Loss of Genetic Variation in Greater Prairie Chickens Following a Population Bottleneck in Wisconsin, U.S.A.

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Abstract: *Over the last century, populations of the Greater Prairie Chicken (*Tympanuchus cupido*) have declined or gone extinct throughout midwestern North America. In Wisconsin the population declined by 50% from 1951 to 1961 and has remained at low (1500 individuals) but fluctuating levels for the past 40 years. We examined historic (1951) and contemporary (1996–1999) populations of prairie chickens in Wisconsin to determine whether there was a loss of genetic variation following the population bottleneck. We compared microsatellite DNA variation at six loci in historic (1951,* n *= 47) and contemporary (1996–1999,* n *= 87) populations. Population mean heterozygosity and number of alleles per locus were significantly lower in the late 1990s than in 1951. This loss of genetic variation following a population bottleneck is consistent with the results of a similar study in Illinois, but we found no evidence of a reduction in hatching success.*

Key Words: ancient DNA, conservation, grouse, microsatellite DNA, population genetics, *Tympanuchus cupido*

Pérdida de Variación Genética en *Tympanuchus cupido* Después de un Cuello de Botella Poblacional en Wisconsin, EE.UU.

Resumen: *A lo largo del último siglo las poblaciones de pollos de la gran pradera (*Tympanuchus cupido*) han disminuido o se han extinguido en el Oeste medio de Norteamérica. En Wisconsin la población disminuyó un 50% entre 1951 y 1961 y se ha mantenido a niveles bajos (1500 individuos) pero fluctuantes durante los últimos 40 años. Examinamos las poblaciones históricas (1951) y contemporáneas (1996–1999) de* Tympanuchus cupido *en Wisconsin para determinar si había una pérdida de variabilidad genética después de un cuello de botella poblacional. Comparamos la variación de ADN microsatélite en seis loci en poblaciones históricas (1951,* n *= 47) y contemporáneas (1996–1999,* n *= 87). La heterocigosidad y el número de alelos por locus fueron significativamente más bajos hacia finales de los años 90 que en 1951. Esta pérdida de variación genética posterior al cuello de botella poblacional es consistente con los resultados de un estudio similar en Illinois; sin embargo, no encontramos evidencia de una reducción en el éxito de incubación.*

Introduction

Populations that have recently undergone large decreases in size are expected to lose genetic variation (Nei et al. 1975; Maruyama & Fuerst 1985). Loss of genetic variation may have important consequences for the long-term viability of a population. Individual fitness, resistance to disease and parasites, and the ability of populations to respond to environmental changes may decrease as a consequence of reduced genetic variation (Lacy 1997). Although severe population declines, or "bottlenecks," often reduce genetic variation (Mundy et al. 1997; Groombridge et al. 2000), evidence for a concomitant loss of fitness remains equivocal (Mitton 1993). Some researchers have demonstrated the importance of genetic variation for survival (McAlpine 1993; Keller

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et al. 1994), clutch size (McAlpine 1993), and growth rate (Alvarez et al. 1989), but other studies have found little or no effect (Ardern & Lambert 1997).

Greater Prairie Chickens (*Tympanuchus cupido*) were once abundant across much of midwestern North America, but over the last century populations have declined as a result of the loss and fragmentation of tallgrass prairie (Schroeder & Robb 1993). In a recent study of Greater Prairie Chickens in Illinois, Westemeier et al. (1998) found that loss of genetic variation was associated with lower hatching success of eggs following a population bottleneck. Greater Prairie Chickens in Illinois declined from over 25,000 birds in 1933 to 2000 in 1962 and finally reached a low of 46 birds in 1994 (Westemeier et al. 1991, 1998). Meanwhile, hatching success declined from 91–100% of eggs in the 1960s to a low of 38% in 1990 (Westemeier et al. 1998). In an attempt to prevent their extinction, managers translocated 271 Greater Prairie Chickens from other populations to Illinois during 1992–1996. Subsequently, hatching success increased from 76% to 94% of eggs (Westemeier et al. 1998). No genetic analysis was made of the population after the translocation, however, so it is not known whether the improvement in hatching success was associated with the introgression of new alleles into the Illinois gene pool.

In Wisconsin, Greater Prairie Chickens once inhabited nearly every county. In 1930, their estimated population size was 54,850 birds (Gross 1930). Over the next 20 years prairie chickens declined to 2500 birds in four management areas within 40 km of Stevens Point. During 1951–1961, the Greater Prairie Chicken population declined by 50% (Hamerstrom & Hamerstrom 1973) and has remained at low (1500 birds) but fluctuating levels over the last 40 years (Anderson & Toepfer 1999). The aim of our study was to determine whether Greater Prairie Chickens in Wisconsin have lost genetic variation as a consequence of a population bottleneck. We were concerned that if the population in Wisconsin had lost genetic variation, then it might also suffer from a loss of fitness, as was observed in Illinois (Westemeier et al. 1998).

