al.* 2009). This does provide a comparative baseline for diversity, albeit tenuous. This is because pre-translocation estimates of heterozygosity are based upon 32 incidental GPCH mortalities that occurred over 19 years, with no analytical consideration made regarding county of origin (Bouzat *et al.* 1998a). This equates to an averaging of heterozygosity over a period during which

great fluctuations occurred in GPCH populations (Figure 1). In contrast, reduced costs and coupled with technological advances have allowed this study to provide a contemporary benchmark for genetic diversity within the Illinois GPCH populations, thus encapsulating a more complete assessment of genetic variation over a much more compressed timeframe.

It is not possible to determine with certainty the effect of translocation on heterozygosity in Marion County, nor is it possible to track changes over the past decade, largely due to the absence of pre- or post-translocation genetic diversity estimates for this population. However, diversity estimates using the same marker panel are available for larger, more contemporary populations in Kansas, Nebraska, Minnesota, and Missouri ($H_E = 0.709-0.763$; Johnson *et al.* 2003) and bottlenecked Wisconsin populations ($H_E = 0.560-0.598$; Bellinger *et al.* 2003; Johnson *et al.* 2003). Neither of the Illinois counties fall within either range, but values for Marion are much closer to the bottlenecked than healthy populations. For both counties, A_R is more similar to that estimated for the bottlenecked populations ($A_R = 4.4-5.6$) than the larger populations ($A_R = 7.3-8.4$) (Bouzat *et al.* 2009).

This disparity in heterozygosities remains among counties when the full msat panel (MSP24) is employed. The value for Marion ($H_E = 0.607$) is still lower than that of Jasper ($H_E = 0.670$), further highlighting the differences in diversity between the two. Measures of A_R are changed but little using the full msat panel (Marion=5.4; Jasper=5.3). This suggests the smaller number of MSP6 markers do not erroneously estimate heterozygosity.

Superficially, the greater heterozygosity estimates for Jasper relative to Marion County seem counterintuitive. The Marion County population was marginally larger (Westemeier 1997) prior to translocations. Current population sizes for both are low, although lek surveys have counted more males in Marion County for 9 (of 13) years post-translocation. Both received

similar numbers of birds, although no Minnesota birds were released in Marion County. Additionally, translocations ended more recently for Marion County, so there has been less time for a loss of genetic diversity to occur (although this may be only 1-2 generations, if a 1.6 year average lifespan for GPCH is assumed: Hamerstrom 1973).

One factor that may underlie the discrepancy is a spatial restriction. Heterozygosity has been found to correlate with island size for some species (Frankham 1996; White & Searle 2007), and Jasper County (at 2346 acres) has twice the protected area relative to Marion County (at 1207.5 acres). Additionally, the connectivity among protected lands in Jasper County is much higher as well (Figure 2). A third possibility is that Marion County translocations had lower initial success, particularly given that Jasper County demonstrated greater numbers of post-translocation GPCH relative to Marion County (Figure 1). Jasper also had two additional years of translocations than did Marion County, thus increasing the probability of introgressive alleles from translocated populations.

4.4 Population Structure and Relatedness

Population structure exists at multiple levels that correspond to county, and more specifically, the family groups they contain. The first major division was identified by ΔK with early all birds unambiguously assigned to one county regardless of model or marker panel. Only one individual at YFM clearly stands out from this pattern by having near equal probability of being assigned to either county.

The Evanno *et al.* (2005) method of determining K will underestimate the true value when hierarchical structure or IBD are present (Waples & Gaggiotti 2006). Both occur with Illinois GPCH, as evidenced by a significant IBD test and greater values of K identified by

posterior probabilities. The 10 populations indicated by the CAF model using MSP24 are differentiated by high F_{ST} values; however this is likely an artifact of population size rather than a reflection of highly divergent subpopulations. None are large. Seven are represented by 8 (or fewer) highly related individuals, corresponding to family groups. Interestingly, these clusters rarely juxtapose with leks, even though individuals show high lek fidelity. The Survey lek is the sole exception, where all but one individual is assigned to the same group. Rather, most groups are evenly distributed among leks within their respective counties – a pattern consistent with natal dispersal.

These patterns are consistent with known GPCH behavior, as well as with that observed in related species. The low number of family groups is explained by the relatively few GPCH that successfully mate. Only 10% of males are responsible for all copulations, and dominance hierarchies among females influence reproductive success as well (Robel 1970). The distribution of closely related individuals across multiple leks corresponds to the natal dispersal patterns witnessed among other grouse species. Approximately half of Greater Sage-Grouse yearlings will disperse to a non-natal lek, and remain faithful to it in subsequent years (Dunn & Braun 1985). Family groups in this species also disperse among multiple leks much like Illinois GPCH, as revealed via genetic structure analysis (Bush *et al.* 2010). Natal dispersal has also been observed in female Capercaillie, (*Tetrao urogallus*) which may disperse up to 30km (Moss *et al.* 2006).

This opens future avenues for exploration, as natal dispersal is often sex-biased. Females of polygynous species such as grouse, where males invest heavily into lek displays (Greenwood 1980), often disperse greater distances from their natal area (Dunn & Braun 1985). Likewise, genetic structure corresponding to leks is found in Black Grouse (*Tetrao tetrix*) as well,

indicating male natal philopatry (Höglund *et al.* 1999). Once sex has been genetically determined for the samples examined in this study, geographic distributions of males within the 10 family groups will be analyzed to identify any sex-biased dispersal.

Despite the underestimation of K by ΔK , it is clear that the two counties represent distinct populations, particularly given the high probabilities of individual assignment at $K=2$ for each model, the low relatedness values found between counties, their significant isolation by distance as detected by the Mantel test, and a lack of inter-county movement as observed through identification of genotypes. Prairie Chickens are known to be capable of traveling the ~60km between the two counties, as evidenced by radio tracking (Westemeier & Jansen 1995) and short distance seasonal migration (Yeatter 1943). Historically, GPCH travelled hundreds of kilometers in a year, but these movements declined with the rise of agriculture (Leopold 1931). While farmland has replaced much of their nesting habitat, GPCH still utilize grasslands interspersed among agricultural lands to rear young (Walk & Warner 1999). Interestingly, GPCH do not perceive roads as barriers, but they will avoid power lines and other tall objects, atop which predators such as hawks may perch (Pruett *et al* 2009). Collisions with power lines are also a source of mortality (Yeatter 1943).

4.5 Marker Panel Performance

Both marker panels displayed comparable performance levels, often converging on similar answers. MSP6 was capable of detecting differences among counties, whereas MSP24 detected more nuanced variances within counties. Although MSP6 tended to overestimate mean relatedness, it still performed surprisingly well despite the inherent difficulty of determining pairwise relatedness with only 6 msats. This is because the issue of relatedness between two

individuals necessitates distinguishing among alleles identical by descent or state, which often requires the confidence ellipse produced by 30-40 msats (Blouin 2003). However, mean relatedness estimates within populations are robust to the inadequacies inherent with reduced msat panels (Queller and Goodnight 1989), which explains the similar answers obtained by MSP6 and MSP24.

However, MSP6 experienced greater shortcomings when used to differentiate populations, particularly their structure. Although both panels converged on similar F_{ST} values, MSP6 had difficulty in finding significant differences among leks within counties. Disparity in STRUCTURE results among the two panels highlights the importance of using an adequate number of molecular markers in evaluating population structure. Although recognized as a key aspect of msat analysis, it is often complicated and therefore overlooked in that it involves interactions between marker number and heterozygosity, as well as additional factors (Kalinowski 2004b). While MSP6 was adequate for detecting population differentiation among counties, it failed to provide clear answers above $K=3$. Taken by itself, these results could be interpreted as a lack of population substructure above $K=3$. However, MSP24 demonstrated additional substructure beyond $K=3$, particularly relating to clusters of closely related individuals.

With this consideration, a marker panel of 24 loci may not be adequate for all studies, depending on goals and scope of work. For this study MSP6 was adequate to reject the hypothesis that Marion and Jasper counties were identical (i.e., one population), but inadequate to identify population substructure. Similarly, Haasl & Payseur (2011) found 25 msats adequate to correctly identify five populations from simulated data using STRUCTURE, whereas 15 msats failed. Larger-scale projects with even more ambitious goals can be even more demanding, such that 25 msats may provide results inadequate for differentiation. For example, the assignment of

humans to their continent of origin with an accuracy exceeding 90% required a panel of 60 msats (Bamshad *et al.* 2003). Panels of 20 msats were only capable of 76-87% accuracy, varying by continent of origin.

4.6 Management Implications

These results of this study highlight two important considerations for the management of GPCH in Illinois: the elevated importance of continued genetic monitoring as a management tool (Schwartz *et al.* 2007), and considerations for behavioral traits and patterns of gene flow in management efforts. The latter could previously be elucidated only through field observations requiring many hours of work and handling of birds, however genetic markers and modern analytical tools allow for much of this to be accomplished in a laboratory rather than disturbing endangered populations (Prugnolle & de Meeus 2002). This has been demonstrated through the application of a large microsatellite panel that identified genetic substructure corresponding to family groups.

Such behaviors are of great consequence in management, because their effects are amplified in small populations (Sutherland 1998). These dispersal behaviors combined with lek mating dynamics lead to small effective population sizes which are detrimental to long-term survival of small populations (Höglund 1996; Pruett *et al.* 2011). These patterns must be closely monitored in Illinois GPCH, since the small populations provide fewer lek options than are available in larger populations (Schroeder 1991). This is further compounded by the fragmented landscape which prevents gene flow between Marion and Jasper counties.

A major success of this study has been the utilization of NIS techniques to gain a more complete understanding of contemporary genetic variation and provide a new baseline for future

studies by linking past genetic assessments to contemporary variation. This has for the first time provided genetic verification that the two counties represent distinct populations, each exhibiting different levels of variation. Furthermore, it highlights the necessity of post-translocation genetic monitoring. A failure to assess genetic diversity prior to management action will compromise any opportunities to evaluate success over time, and to make an informed management recommendation that will benefit both resource and approach. This is most apparent for the Marion County population. Loss of genetic diversity cannot be taken lightly, particularly in small populations where the risk of extinction is heightened (Frankham 2005). Thus, it is of critical importance that genetic monitoring of the Illinois GPCH continues unabated. Only in this way can estimates of diversity be gauged, and temporal variability quantified.