Methods

Prairie chicken wings were collected by Fredrick and Frances Hamerstrom from hunters at Buena Vista Marsh, Portage County, Wisconsin, during the last hunting season in 1951 (Hamerstrom & Hamerstrom 1973). Forty-seven wings from adult birds (within Township 22N, Ranges 7 and 8 E) were used for comparison with contemporary (1996–1999) specimens from the same location (T21–22N, R 6–8E). Birds from the same location were used to minimize differences in allele frequencies arising from geographic rather than temporal differences.

The sample size from the historic population of individuals ranged from 40 to 45 depending on the locus; some individuals could not be amplified at all loci (Table 1). To eliminate any potential contamination, extraction of historic DNA from feathers (two or three per bird) was performed in a laboratory located on a different floor than the laboratory in which contemporary samples were extracted. Feather tips were digested for 24–48 ha at 55° C in 750 μL of buffer contained 10 mg/mL DTT, 1 mg/mL proteinase K, 1% SDS, 10 mM Tris, 2 mM EDTA, and 10 mM $NaCl_2$. Two phenol extractions and one chloroform extraction were used to extract the DNA. Contemporary samples of adult prairie chickens from Buena Vista Marsh (n = 87) were obtained from blood collected during 1996–1999. The DNA from blood samples was extracted by salt extraction (Miller et al. 1988).

Microsatellite Analysis

Five Red Grouse (*Lagopus lagopus scoticus*; Piertney & Dallas 1997) and six Domestic Chicken primers (*Gallus gallus*; Bouzat et al. 1998*a*) were tested for suitability as microsatellite markers. We had difficulty with three of the primers (ADL23, ADL42, ADL162) used by Bouzat et al. (1998*a*) and consequently used only ADL 44, ADL146, and ADL 230. Three Red Grouse primers were optimized for use: LLST1, LLSD4, and LLSD9 (Piertney & Dallas 1997). All microsatellites were dinucleotide repeats, and ADL 44 and LLST1 had imperfect core repeats. The PCR amplifications contained the following reagents in a 10-μL reaction: 20–75 ng/μL of genomic DNA, 0.25 μM (Red Grouse) or 0.50 μM (Domestic Chicken) fluorescently labeled forward and unlabeled reverse primer, 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM $MgCl_2$ (except ADL44, which required 3.0 mM), 1 unit of Taq polymerase, and 0.2 mM dNTP. The PCR conditions were one denaturing cycle at 94° C for 3 minutes, followed by 32–40 cycles at 94° C for 30 seconds, 30 seconds at annealing temperature (48° C for all primers except ADL230 at 46° C, LLSD9 and ADL44 at 49° C), and a 30-second extension step at 72° C. This was followed by an extension step at 72° C for 5 minutes. Amplified PCR products were run on 6% polyacrylamide gels with an ABI Prism 370 automated sequencer (Perkin Elmer). A fluorescently labeled ladder (Genescan 350 Tamra) was run in every lane of the gel to determine allele sizes, and a known prairie chicken size standard was run on every gel to ensure consistency between gels. We collected data on fragment size and analyzed them using Genescan 2.0 and Genotyper 2.1 software (ABI).

There was no evidence of contamination from the historic feathers. Approximately half of all samples were amplified twice in independent PCR reactions and run on the ABI 370 sequencer. In all cases the first reactions were fully repeatable (i.e., no evidence of allele dropout), and in no cases were more than two bands detected. We

Table 1. Number of alleles per locus and average population heterozygosity of historic (1951) and contemporary (1996–1999) Greater Prairie Chickens in Wisconsin.

Locus	Historic sample size (n)	Number of alleles per locus		Mean observed (expected) heterozygosity	
		historic	contemporary	historic	contemporary
ADL 44	42	9	6	0.81 (0.82)	0.71 (0.71)
ADL 146	45	5	3	0.49 (0.57)	0.16 (0.17)
ADL 230	44	10	9	0.89 (0.87)	0.76 (0.76)
LLST 1	40	7	5	0.40 (0.50)	0.21 (0.22)
LLSD 4	43	16	12	0.84 (0.88)	0.91 (0.86)
LLSD 9	45	8	7	0.82 (0.72)	0.60 (0.63)
Mean ± SE		9.17 ± 1.53[a,b]	7.0 ± 1.29[a,b]	0.71 ± 0.08[a,c] (0.73 ± 0.07)	0.56 ± 0.13[a,c] (0.56 ± 0.12)

[a]*Comparison of historic and contemporary populations.*
[b]*Z-corrected for ties = 2.21,* p = *0.027.*
[c]*Z-corrected for ties = −1.992,* p = *0.046.*

included a negative control in every PCR reaction and visualized it on an agarose gel to rule out contamination during PCR. Additionally, we amplified all alleles unique to the pre- or post-bottleneck sample and ran them at least twice in lanes adjacent to similar-sized alleles (on the sequencer) to verify that they were different in size.

Statistical Analyses

We tested changes in mean heterozygosity and mean number of alleles per locus (allelic diversity) using a Wilcoxon signed rank test, which pairs the data by locus. We also examined allelic richness, which is another measure of allelic variation that takes into account unequal sample sizes using the technique of rarefaction (Petit et al. 1998). Allelic richness was calculated with the program FSTAT (Goudet 1995). Deviations from Hardy-Weinberg equilibrium and linkage disequilibrium were assessed by genetic data analysis (Lewis & Zaykin 2000). All means for number of alleles per locus and heterozygosity calculations are presented with their SE.

Simulation Analyses and Estimation of Effective Population Size

During a population bottleneck, alleles are lost more rapidly than heterozygosity (Nei et al. 1975; Maruyama & Fuerst 1985; Spencer et al. 2000). This occurs because alleles present in low frequencies are lost first, and the initial reduction in allele number has relatively little effect on overall heterozygosity. As a consequence, the heterozygosity estimated from a bottlenecked population (H_e) is larger than the heterozygosity expected based on the observed (low) number of alleles, assuming that the population is in mutation-drift equilibrium (H_{eq}; Cornuet & Luikart 1996). Thus, a method for detecting recently bottlenecked populations is to compare the Hardy-Wienberg expected heterozygosity (H_e) in a sample with the heterozygosity expected at mutation-drift equilibrium. If $H_e > H_{eq}$ at the majority of loci, then there is a heterozygosity "excess" that suggests a recent population bottleneck (Cornuet & Luikart 1996). This heterozygosity excess should be detectable for approximately 0.2–4 N_e generations, where N_e is the effective population size. We tested for a heterozygote excess with a one-tailed Wilcoxon sign-rank test with the program Bottleneck (Piry et al. 1999), which uses the observed number of alleles and sample size under the two-phase mutation model. The exact nature of microsatellite mutation is unclear, but it is thought to most closely follow the two-phase model (TPM) or stepwise mutation model (SMM) and least likely to follow the infinite alleles model (Primmer et al. 1998; Estoup & Cornuet 2000). The SMM predicts that all mutations are of single base-pair repeats, whereas the TPM predicts that the majority of mutations are single, base-pair repeats, with occasional multiple repeats.

We used the computer program Geneloss (England & Osler 2001) to simulate a population bottleneck similar to that observed in Greater Prairie Chickens in Wisconsin. The simulation allowed us to determine whether the observed losses of genetic variation were consistent with a simulated scenario of genetic drift. Geneloss uses Monte Carlo sampling to simulate the effects of a population bottleneck on allelic diversity and heterozygosity at neutral loci with known allele frequencies. It assumes random mating and can simulate bottlenecks lasting one or more generations. The initial conditions of the simulation were based on the allele frequencies in the historic sample of Greater Prairie Chickens in Wisconsin. We modeled bottlenecks of 77 and 416 individuals, which were our high and low estimates of effective population size for up to 50 generations (1000 iterations per simulation). Prairie chickens have a generation time of

approximately 2 years, so we compared the twenty-fifth simulated generation with the contemporary sample.

The effective population size (N_e) of Greater Prairie Chickens was calculated as the harmonic mean of annual census counts of males for each year during the bottleneck (1952–1997), assuming a 1:1 sex ratio and similar mortality rates for males and females (Hamerstrom & Hamerstrom 1973; Anderson & Toepfer 1999). For simplicity, we assumed that generations did not overlap, although Greater Prairie Chickens live an average of 1.6 years and females mate at age one (Hamerstrom & Hamerstrom 1973; Schroeder & Robb 1993). We also estimated an N_e that took into account the observed skew in male mating success on leks, by assuming that 10% of males on booming grounds mated with females (Robel 1970). The N_e incorporating this reproductive skew was calculated as the harmonic mean of $4N_m\mathrm{N_f}/(N_m+N_f)$ (Hartl & Clark 1997), where N_f is the number of females (we assumed a 1:1 sex ratio and used the number of males seen during census counts) and N_m is 10% of the number of males counted on booming grounds in Buena Vista Marsh (Fig. 1; Anderson & Toepfer 1999). Based on these calculations, the effective population size from 1952 to 1997 was 77 birds when we assumed that 10% of males bred and 416 birds when we assumed that all males and females bred.