FIGURES AND TABLES

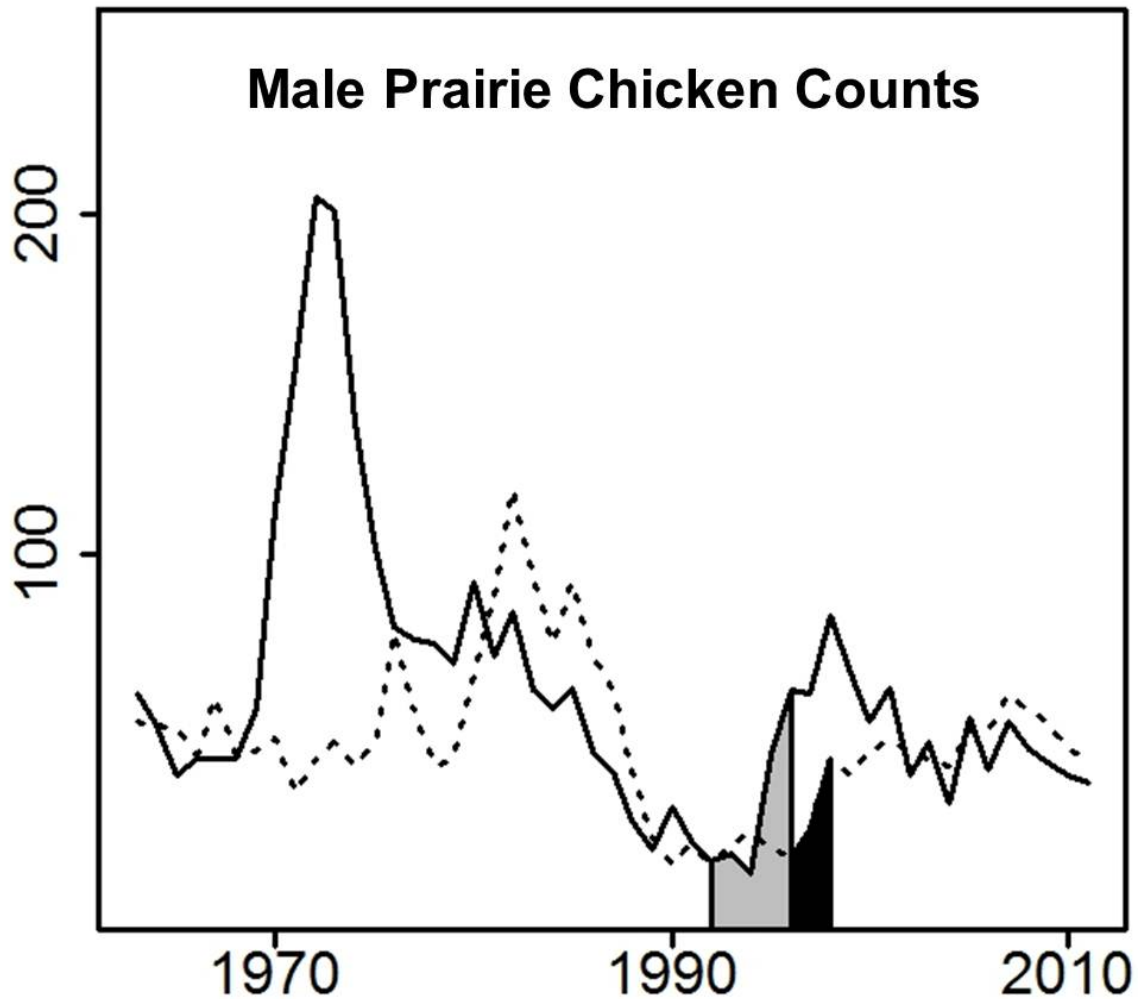


Figure 1. Male Greater Prairie Chicken (GPCH) counted by year in Marion (broken line) and Jasper (solid line) counties, Illinois. Lek surveys have been conducted annually since 1963 (X axis), with only male GPCH being counted (Y axis). Translocation programs occurred from 1992-1996 (Jasper – shaded grey) and 1996-1998 (Marion – shaded black). Data provided by S. Simpson, IL Department of Natural Resources.

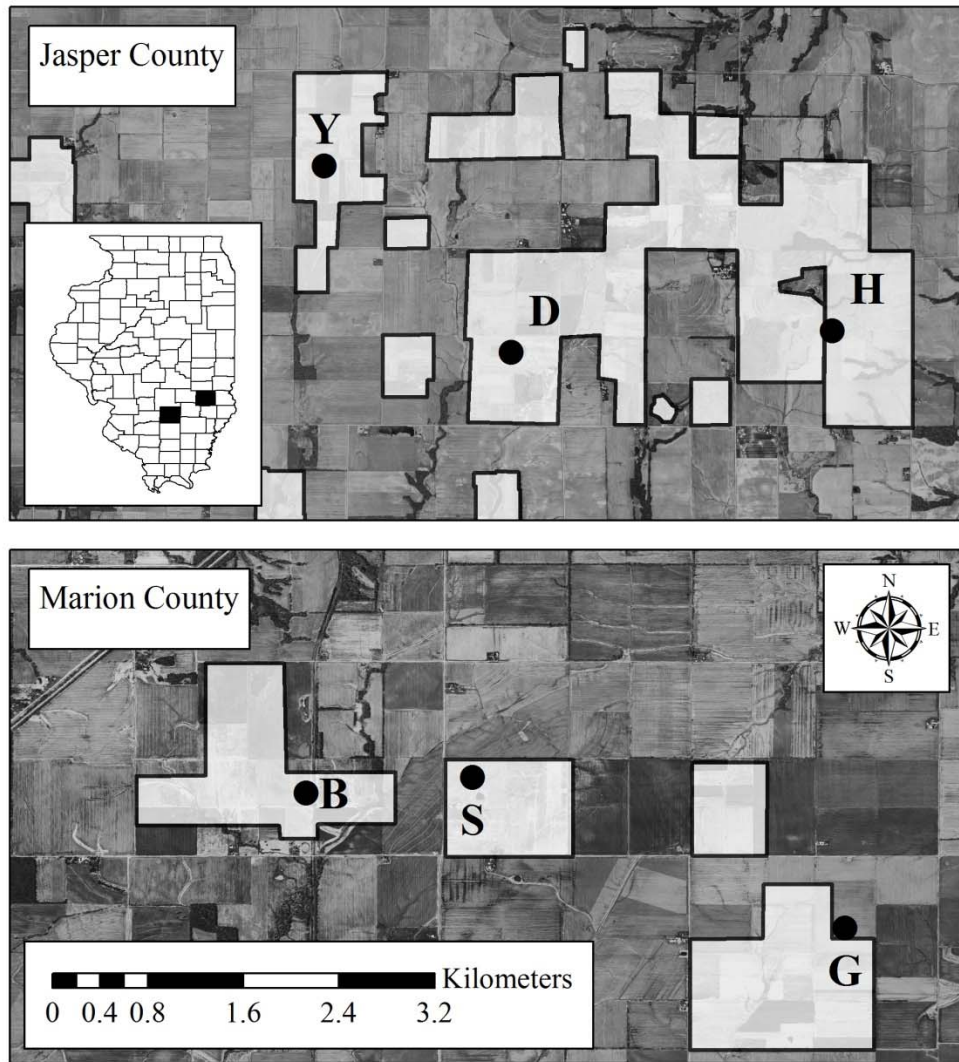


Figure 2. Greater Prairie Chicken Leks found in two Illinois counties, Jasper (top) and Marion (bottom) (see insert for locations in state, with Jasper County east of Marion County). Feathers for genotyping were collected from six leks: Bainbridge (B), Guymon (G) and Survey (S) in Marion County, and Donsbach (D), Hunt (H), and YFM (Y) in Jasper County. Polygons shaded in grey represent land managed by the Illinois Department of Natural Resources.

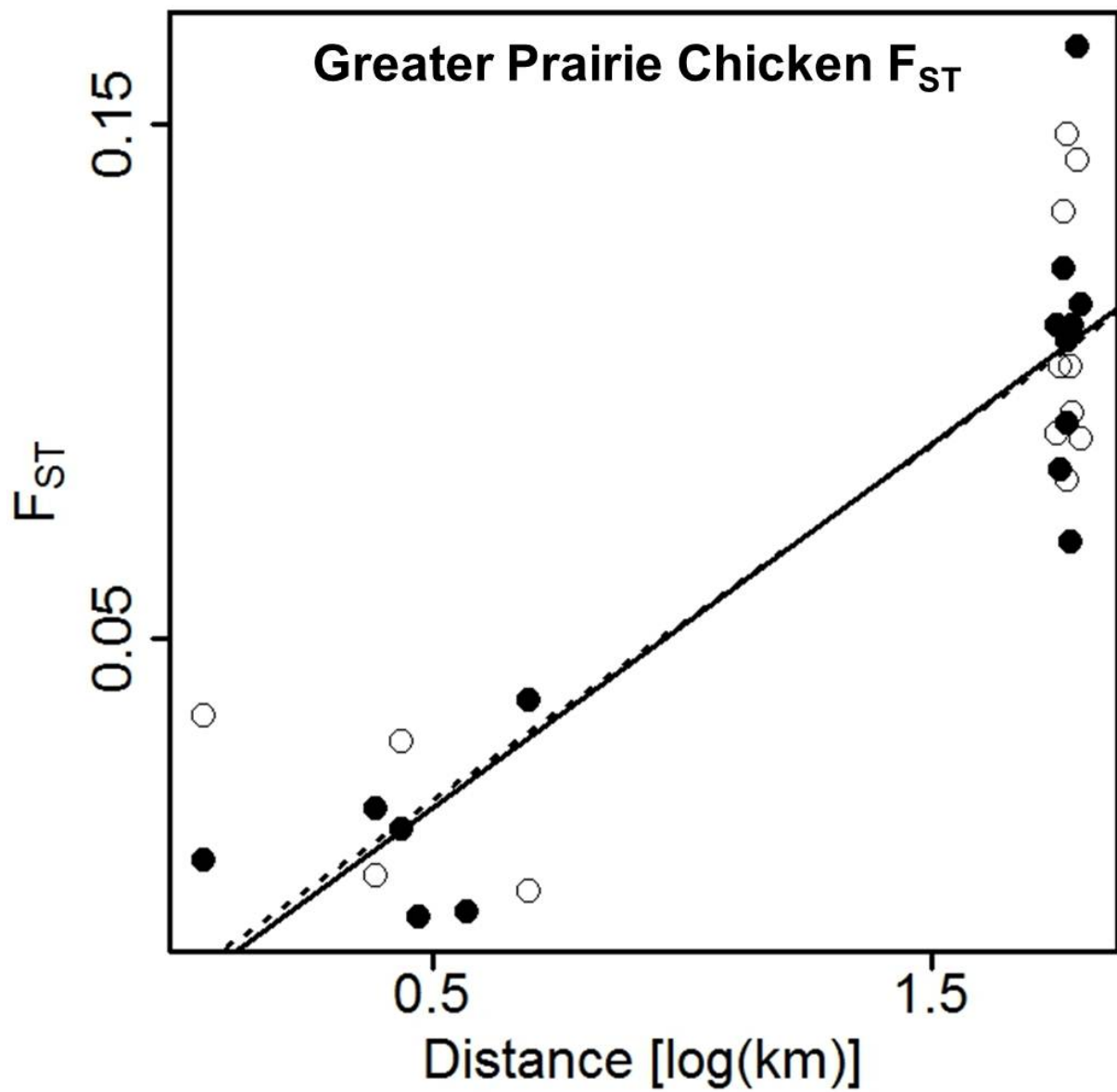


Figure 3. Pairwise F_{ST} values among Greater Prairie Chicken leks in Illinois, as calculated from the 24-microsatellite DNA panel (=MSP24 - open circles) and the reduced 6 microsatellite DNA panel (MSP6 - filled circles) plotted against linear distances [$\log_{10}(\text{km})$] separating leks.

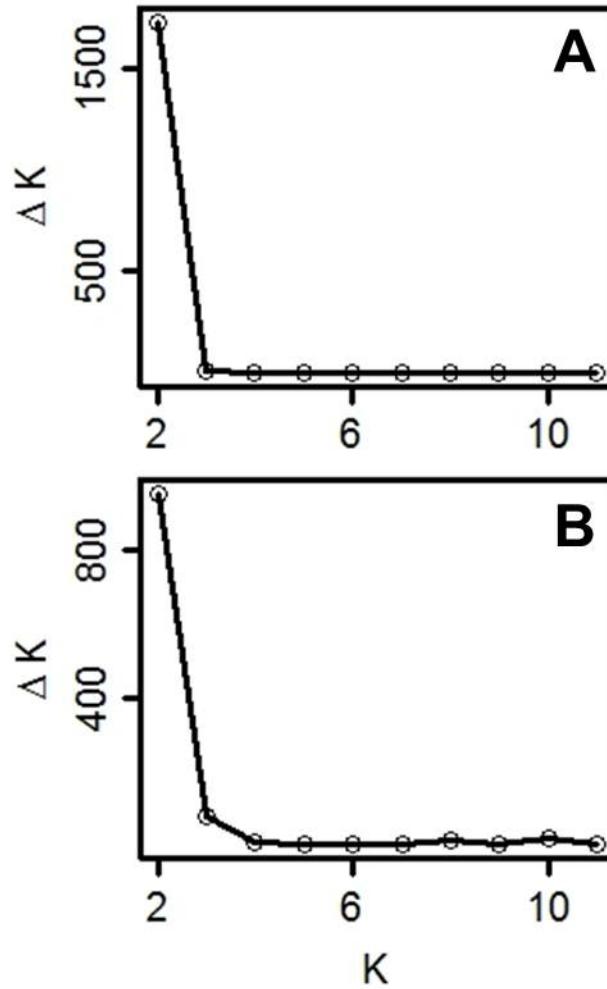


Figure 4. Graphs depicting the most appropriate K -value with which to group Greater Prairie Chicken in Illinois, determined by the Evanno ΔK method. Two models of microsatellite DNA evolution [i.e., correlated allele frequencies (=CAF) and independent allele frequencies (=IAF)] were applied in STRUCTURE 2.3.3 using the full microsatellite DNA panel (=24 loci: MSP24) and the reduced microsatellite DNA panel (=6 loci: MSP6). Plots represent: (A) CAF-MSP24 and (B) CAF-MSP6. IAF models returned equivalent results for respective panels (not depicted).

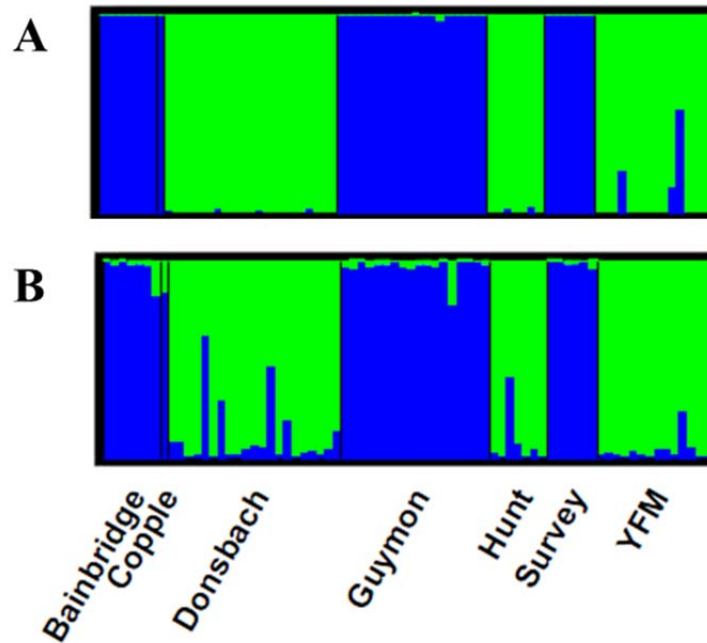


Figure 5. Probabilities of assigning individual Greater Prairie Chicken to group (K) determined by the Evanno ΔK method. Correlated allele frequencies (=CAF) and independent allele frequencies (=IAF) models were applied in STRUCTURE 2.3.3 using the complete microsatellite DNA panel (24 loci: MSP24) and the reduced microsatellite panel (6 loci: MSP6). K values of 1 through 12 were evaluated. $K=2$ was selected by ΔK as the best explanation of population structure, and corresponds to Marion County (blue) and Jasper County (green). Each vertical bar corresponds to an individual, with the X axis indicating lek of origin. (A) = CAF-MSP24; (B) CAF-MSP6. Equivalent results for each microsatellite panel were obtained for the IAF model (not pictured).

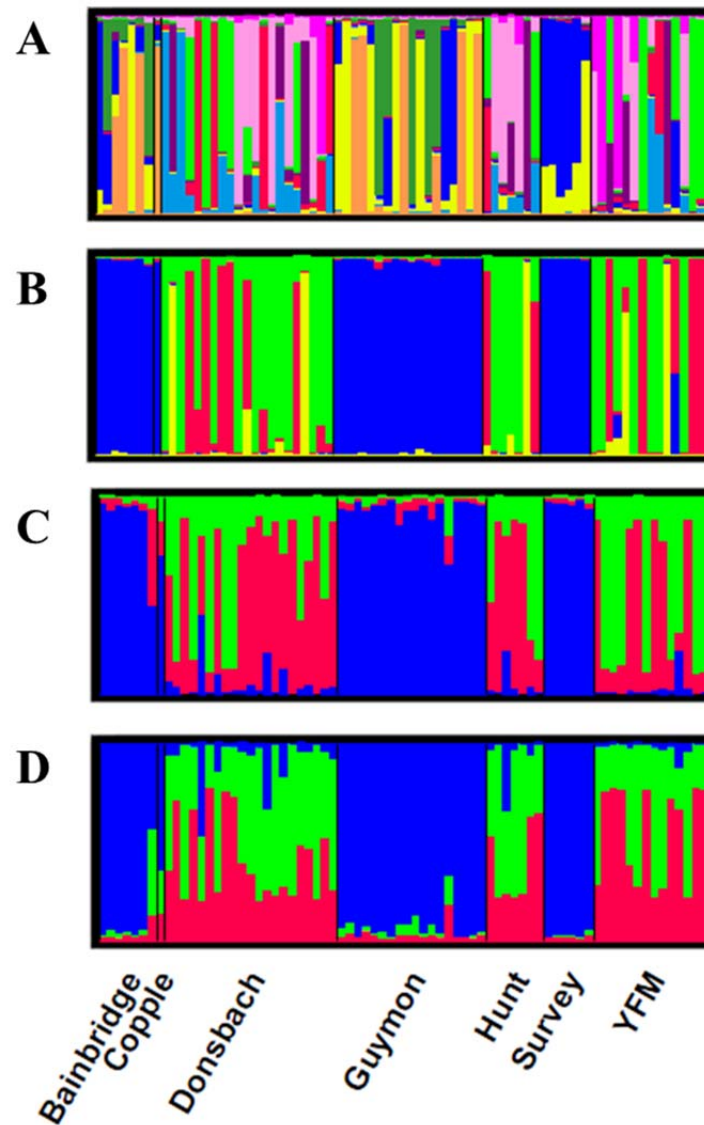


Figure 6. Probabilities of assigning individual Greater Prairie Chicken to group (K) as determined by posterior probability [$\text{Pr}(K)$]. Correlated allele frequencies (=CAF) and independent allele frequencies (=IAF) models were applied in STRUCTURE 2.3.3 using the complete microsatellite DNA panel (24 loci: MSP24) and the reduced microsatellite panel (6 loci: MSP6). Different K values were favored by $\text{Pr}(K)$ contingent upon model and marker panel. $K=10$ (A) was the best grouping for the CAF model and MSP24 panel. $K=4$ (B) was selected for the IAF model and MSP24 panel. $K=3$ was the best value for MSP6 for both the CAF (C) and IAF (D) models. Each vertical bar corresponds to an individual, with the X axis indicating lek of origin.

Table 1. Annual Greater Prairie Chicken translocations (1992-1998) in Illinois. The number of birds released each year is provided by county of release (A = Jasper; B = Marion), sex, and source population (Kansas, Minnesota, or Nebraska).

A.

Year	Kansas		Minnesota		Nebraska	
	Male	Female	Male	Female	Male	Female
1992	-	-	-	15	-	-
1993	-	-	8	4	-	-
1994	46	50	-	-	-	-
1995	48	50	-	-	-	-
1996	-	-	-	-	25	25

B.

Year	Kansas		Minnesota		Nebraska	
	Male	Female	Male	Female	Male	Female
1996	-	-	-	-	24	24
1997	45	47	-	-	-	-
1998	46	49	-	-	-	-

Table 2. Numbers of Greater Prairie Chicken feathers by year from which DNA was extracted in this study. Bainbridge, Guymon, and Survey = Marion County leks; Donsbach, Hunt, and YFM = Jasper County leks; Total = total.

	Bainbridge	Donsbach	Guymon	Hunt	Survey	YFM	Total
2010	198	297	55	51	45	215	861
2011	1	120	388	38	170	148	865
Total	199	417	443	89	215	363	1726

Table 3. Summary of the microsatellite DNA markers used to genotype 74 Greater Prairie Chicken. Listed for each locus are Motif = microsatellite repeat motif; MP = multiplex number (1-4, 6, 11); Dye = ABI fluorescent dye (6-FAM, NED, PET, VIC); A = number of alleles at each locus; Size Range = expected DNA fragment length in base pairs (bp); HO = observed heterozygosity; HE = expected heterozygosity.

Locus	Motif	MP	Dye	A	Size Range	H _O	H _E
ADL44	Di	11	PET	6	176 - 184	0.703	0.734
ADL146	Di	1	NED	5	184 - 196	0.338	0.343
ADL230	Di	6	PET	8	104 - 118	0.770	0.786
ADL260	Di	3	PET	3	117 - 121	0.378	0.525
BG12	Tetra	2	VIC	5	143 - 159	0.797	0.759
BG14	Tetra	4	6-FAM	9	195 - 239	0.877	0.843
BG16	Tetra	6	VIC	6	143 - 163	0.730	0.766
CUAAGG37	Tetra	1	VIC	5	148 - 217	0.527	0.646
LLSD4	Di	6	NED	12	181 - 217	0.878	0.885
LLSD7	Di	3	VIC	15	152 - 192	0.726	0.849
LLSD9	Di	11	VIC	9	135 - 155	0.500	0.791
LLST1	Di	11	6-FAM	5	117 - 129	0.635	0.584
LLST4	Tetra	2	6-FAM	26	356 - 562	0.986	0.910
MCW126	Tetra	4	NED	5	149 - 175	0.135	0.153
MCW146	Di	6	6-FAM	9	153 - 169	0.931	0.841
MNT12	Di	3	6-FAM	15	87 - 135	0.946	0.895
SR3	Di	1	6-FAM	2	285 - 287	0.270	0.255
TTD1	Di	1	PET	6	146 - 166	0.716	0.670
TTD4	Di	4	VIC	3	182 - 186	0.311	0.349
TTD6	DI	3	NED	7	111 - 133	0.811	0.776
TTT1	Tetra	2	PET	7	210 - 234	0.878	0.834
TTT2	Tetra	11	NED	5	171 - 191	0.589	0.758
TUD3	Di	2	NED	6	167 - 203	0.500	0.572
TUT3	Tetra	4	PET	7	153 - 177	0.757	0.769

Table 4. Greater Prairie Chicken detected by year at each of six Illinois leks. Shared = individuals resident for both years.

Year	Bainbridge	Donsbach	Guymon	Hunt	Survey	YFM
2010	7	14	4	4	1	6
2011	0	2	11	2	1	2
Shared	0	5	4	1	5	6
Total	7	21	18	7	6	14

Table 5. Greater Prairie Chicken population size estimates. The Jolly-Seber model was applied to estimate the number of GPCH visiting leks in Marion and Jasper counties each year. Estimates for 2010 encompass 6 leks (Bainbridge, Donsbach, Guymon, Hunt, Survey, YFM). Estimates for 2011 exclude Bainbridge lek. "Year" = year, "Count" = number of males observed on the leks, "Genotype" = the total number of birds identified by genotype, "Estimate" = Jolly-Seber model population size estimate, "SE" = standard error for estimate, "d. f." = degrees of freedom, "P" = p-value for model goodness of fit test.

Year	Count	Genotype	Estimate	SE	d. f.	P
2010	63	57	64.1	3.7	49	0.24
2011	46	37	38.3	1.4	50	0.43

Table 6. Pair-wise comparison of linkage disequilibrium (LD) for 24 microsatellite DNA loci evaluated across six study leks of Greater Prairie Chicken in Illinois. Significant LD was detected for 28 pair of loci and three leks: 18 at Donsbach (d); six at YFM (y); four at Guymon (g); none at Bainbridge, Hunt, and Survey. Only 2 loci-pair (LLSD7/ MNT12; TTT2/ BG16) demonstrated LD at multiple leks. Significance gauged at Bonferroni adjusted P=0.0021 (24 comparisons).

	SR	CUR	TTD	ADL	MNT	LLSD	ADL	TTD	BG	TTD	TUT	MCW	MCW	BG	ADL	LLSD	LLST	LLSD	ADL	TTT	LLST	BG	TTT	TUD
	3	6	1	146	12	7	260	6	14	4	3	126	146	16	230	4	1	9	44	2	4	12	1	3
SR03	*																							
CUR06	-	*																						
TTD01	-	-	*																					
ADL146	-	-	-	*																				
MNT12	-	-	-	-	*																			
LLSD7	-	-	-	-	d,g,y	*																		
ADL260	-	-	-	-	-	-	*																	
TTD06	-	d	-	-	-	-	-	*																
BG14	-	-	-	-	d	-	-	-	*															
TTD04	-	-	-	-	-	-	-	-	-	*														
TUT03	-	-	-	-	-	-	-	-	-	-	*													
MCW126	-	d	-	-	-	-	-	d	-	-	-	*												
MCW146	-	-	-	-	-	-	-	-	-	-	-	-	*											
BG16	-	-	-	-	-	-	-	-	-	-	-	-	-	*										
ADL230	-	-	-	-	-	-	-	-	-	-	-	-	-	y	*									
LLSD4	-	-	-	-	-	-	-	-	d	-	d	-	-	d	-	*								
LLST1	g	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*							
LLSD9	-	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	*						
ADL44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*					
TTT2	-	d	-	-	-	-	-	-	-	-	-	-	-	d,g,y	-	d	-	-	-	*				
LLST4	-	-	-	y	d	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*			
BG12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	*		
TTT1	-	-	-	y	-	-	-	-	d	-	-	-	-	-	-	-	-	-	-	d	d	y	*	
TUD3	-	-	-	-	-	-	-	g	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*

Table 7. Genetic diversity estimates for Greater Prairie Chickens found within leks and counties in Illinois. All estimates were derived using the full 24 microsatellite DNA marker panel (A and C) and a reduced panel of 6 markers (B and D). Estimates for the 2003 Jasper County and Pre-translocation populations were retrieved from Bouzat et al. (2009). The following were calculated using each marker panel: N = number of individuals; A_M = mean number of alleles per locus; A_R = allelic richness; A_{pr} = private alleles; H_O = observed heterozygosity; H_E = Expected heterozygosity; SE = standard error associated with H_O and H_E; F = inbreeding coefficient; “-“ = data unavailable.

A.

Lek	N	A _M	A _R	A _{pr}	H _O	SE	H _E	SE	F
Bainbridge	7	4.3	3.8	4	0.605	0.046	0.636	0.043	0.099
Donsbach	21	5.3	3.8	4	0.681	0.048	0.663	0.036	0.088
Guymon	18	5.5	3.9	11	0.613	0.056	0.611	0.053	0.025
Hunt	7	4.3	3.8	0	0.667	0.064	0.660	0.045	0.061
Survey	6	2.9	2.8	0	0.625	0.079	0.520	0.061	0.047
YFM	14	5.3	4.0	2	0.690	0.054	0.684	0.042	0.056

B.

Lek	N	A _M	A _R	A _{pr}	H _O	SE	H _E	SE	F
Bainbridge	7	4.3	3.7	0	0.619	0.080	0.621	0.084	0.138
Donsbach	21	5.8	3.9	1	0.635	0.100	0.667	0.077	0.212
Guymon	18	5.2	3.7	3	0.565	0.090	0.609	0.106	0.106
Hunt	7	4.0	3.7	0	0.595	0.158	0.636	0.089	0.147
Survey	6	3.3	3.2	0	0.611	0.159	0.558	0.124	0.171
YFM	14	5.7	4.2	0	0.750	0.080	0.724	0.042	0.102

C.

County	N	A _M	A _R	A _{pr}	H _O	SE	H _E	SE	F
Marion 2010-11	32	6.0	5.4	42	0.617	0.056	0.607	0.052	0.077
Jasper 2010-11	42	6.0	5.3	41	0.682	0.049	0.670	0.037	0.057

D.

County	N	A _M	A _R	A _{pr}	H _O	SE	H _E	SE	F
Marion 2010-11	32	5.7	5.1	8	0.599	0.096	0.611	0.101	0.169
Jasper 2010-11	42	6.2	5.6	11	0.667	0.100	0.688	0.060	0.136
Jasper 2003	18	5.5	5.5	-	-	-	0.676	0.058	0.099
Pre-Translocation	32	5.2	4.7	-	-	-	0.654	0.055	0.200

Table 8. Fst values calculated among leks of Illinois Greater Prairie Chicken using the full microsatellite DNA panel of 24 loci (above diagonal) and the reduced panel of 6 loci (below diagonal) panels. Statistical significance is displayed with a star (*).

	Bainbridge	Donsbach	Guymon	Hunt	Survey	YFM
Bainbridge	-	0.069*	-0.003	0.115*	0.007	0.092*
Donsbach	0.103*	-	0.083*	-0.004	0.108*	0.017
Guymon	-0.003	0.103*	-	0.111*	0.013	0.111*
Hunt	0.089*	-0.004	0.094*	-	0.165*	0.038
Survey	0.035*	0.148*	0.030*	0.143*	-	0.122*
YFM	0.081*	0.004	0.090*	0.001	0.133*	-

Table 9. Mean relatedness (r) within 6 Greater Prairie Chicken leks in Illinois calculated for (A) the full microsatellite DNA panel of 24 loci and (B) the reduced panel of 6 loci, following methods of Wang (2007). All relatedness values are significantly different from zero within leks (one-sample t-test). Relatedness >1/2-sib=***; > 1st cousin=**; > 2nd cousin=*

A.

Lek	r	Variance	t	d. f.	P
Bainbridge	0.146**	0.025	4.106	20	0.001
Donsbach	0.139**	0.025	12.596	209	0.000
Guymon	0.128**	0.018	11.687	152	0.000
Hunt	0.122*	0.012	5.025	20	0.000
Survey	0.379***	0.038	7.280	14	0.000
YFM	0.112*	0.031	6.047	90	0.000

B.

Lek	r	Variance	t	d. f.	P
Bainbridge	0.200**	0.024	5.821	20	0.000
Donsbach	0.188**	0.038	13.940	209	0.000
Guymon	0.224**	0.047	7.199	152	0.000
Hunt	0.264***	0.054	5.069	20	0.000
Survey	0.342***	0.030	7.369	14	0.000
YFM	0.167*	0.048	12.810	90	0.000

Table 10. Pairwise relatedness estimates calculated among leks of Greater Prairie Chicken in Illinois using methods of Wang (2007). Calculations were based on the full microsatellite DNA panel of 24 loci (below diagonal) and the reduced panel of 6 loci (above diagonal). Relatedness >1/2-sib=***; > 1st cousin=**; > 2nd cousin=*

	Bainbridge	Donsbach	Guymon	Hunt	Survey	YFM
Bainbridge	*	0.107**	0.234**	0.113*	0.242**	0.0734*
Donsbach	0.014	*	0.084*	0.229**	0.0812*	0.176**
Guymon	0.139**	0.013	*	0.098*	0.229**	0.059*
Hunt	0.029	0.129**	0.018	*	0.054*	0.175**
Survey	0.166**	0.008	0.156**	0.016	*	-0.136
YFM	0.025	0.119*	0.112*	0.118*	0.013	*

Table 11. Relatedness values within (diagonal) and among (off-diagonal) the 10 populations of Greater Prairie Chicken identified in Illinois using the correlated allele frequency (=CAF) model of program Structure. Six (of 10) were confined to Jasper County (=J1-J6) while the remaining four were in Marion County (=M1-M4). Relatedness >1/2-sib=***; >1st cousin=**; >2nd cousin=.

	J1	J2	J3	J4	J5	J6	M1	M2	M3	M4
J1	0.425***									
J2	0.122*	0.406***								
J3	0.083*	0.076*	0.363***							
J4	0.089*	0.115*	0.060*	0.387***						
J5	0.133**	0.051*	0.080*	0.061*	0.222**					
J6	0.071*	0.144**	0.047*	0.056*	0.063*	0.487***				
M1	0.012*	0.007	0.015	0.008	0.012	0.006	0.227**			
M2	0.016*	0.027	0.026	0.011	0.026	0.018	0.079*	0.307***		
M3	0.002	0.001	0.008	0.019	0.007	0.013	0.114*	0.171**	0.426***	
M4	0.033*	0.021	0.006	0.022	0.016	0.013	0.081*	0.067*	0.116*	0.251***

Table 12. Pairwise Fst values for 10 populations of Illinois Greater Prairie Chicken identified with the correlated allele frequency (=CAF) model of program Structure. Six were confined to Jasper County (=J1-J6) while an additional four (=M1-M4) were in Marion County. None correspond to recognized leks. All Fst values were significant at P=0.05.

	J1	J2	J3	J4	J5	J6	M1	M2	M3	M4
J1	*									
J2	0.116	*								
J3	0.160	0.142	*							
J4	0.141	0.128	0.167	*						
J5	0.068	0.123	0.116	0.132	*					
J6	0.178	0.129	0.201	0.178	0.151	*				
M1	0.137	0.168	0.169	0.204	0.146	0.193	*			
M2	0.186	0.162	0.195	0.225	0.140	0.224	0.094	*		
M3	0.223	0.225	0.236	0.242	0.190	0.245	0.081	0.092	*	
M4	0.135	0.162	0.205	0.183	0.132	0.194	0.074	0.110	0.098	*

Table 13. Relatedness values within and among four populations of Illinois Greater Prairie Chicken identified by program Structure using the independent allele frequency (=IAF) model. Three are confined to Jasper County (=J1-J3) while a single population is in Marion County (=M1). Relatedness >1/2-sib=***; > 1st cousin=**; > 2nd cousin=*.

	J1	J2	J3	M1
J1	0.363***			
J2	0.074*	0.325***		
J3	0.069*	0.070*	0.157**	
M1	0.014	0.014	0.015	0.150*

Table 14. Pairwise F_{st} values for 4 populations of Illinois Greater Prairie Chicken using the independent allele frequency (=IAF) model of program Structure. Three are confined to Jasper County (=J1-J3) while a single population is in Marion County (=M1). None correspond to recognized leks. All F_{st} values are significant at $P=0.05$.

	J1	J2	J3	M1
J1	*			
J2	0.142	*		
J3	0.111	0.096	*	
M1	0.171	0.173	0.102	*

REFERENCES

- Alda F, Sastre P, De La Cruz-Cardiel PJ, Doadrio I (2011) Population genetics of the endangered Cantabrian capercaillie in northern Spain. *Animal Conservation* **14**, 249-260.
- Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of conservation genetics. *Nature Reviews Genetics* **11**, 697-709.
- Anthony LL, Blumstein DT (2000) Integrating behaviour into wildlife conservation: the multiple ways that behaviour can reduce N_e . *Biological Conservation* **95**, 303-315.
- Baillargeon S, Rivest LP (2007) RCAPTURE: loglinear models for capture-recapture in R. *Journal of Statistical Software* **19**, 1-31.
- Bamshad MJ, Wooding S, Watkins WS, Ostler CT, Batzer MA, Jorde LB (2003) Human population genetic structure and inference of group membership. *American Journal of Human Genetics* **72**, 578-589.
- Baratti M, Alberti A, Groenen M, Veenendaal T, Fulgheri FD (2001) Polymorphic microsatellites developed by cross-species amplifications in common pheasant breeds. *Animal Genetics* **32**, 222-225.
- Bech N, Novoa C, Allienne JF, Boissier J (2010) Transferability of microsatellite markers among economically and ecologically important galliform birds. *Genetics and Molecular Research* **9**, 1121-1129.
- Bellinger MR, Johnson JA, Toepfer J, Dunn P (2003) Loss of genetic variation in greater prairie chickens following a population bottleneck in Wisconsin, USA. *Conservation Biology* **17**, 717-724.
- Blouin MS (2003) DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends in Ecology and Evolution* **18**, 503-511.
- Boulanger J, Stenhouse G, Munro R (2004) Sources of heterogeneity bias when DNA mark-recapture sampling methods are applied to grizzly bear (*Ursus arctos*) populations. *Journal of Mammalogy* **85**, 618-624.
- Bouzat JL (2000) The importance of control populations for the identification and management of genetic diversity. *Genetica* **110**, 109-115.
- Bouzat JL, Cheng HH, Lewin HA, Westemeier RL, Brawn JD, Paige KN (1998a) Genetic evaluation of a demographic bottleneck in the greater prairie chicken. *Conservation Biology* **12**, 836-843.

- Bouzat JL, Johnson JA, Toepfer JE, Simpson SA, Esker TL, Westemeier RL (2009) Beyond the beneficial effects of translocations as an effective tool for the genetic restoration of isolated populations. *Conservation Genetics* **10**, 191-201.
- Bouzat JL, Lewin HA, Paige KN (1998b) The ghost of genetic diversity past: Historical DNA analysis of the greater prairie chicken. *American Naturalist* **152**, 1-6.
- Boyce WM, Weisenberger ME, Penedo MCT, Johnson CK (2011) Wildlife translocation: the conservation implications of pathogen exposure and genetic heterozygosity. *BMC Ecology* **11**, 1-7.
- Breckenridge WJ (1929) The booming of the prairie chicken. *Auk* **46**, 540-543.
- Brownstein MJ, Carpten JD, Smith JR (1996) Modulation of non-templated nucleotide addition by *Taq* DNA polymerase: primer modifications that facilitate genotyping. *Biotechniques* **20**, 1004-1010.
- Bush KL, Aldridge CL, Carpenter JE, Paszkowski CA, Boyce MS, and Coltman DW (2010) Birds of a feather do not always lek together: genetic diversity and kinship structure of greater sage-grouse (*Centrocercus urophasianus*) in Alberta. *Auk* **127**, 343-353.
- Bush KL, Vinsky MD, Aldridge CL, Paszkowski CA (2005) A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater sage grouse (*Centrocercus urophasianus*). *Conservation Genetics* **6**, 867-870.
- Buyse K, Verbeke A (2003) Proactive environmental strategies: a stakeholder management perspective. *Strategic Management Journal* **24**, 453-470.
- Caizergues A, Dubois S, Loiseau A, Mondor G, Rasplus JY (2001) Isolation and characterization of microsatellite loci in black grouse (*Tetrao tetrix*). *Molecular Ecology Notes* **1**, 36-38.
- Campbell N, Reece J (2005) *Biology*, 7th edn. Pearson Benjamin Cummings, Boston, MA.
- Carroll SP, Hendry AP, Reznick DN, Fox CW (2007) Evolution on ecological time-scales. *Functional Ecology* **21**, 387-393.
- Cheng HH, Crittenden LB (1994) Microsatellite markers for genetic mapping in the chicken. *Poultry Science* **73**, 539-546.
- Cheng HH, Levin I, Vallejo RL, Khatib H, Dodgson JB, Crittenden LB, and Hillel J (1995) Development of a genetic map of the chicken with markers of high utility. *Poultry Science* **74**, 1855-1874.
- Connelly JW, Gratson MW, Reese KP (1998) Sharp-tailed grouse (*Tympanuchus phasianellus*). In: *The Birds of North America Online* (ed. Poole A). Cornell Lab of Ornithology, Ithaca.

- Crow JF (1948) Alternative hypotheses of hybrid vigor. *Genetics* **33**, 477-487.
- Dieringer D, Schlötterer C (2003) MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes* **3**, 167-169.
- Drovetski SV, Ronquist F (2003) Plio-Pleistocene climatic oscillations, Holarctic biogeography and speciation in an avian subfamily. *Journal of Biogeography* **30**, 1173-1181.
- Drummer TD, Corace III RG, Sjogren SJ (2011) Sharp-tailed grouse lek attendance and fidelity in Upper Michigan. *Journal of Wildlife Management* **75**, 311-318.
- Dunn PO, Braun CE (1985) Natal dispersal and lek fidelity of sage grouse. *Auk* **102**, 621-627.
- Earl DA, vonHoldt BM (2011) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*: doi 10.1007/s12686-011-9548-7.
- Edmands S (2007) Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology* **16**, 463-475.
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**, 2611-2620.
- Evrard JO (2004) The greater prairie chicken in Crex Meadows: an unsuccessful restoration attempt. *Passenger Pigeon* **66**, 199-209.
- Ewers RM, Didham RK (2006) Confounding factors in the detection of species responses to habitat fragmentation. *Biological Reviews* **81**, 117-142.
- Excoffier L, Lischer HEL (2010) ARLEQUIN suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564-567.
- Ferrero ME, González-Jara P, Blanco-Aguilar JA, Sánchez-Barbudo I, Dávila JA (2007) Sixteen new polymorphic microsatellite markers isolated for red-legged partridge (*Alectoris rufa*) and related species. *Molecular Ecology Notes* **7**, 1349-1351.
- Fischer J, Lindenmayer DB (2000) An assessment of the published results of animal relocations. *Biological Conservation* **96**, 1-11.
- Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR, Chapin FS, Coe MT, Daily GC, Gibbs HK, Helkowski JH, Holloway T, Howard EA, Kucharik CJ, Monfreda C, Patz JA, Prentice IC, Ramankutty N, Snyder PK (2005) Global consequences of land use. *Science* **309**, 570-574.

- Frankham R (1996) Relationship of genetic variation to population size in wildlife. *Conservation Biology* **10**, 1500-1508.
- Frankham R (2005) Genetics and extinction. *Biological Conservation* **126**, 131-140.
- Frankham R, Ballou JD, Eldridge MDB, Lacy RC, Ralls K, Dudash MR, and Fenster CB (2011) Predicting the probability of outbreeding depression. *Conservation Biology* **25**, 465-475.
- Freeman S, Herron JC (2004) *Evolutionary Analysis*, 3rd edn. Pearson Prentice Hall, Upper Saddle River, NJ.
- Gibson RM, Pires D, Delaney KS, Wayne RK (2005) Microsatellite DNA analysis shows that greater sage grouse leks are not kin groups. *Molecular Ecology* **14**, 4453-4459.
- Gienapp P, Teplitsky C, Alho JS, Mills JA, Merilä J (2008) Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology* **17**, 167-178.
- Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour* **28**, 1140-1162.
- Griffiths R, Double MC, Orr K, Dawson RJG (1998) A DNA test to sex most birds. *Molecular Ecology* **7**, 1071-1075.
- Gross AO (1930) *Progress Report of the Wisconsin Prairie Chicken Investigation*. Wisconsin Conservation Commission, Madison, WI.
- Haasl RJ, Payseur BA (2011) Multi-locus inference of population structure: a comparison between single nucleotide polymorphisms and microsatellites. *Heredity* **106**, 158-171.
- Hamerstrom FN (1939) A study of Wisconsin prairie chicken and sharp-tailed grouse. *Wilson Bulletin* **51**, 105-120.
- Hamerstrom FN (1973) The prairie chicken in Wisconsin. Highlights of a 22-year study of counts, behavior, movements, turnover and habitat. *Technical Bulletin (Wisconsin Department of Natural Resources)* **64**, 1-52.
- Hedrick PW, Kalinowski ST (2000) Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics* **31**, 139-162.
- Hogan FE, Cooke R, Burrridge CP, Norman JA (2008) Optimizing the use of shed feathers for genetic analysis. *Molecular Ecology Resources* **8**, 561-567.
- Höglund J (1996) Can mating systems affect local extinction risks? Two examples of lek-breeding waders. *Oikos* **77**, 184-188.

- Höglund J, Alatalo RV, Lundberg A, Rintamäki PT, Lindell J (1999) Microsatellite markers reveal the potential for kin selection on black grouse leks. *Proceedings of the Royal Society of London Series B-Biological Sciences* **266**, 813-816.
- Horváth MB, Martínez-Cruz B, Negro JJ, Kalmár L, Godoy JA (2005) An overlooked DNA source for non-invasive genetic analysis in birds. *Journal of Avian Biology* **36**, 84-88.
- Jamison BE, Alleger MR (2009) Status of Missouri greater prairie chicken populations and preliminary observations from ongoing translocations and telemetry. *Grouse News* **38**, 15-24.
- Johnsgard PA (2002) *Grassland Grouse and Their Conservation*. Smithsonian Institution Press, Washington, DC.
- Johnson JA (2008) Recent range expansion and divergence among North American prairie grouse. *Journal of Heredity* **99**, 165-173.
- Johnson JA, Dunn PO (2006) Low genetic variation in the heath hen prior to extinction and implications for the conservation of prairie chicken populations. *Conservation Genetics* **7**, 37-48.
- Johnson JA, Schroeder MA, Robb LA (2011) Greater prairie chicken (*Tympanuchus cupido*). In: *The Birds of North America Online* (ed. Poole A). Cornell Lab of Ornithology, Ithaca.
- Johnson JA, Toepfer JE, Dunn PO (2003) Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie chickens. *Molecular Ecology* **12**, 3335-3347.
- Kalinowski ST (2004a) Counting alleles with rarefaction: private alleles and hierarchical sampling designs. *Conservation Genetics* **5**, 539-543.
- Kalinowski ST (2004b) Genetic polymorphism and mixed-stock fisheries analysis. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 1075-1082.
- Krakauer AH, Kimball RT (2009) Interspecific brood parasitism in galliform birds. *Ibis* **151**, 373-381.
- Law B, Buckleton JS, Triggs CM, Weir BS (2003) Effects of population structure and admixture on exact tests for association between loci. *Genetics* **164**, 381-387.
- Leberg PL (1990) Genetic considerations in the design of introduction programs. *Transactions of the North American Wildlife and Natural Resources Conference* **55**, 609-619.
- Leopold A (1931) *Report on a Game Survey of the North Central States*. Democrat Printing Company, Madison, WI.

- Link WA (2003) Nonidentifiability of population size from capture-recapture data with heterogeneous detection probabilities. *Biometrics* **59**, 1123-1130.
- Martínez-Abraín A, Regan HM, Viedma C, Villuendas E, Bartolomé MA, Gómez JA, and Oro D (2011) Cost-effectiveness of translocation options for a threatened waterbird. *Conservation Biology* **25**, 726-735.
- McCarty JP (2001) Ecological consequences of recent climate change. *Conservation Biology* **15**, 320-331.
- McKee GW, Ryan MR, Mechlin LM (1998) Predicting greater prairie chicken nest success from vegetation and landscape characteristics. *Journal of Wildlife Management* **62**, 314-321.
- McKee JK, Sciulli PW, Fooce CD, Waite TA (2004) Forecasting global biodiversity threats associated with human population growth. *Biological Conservation* **115**, 161-164.
- Mock KE, Latch EK, Rhodes OE (2004) Assessing losses of genetic diversity due to translocation: long-term case histories in Merriam's turkey (*Meleagris gallopavo merriami*). *Conservation Genetics* **5**, 631-645.
- Morrow ME, Rossignol TA, Silvy NJ (2004) Federal listing of prairie grouse: Lessons from the Attwater's prairie chicken. *Wildlife Society Bulletin* **32**, 112-118.
- Moss R, Picozzi N, Catt DC (2006) Natal dispersal of capercaillie *Tetrao urogallus* in northeast Scotland. *Wildlife Biology* **12**, 227-232.
- Paetkau D (2003) An empirical exploration of data quality in DNA-based population inventories. *Molecular Ecology* **12**, 1375-1387.
- Paetkau D, Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology* **3**, 489-495.
- Palkovacs EP, Oppenheimer AJ, Gladyshev E, Toepfer JE, Amato G, Chase T, Caccone A (2004) Genetic evaluation of a proposed introduction: the case of the greater prairie chicken and the extinct heath hen. *Molecular Ecology* **13**, 1759-1769.
- Palsbøll PJ (1999) Genetic tagging: contemporary molecular ecology. *Biological Journal of the Linnean Society* **68**, 3-22.
- Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288-295.
- Pertoldi C, Bijlsma R, Loeschcke V (2007) Conservation genetics in a globally changing environment: present problems, paradoxes and future challenges. *Biodiversity and Conservation* **16**, 4147-4163.

- Piertney SB, Dallas JF (1997) Isolation and characterization of hypervariable microsatellites in the red grouse *Lagopus lagopus scoticus*. *Molecular Ecology* **6**, 93-95.
- Piertney SB, Höglund J (2001) Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). *Molecular Ecology Notes* **1**, 303-304.
- Piertney SB, MacColl ADC, Bacon PJ, Dallas JF (1998) Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. *Molecular Ecology* **7**, 1645-1654.
- Pimm SL, Dollar L, Bass Jr. OL (2006) The genetic rescue of the Florida panther. *Animal Conservation* **9**, 115-122.
- Pimm SL, Russell GJ, Gittleman JL, Brooks TM (1995) The future of biodiversity. *Science* **269**, 347-350.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945-959.
- Pruett CL, Johnson JA, Larsson LC, Wolfe DH, Patten MA (2011) Low effective population size and survivorship in a grassland grouse. *Conservation Genetics* **12**, 1205-1214.
- Pruett CL, Patten MA, Wolfe DH (2009) Avoidance behavior by prairie grouse: implications for development of wind energy. *Conservation Biology* **23**, 1253-1259.
- Prugnolle F, de Meeus T (2002) Inferring sex-biased dispersal from population genetic tools: a review. *Heredity* **88**, 161-165.
- Purvis A, Gittleman JL, Cowlshaw G, Mace GM (2000) Predicting extinction risk in declining species. *Proceedings of the Royal Society of London Series B-Biological Sciences* **267**, 1947-1952.
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* **43**, 258-275.
- Ramankutty N, Foley JA (1999) Estimating historical changes in land cover: North American croplands from 1850 to 1992. *Global Ecology and Biogeography* **8**, 381-396.
- Raymond M, Rousset F (1995) GENEPOP (version 1.2) - population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-249.
- R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.
- Ridgway R (1873) A new variety of prairie chicken. *Bulletin of the Essex Institute* **5**, 199.

- Ridgway R (1885) Some amended names of North American birds. *Proceedings of United States Museum* **8**, 354-357.
- Robel RJ (1970) Possible role of behavior in regulating greater prairie chicken populations. *Journal of Wildlife Management* **34**, 306-312.
- Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes* **4**, 137-138.
- Rousset F (2008) GENEPOP '007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* **8**, 103-106.
- Sala OE, Chapin III FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke F, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. *Science* **287**, 1770-1774.
- Sarrazin F, Barbault R (1996) Reintroduction: challenges and lessons for basic ecology. *Trends in Ecology and Evolution* **11**, 474-478.
- Schroeder MA (1991) Movement and lek visitation by female greater prairie chickens in relation to predictions of Bradbury's female preference hypothesis of lek evolution. *Auk* **108**, 896-903.
- Schroeder MA, Robb LA (2003) Fidelity of greater sage-grouse *Centrocercus urophasianus* to breeding areas in a fragmented landscape. *Wildlife Biology* **9**, 291-299.
- Schwartz CW (1945) *The Ecology of the Prairie Chicken in Missouri*. University of Missouri, Columbia, MO.
- Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology and Evolution* **22**, 25-33.
- Segelbacher G, Paxton RJ, Steinbrück G, Trontelj P, Storch I (2000) Characterization of microsatellites in capercaillie *Tetrao urogallus* (Aves). *Molecular Ecology* **9**, 1934-1935.
- Simberloff D (1998) Flagships, umbrellas, and keystones: Is single-species management passé in the landscape era? *Biological Conservation* **83**, 247-257.
- Snyder JW, Pelren EC, Crawford JA (1999) Translocation histories of prairie grouse in the United States. *Wildlife Society Bulletin* **27**, 428-432.
- Soulé ME (1985) What is conservation biology? *Bioscience* **35**, 727-734.

- Sparling DW (1979) Restoration of greater prairie chickens in southwestern Illinois: project summary October 1978 - July 1979. Southern Illinois University Cooperative Wildlife Research Laboratory, Carbondale, IL.
- Storch I (2007) Conservation status of grouse worldwide: an update. *Wildlife Biology* **13**, 5-12.
- Storfer A (1999) Gene flow and endangered species translocations: a topic revisited. *Biological Conservation* **87**, 173-180.
- Sutherland WJ (1998) The importance of behavioural studies in conservation biology. *Animal Behaviour* **56**, 801-809.
- Svedarsky WD (1988) Reproductive ecology of female greater prairie chickens in Minnesota. In: *Adaptive Strategies and Population Ecology of Northern Grouse*. (eds. Bergerud AT, Gratson MW), pp. 193-239. University of Minnesota Press, Minneapolis, MN.
- Svedarsky WD, Westemeier RL, Robel RJ, Gough S, Toepfer JE (2000) Status and management of the greater prairie chicken *Tympanuchus cupido pinnatus* in North America. *Wildlife Biology* **6**, 277-284.
- Svobodová J, Segelbacher G, Höglund J (2011) Genetic variation in black grouse populations with different lekking systems in the Czech Republic. *Journal of Ornithology* **152**, 37-44.
- Taylor SE, Oyler-McCance SJ, Quinn TW (2003) Isolation and characterization of microsatellite loci in greater sage-grouse (*Centrocercus urophasianus*). *Molecular Ecology Notes* **3**, 262-264.
- Templeton AR (1986) Coadaptation and outbreeding depression. In: *Conservation Biology: The Science of Scarcity and Diversity*. (ed. Soulé ME), pp. 105-116. Sinauer Associates, Sunderland, MA.
- Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, de Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Peterson AT, Phillips OL, Williams SE (2004) Extinction risk from climate change. *Nature* **427**, 145-148.
- Turner RE, Rabalais NN (2003) Linking landscape and water quality in the Mississippi River Basin for 200 years. *Bioscience* **53**, 563-572.
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535-538.
- Walk JW, Warner RE (1999) Effects of habitat area on the occurrence of grassland birds in Illinois. *American Midland Naturalist* **141**, 339-344.

- Wang J (2007) Triadic IBD coefficients and applications to estimating pairwise relatedness. *Genetics Research* **89**, 135-153.
- Wang J (2011) A new likelihood estimator and its comparison with moment estimators of individual genome-wide diversity. *Heredity* **107**, 433-443.
- Wang JL, Caballero A, Hill WG (1998) The effect of linkage disequilibrium and deviation from Hardy-Weinberg proportions on the changes in genetic variance with bottlenecks. *Heredity* **81**, 174-186.
- Wang N, Chang J, Gu LY, Zhang ZW (2009) Polymorphic microsatellites in the Reeves's pheasant developed by cross-species amplification. *European Journal of Wildlife Research* **55**, 627-629.
- Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology* **15**, 1419-1439.
- Watt PG (1973) Life history and behavior of greater prairie chickens. In: *The Prairie Chicken in Minnesota Conference Proceedings* (eds. Svedarsky WD, Wolfe T), pp. 5-14. University of Minnesota, Crookston, MN.
- Westemeier RL (1997) Grassland for prairie chickens: how much is enough? *Illinois Natural History Survey Reports* **343**, 1-2.
- Westemeier RL, Brawn JD, Simpson SA, Esker TL, Jansen RW, Walk JW, Kershner EL, Bouzat JL, Paige KN (1998a) Tracking the long-term decline and recovery of an isolated population. *Science* **282**, 1695-1698.
- Westemeier RL, Buhnerkempe JE, Edwards WR, Brawn JD, Simpson SA (1998b) Parasitism of greater prairie chicken nests by ring-necked pheasants. *Journal of Wildlife Management* **62**, 854-863.
- Westemeier RL, Jansen RW (1995) Nonresident prairie chickens in Illinois. *Illinois Natural History Survey Reports* **332**, 1-9.
- Westemeier RL, Simpson SA, Cooper DA (1991) Successful exchange of prairie chicken eggs between nests in two remnant populations. *Wilson Bulletin* **103**, 717-720.
- White TA, Searle JB (2007) Genetic diversity and population size: island populations of the common shrew, *Sorex araneus*. *Molecular Ecology* **16**, 2005-2016.
- Wilberg MJ, Dreher BP (2004) GENECAP: a program for analysis of multilocus genotype data for non-invasive sampling and capture-recapture population estimation. *Molecular Ecology Notes* **4**, 783-785.

Yeatter RE (1943) The prairie chicken in Illinois. *Bulletin of the Illinois Natural History Survey* **22**, 375-416.

Yeatter RE (1963) Population responses of prairie chickens to land-use changes in Illinois. *Journal of Wildlife Management* **27**, 739-757.

APPENDIX A: STUDY ORGANISM

The Greater (GPCH; *Tympanuchus cupido pinnatus*) and Lesser Prairie Chicken (LPCH; *T. pallidicinctus*) originally comprised a single species before each was described separately (Ridgway 1873, 1885). These and the Sharp-Tailed Grouse (*T. phasianellus*) are sole representatives of *Tympanuchus* (Connelly *et al.* 1998). Although superficially similar and overlapping at the extremes of their ranges, all are demarcated by habitat preference, appearance and behavior (Johnson *et al.* 2011). Low genetic variability among the three suggests a recent Pleistocene divergence, which has in turn caused difficulty in resolving their phylogenetic relationships (Drovetski & Ronquist 2003; Johnson 2008).

Three subspecies of *T. cupido* are recognized, with the GPCH (*T. c. pinnatus*) being the sole Illinois representative (Johnson *et al.* 2011). The federally endangered Attwater's Prairie Chicken (*T. c. attwateri*) is native to the Gulf Coast of Texas and Louisiana but as of 2007 had fewer than 50 wild birds in two Texas populations that have persisted since 1996 due to captive-reared supplementation (Morrow *et al.* 2004; Storch 2007). The Heath Hen (*T. c. cupido*) has been extinct since 1932 but was previously native to the Atlantic coast with a range stretching from New Hampshire to Virginia and perhaps into the Carolinas (Johnson & Dunn 2006; Palkovacs *et al.* 2004).

GPCH exhibit a unique behavior in which males aggregate and defend small territories within larger leks where they display for prospective mates by strutting and producing their characteristic “booming” sound, and occasionally engage in male-male combat (Breckenridge 1929). Lekking typically occurs in the early morning hours during March and April but perhaps as early as January, with courtship decreasing as hens begin to incubate nests (Gross 1930;

Yeatter 1943). GPCH are ground-nesters, preferring tall grasses and weeds for cover (Yeatter 1963). Nests may contain 20 or more eggs, and hens incubate then brood the precocious young while males provide no parental care (Schwartz 1945; Yeatter 1943).

Mating success is variable for male GPCH, often with only one or two males accounting for all copulations on a lek (Watt 1973). Likewise, Robel (1970) found that only 10% of males mate each year, with these typically representing the oldest individuals. Subordinate males control little or no territory and rarely mate. Given that GPCH live an average of 1.6 years (Hamerstrom 1973), some males may never sire offspring if unable to mate in their first breeding season. Additionally, dominant females tend to mate early in the breeding season, leading to earlier nesting and greater success relative to subordinate hens that mate later in the season (Robel 1970; Svedarsky 1988).

APPENDIX B: CONSERVATION STATUS

Grouse worldwide are subject to diminishing ranges and populations, with the GPCH no exception (Storch 2007). During the late 19th century, the GPCH was abundant throughout its range and served as an important game species (Johnsgard 2002). It initially flourished with the arrival of European settlers because agricultural fields intermixed with existing prairies provided adequate habitat and an important winter food source (Svedarsky *et al.* 2000). Anthropogenic factors, especially habitat destruction or fragmentation and introductions of exotic species, are now leading factors for its decline (Storch 2007; Westemeier *et al.* 1998b).

In the 20th century, GPCH populations were subjected to continued attrition stemming from intensified agriculture and sustained destruction of habitat (Storch 2007). GPCH are highly sensitive to excessive litter accumulation, habitat fragmentation, and increased tree cover, all of which negatively impact nesting success (McKee *et al.* 1998; Yeatter 1963). Introduction of Ring-necked Pheasant (*Phasianus colchicus*) has also negatively impacted GPCH populations (Westemeier *et al.* 1998b; Krakauer & Kimball 2009).

In several states, the GPCH became one of, if not the earliest, species protected from hunting (Johnson *et al.* 2011). Illinois, Iowa, Missouri, and Wisconsin closed their GPCH season prior to 1951 (Bellinger *et al.* 2003; Jamison & Alleger 2009; Yeatter 1943) while the Oklahoma season closed in 1997 due to low harvest. Only Colorado, Kansas, Minnesota, Nebraska, North and South Dakota sustain huntable populations (Svedarsky *et al.* 2000).

APPENDIX C: TRANSLOCATION AS A MANAGEMENT TOOL

Translocation has become an essential tool for managing endangered species that encompasses a broad range of management efforts, including both reintroduction of an extirpated species to its former range and supplementation of remnant populations via artificial gene flow from natural or captive-raised populations (Storfer 1999). Much additional work is required post-translocation to inform future decisions by better understanding successes and failures. These require both population monitoring and close collaboration between wildlife managers and ecologists to achieve maxim benefit (Sarrazin & Barbault 2004). Genetic monitoring has become a vital component of translocation programs as its impacts on evolutionary processes have become more fully realized (Schwartz *et al.* 2007). This work is necessary to better understand the many potential complications.

Translocation as a management tool can lead to unpredictable outcomes, thus requiring a thorough situational evaluation before actions can be taken (Fischer & Lindenmayer 2000). Genetic considerations are often overlooked, but paramount to long-term success. Crossing geographically isolated populations may yield positive results for bottlenecked populations (i.e., “hybrid vigor.” Crow 1948). In other cases effects can be detrimental, the least of which include obfuscation of once genetically distinct lineages (Leberg 1990; Pimm *et al.* 2006). More serious ramifications include a loss of fitness from outbreeding depression (Templeton 1986). This has been observed in a wide range of taxa (Edmands 2007) and its occurrence is difficult to predict (Frankham *et al.* 2011). The act of translocation itself can reduce genetic diversity in the introduced population via a founder effect (Mock *et al.* 2004). Transmission of pathogens between populations is also a concern with a genetic basis (Boyce *et al.* 2011). With these

factors in mind, both monetary and biological costs must be carefully weighed against the benefits of any translocation program (Martínez-Abraín *et al.* 2011).

Management via translocation has been applied to several bottlenecked populations of grouse species, with mixed results. Failed translocations have been twice as prevalent as successes, with the underlying determinants of success remaining elusive (Snyder *et al.* 1999). Most failures are attributed to a poor timing of release or lack of suitable habitat (Evrard 2004), with summer translocations most effective (Svedarsky *et al.* 2000). Successes involve larger releases of birds (> 100) or programs continued for multiple years (Westemeier *et al.* 1998a). As more knowledge is gained through monitoring and experimentation, management practices will be refined to increase the effectiveness of translocation (Sarrazin & Barbault 2004).

APPENDIX D: MODIFIED EXTRACTION PROTOCOL

For each feather, conduct the following steps:

Day 1

- I. Cut a 2-5 cm section of feather, including the superior umbilicus.
- II. Cut the feather into smaller pieces.
- III. Add 300µl ATL buffer, 20µl Proteinase K, and 20µl DTT to each sample, then vortex for 10 seconds.
- IV. Incubate at 56°C for 24 hours.

Day 2

- I. Add 300µl Buffer AL and vortex 15 seconds.
- II. Add 300µl ethanol and vortex 15 seconds.
- III. Incubate at room temperature for 5 minutes.
- IV. Centrifuge samples at 13,000 x g for 1 minute to pellet any undigested material.
- V. Transfer supernatant onto QIAamp MinElute Column and centrifuge at 6,000 x g for 1 minute.
- VI. Discard flow-through and collection tube.
- VII. Place the spin column onto a new collection tube, pipet 500µl Buffer AW1 onto the spin column, and centrifuge at 6,000 x g for 1 minute.
- VIII. Discard flow-through and collection tube.
- IX. Place the spin column onto a new collection tube, pipet 500µl Buffer AW2 onto the spin column and centrifuge at 6,000 x g for 1 minute.
- X. Centrifuge at 20,000 x g for 3 minutes to dry the column, then discard the flow-through and collection tube.
- XI. Place the spin column onto a 1.5ml microcentrifuge tube, and pipet 100µl Buffer AE directly onto the membrane. Incubate for 1 minute, then centrifuge for 1m at 6,000 x g to elute.
- XII. Pipette the eluate onto the membrane, incubate at room temperature for 1 minute, and centrifuge for 1 minute at 6,000 x g.

APPENDIX E: MICROSATELLITE AMPLIFICATION SUCCESS

Eighty-three microsatellite DNA loci previously developed for Galliform birds by other researchers (= Reference) and cross-amplification in Illinois Greater Prairie Chicken. Results are summarized for each locus (=Locus). Amp = status of amplification, with "-" = failure; "+" = success (but not tested for diversity); "P" = polymorphic; "M" = monomorphic; "X" = presence of spurious fragments that interfered with assessment; "L" = too large to be sized. TA = annealing temperature in °C. Reference = primary literature citation for locus.

Locus	Amp	TA	Reference
ADL44	P	50	Cheng & Crittendon 1994
ADL146	P	54	Cheng <i>et al.</i> 1995
ADL230	P	50	Cheng <i>et al.</i> 1995
ADL260	P	59	Bech <i>et al.</i> 2010
ADL292	M	62	Bech <i>et al.</i> 2010
Aru1A1	X	56	Ferrero <i>et al.</i> 2007
Aru1B3	-	59	Ferrero <i>et al.</i> 2007
Aru1D39	M	58	Ferrero <i>et al.</i> 2007
Aru1E7	M	55	Ferrero <i>et al.</i> 2007
Aru1E93	M	56	Ferrero <i>et al.</i> 2007
Aru1E97	-	58	Ferrero <i>et al.</i> 2007
Aru1E102	-	58	Ferrero <i>et al.</i> 2007
Aru1F25	-	60	Ferrero <i>et al.</i> 2007
Aru1F114	-	56	Ferrero <i>et al.</i> 2007
Aru1G4	M	58	Ferrero <i>et al.</i> 2007
Aru1G49	-	62	Ferrero <i>et al.</i> 2007
Aru1I121	X	62	Ferrero <i>et al.</i> 2007
Aru1J76	-	63	Ferrero <i>et al.</i> 2007
Aru1K10	L	52	Ferrero <i>et al.</i> 2007
Aru1M12	X	56	Ferrero <i>et al.</i> 2007
BG4	P	54	Piertney & Hoglund 2001
BG6	P	54	Piertney & Hoglund 2001
BG10	P	54	Piertney & Hoglund 2001
BG12	P	54	Piertney & Hoglund 2001
BG14	P	54	Piertney & Hoglund 2001
BG15	P	54	Piertney & Hoglund 2001
BG16	P	50	Piertney & Hoglund 2001
BG18	P	54	Piertney & Hoglund 2001

APPENDIX E (cont.)

Locus	Amp	TA	Reference
BG19	+	54	Piertney & Hoglund 2001
BG20	P	54	Piertney & Hoglund 2001
CUAAAG43	-	56	Gibson <i>et al.</i> 2005
CUAACC46a	+	55	Gibson <i>et al.</i> 2005
CUAACC8b	-	55	Gibson <i>et al.</i> 2005
CUAAGG37	P	54	Gibson <i>et al.</i> 2005
LLSD4	P	50	Piertney & Dallas 1997
LLSD6	-	62	Piertney & Dallas 1997
LLSD7	P	59	Piertney & Dallas 1997
LLSD9	P	50	Piertney & Dallas 1997
LLSD10	-	60	Piertney & Dallas 1997
LLST1	P	50	Piertney & Dallas 1997
LLST2	-	52	Piertney <i>et al.</i> 1998
LLST3	+	52	Piertney <i>et al.</i> 1998
LLST4	P	54	Piertney <i>et al.</i> 1998
LLST5	+	52	Piertney <i>et al.</i> 1998
LLST6	-	52	Piertney <i>et al.</i> 1998
LLST7	L	54	Piertney <i>et al.</i> 1998
MCW36	M	52	Baratti <i>et al.</i> 2001
MCW55	M	51	Baratti <i>et al.</i> 2001
MCW91	M	50	Baratti <i>et al.</i> 2001
MCW97	X	50	Baratti <i>et al.</i> 2001
MCW126	P	54	Baratti <i>et al.</i> 2001
MCW146	P	50	Baratti <i>et al.</i> 2001
MCW151	M	50	Baratti <i>et al.</i> 2001
MCW230	-	62	Bech <i>et al.</i> 2010
MNT12	P	59	Bech <i>et al.</i> 2010
MNT45	-	60	Bech <i>et al.</i> 2010

APPENDIX E (cont.)

Locus	Amp	TA	Reference
MNT404	-	62	Bech <i>et al.</i> 2010
MNT408	-	57	Bech <i>et al.</i> 2010
MNT412	-	62	Bech <i>et al.</i> 2010
MNT467	M	62	Bech <i>et al.</i> 2010
MNT477	M	57	Bech <i>et al.</i> 2010
SGCA6	P	54	Taylor <i>et al.</i> 2003
SGCA9	X	54	Taylor <i>et al.</i> 2003
SGCA11	-	62	Taylor <i>et al.</i> 2003
SGCTAT1	X	55	Taylor <i>et al.</i> 2003
SR01	-	55	Wang <i>et al.</i> 2009
SR03	P	54	Wang <i>et al.</i> 2009
SR08	M	55	Wang <i>et al.</i> 2009
SR11	-	55	Wang <i>et al.</i> 2009
SR13	-	55	Wang <i>et al.</i> 2009
SR17	-	55	Wang <i>et al.</i> 2009
SR18	-	55	Wang <i>et al.</i> 2009
SR19	M	55	Wang <i>et al.</i> 2009
SR20	M	55	Wang <i>et al.</i> 2009
TTD1	P	54	Caizergues <i>et al.</i> 2001
TTD4	P	54	Caizergues <i>et al.</i> 2001
TTD6	P	59	Caizergues <i>et al.</i> 2001
TTT1	P	54	Caizergues <i>et al.</i> 2001
TTT2	P	50	Caizergues <i>et al.</i> 2001
TUD3	P	54	Segelbacher <i>et al.</i> 2000
TUT1	+	60	Segelbacher <i>et al.</i> 2000
TUT2	P	50	Segelbacher <i>et al.</i> 2000
TUT3	P	54	Segelbacher <i>et al.</i> 2000

APPENDIX F: MULTIPLEX CONDITIONS

The 24 microsatellite DNA markers used to evaluate Illinois Greater Prairie Chickens were amplified with multiplex polymerase chain reaction (PCR) conditions listed in (A). Multiplex = 1-4, 6 & 11; ABI Dye-labeled Primer (in micromolar) = 6-FAM, VIC, NED, PET; MgCl₂ = magnesium chloride concentration (in millimolar); taq = Taq polymerase (in units); TA = annealing temperature (in °C); PCR Cycles = total PCR cycles to boost overall signal strength; Size Standard = LIZ-500 or LIZ-600. Microsatellite DNA loci that composed each Multiplex are listed in (B) by Multiplex and ABI Dye-labeled Primer.

A.

Multiplex	ABI Dye-labeled Primer [μM]				MgCl ₂ [mM]	taq (u)	TA (°C)	PCR Cycles	Size Standard
	6-FAM	VIC	NED	PET					
1	0.043	0.031	0.052	0.038	2.62	0.75	54	47	LIZ-500
2	0.052	0.044	0.025	0.046	2.74	0.75	54	45	LIZ-600
3	0.043	0.050	0.043	0.038	2.38	0.75	59	43	LIZ-500
4	0.058	0.030	0.053	0.040	2.75	0.5	54	45	LIZ-500
6	0.033	0.033	0.053	0.055	2.69	0.5	50	43	LIZ-500
11	0.055	0.035	0.043	0.050	2.63	0.5	50	45	LIZ-500

B.

Multiplex	ABI Dye-labeled Primer			
	6-FAM	VIC	NED	PET
1	SR03	CUAAGG37	ADL146	TTD01
2	LLST4	BG12	TUD3	TTT1
3	MNT12	LLSD7	TTD6	ADL260
4	BG14	TTD4	MCW126	TUT3
6	MCW146	BG16	LLSD4	ADL230
11	LLST1	LLSD9	TTT2	ADL44