Results

All of the 12 population and locus combinations were in Hardy-Weinberg equilibrium after the significance (alpha) level was adjusted for the number of comparisons with a Bonferroni correction (Rice 1989). Exact tests for linkage disequilibrium revealed no linkage between loci. The mean number of alleles per locus was greater in the historic (9.17 ± 1.54) than the contemporary (7.00 ± 1.29) population (Wilcoxon signed rank test, $Z = 2.21$, $p = 0.027$; Table 1). Similarly, allelic richness was greater in the historic ($R_t = 9.032$) than the contemporary ($R_t = 6.55$) population (Wilcoxon signed rank test, $Z = -2.2$, $p = 0.028$). All six microsatellite loci were polymorphic, with the number of alleles ranging from 5 (ADL146) to 16 (LLSD4; Fig. 2).

Among all six loci, 16 alleles were detected in the historic population that were not detected in the current population (Fig. 1), a loss of 29% (16/55) of alleles across all six loci. All alleles lost since 1951 were rare (frequency ≤0.15) in the historic population. Three alleles were detected in the current but not in the historic population. Detection of rare alleles is a direct function of sample size, and alleles detected in the contemporary but not in the historic samples were present at a frequency of <3%. Thus, the most likely explanation for these "novel" alleles is probably the difference in sample size between the historic ($n = 47$) and contemporary

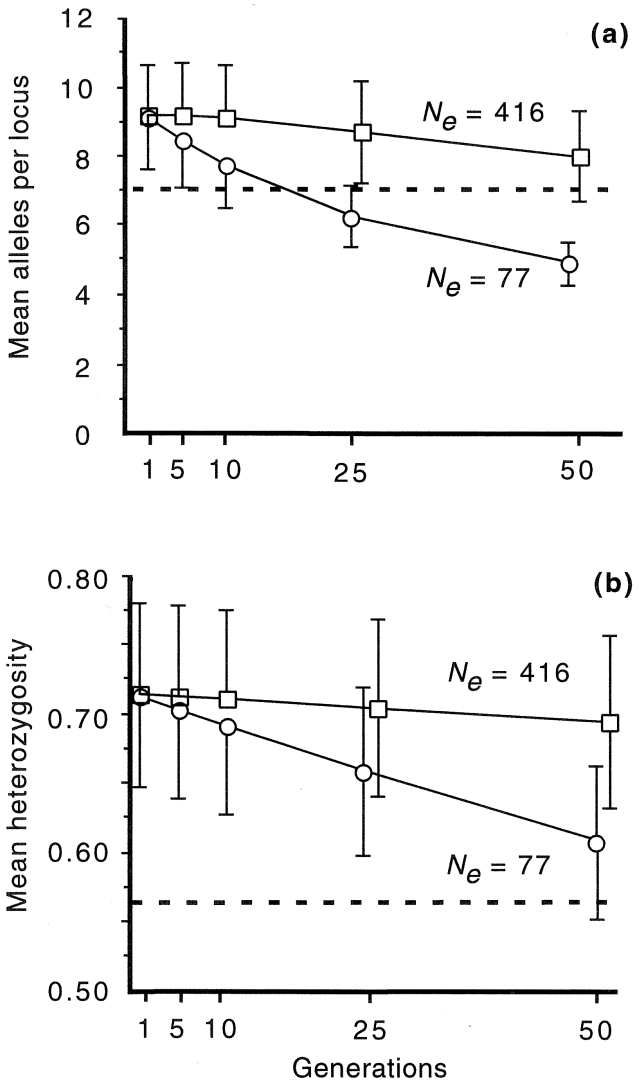

Figure 1. Geneloss computer simulations of (a) the number of alleles per locus and (b) heterozygosity at 1, 5, 10, 25, and 50 generations, with effective population sizes (N_e) of 77 and 416 birds. The simulation began with estimates of allele frequencies from the historic Greater Prairie Chicken population at six microsatellite loci. The contemporary population is at approximately 25 generations, and horizontal lines indicate mean values from the contemporary population. Vertical lines are standard errors from the six loci. For clarity, SE bars are shown in only one direction at <25 generations, and symbols are shifted slightly at 25 and 50 generations.

($n = 87$) populations. Alternatively, they could be new mutations; mutation rates of microsatellites in birds have been reported to be as high as 2.7–7.1% (Primmer et al 1998). A third explanation is that these alleles were introduced into the population by immigrants. Movements of birds between the four management areas in Wiscon-

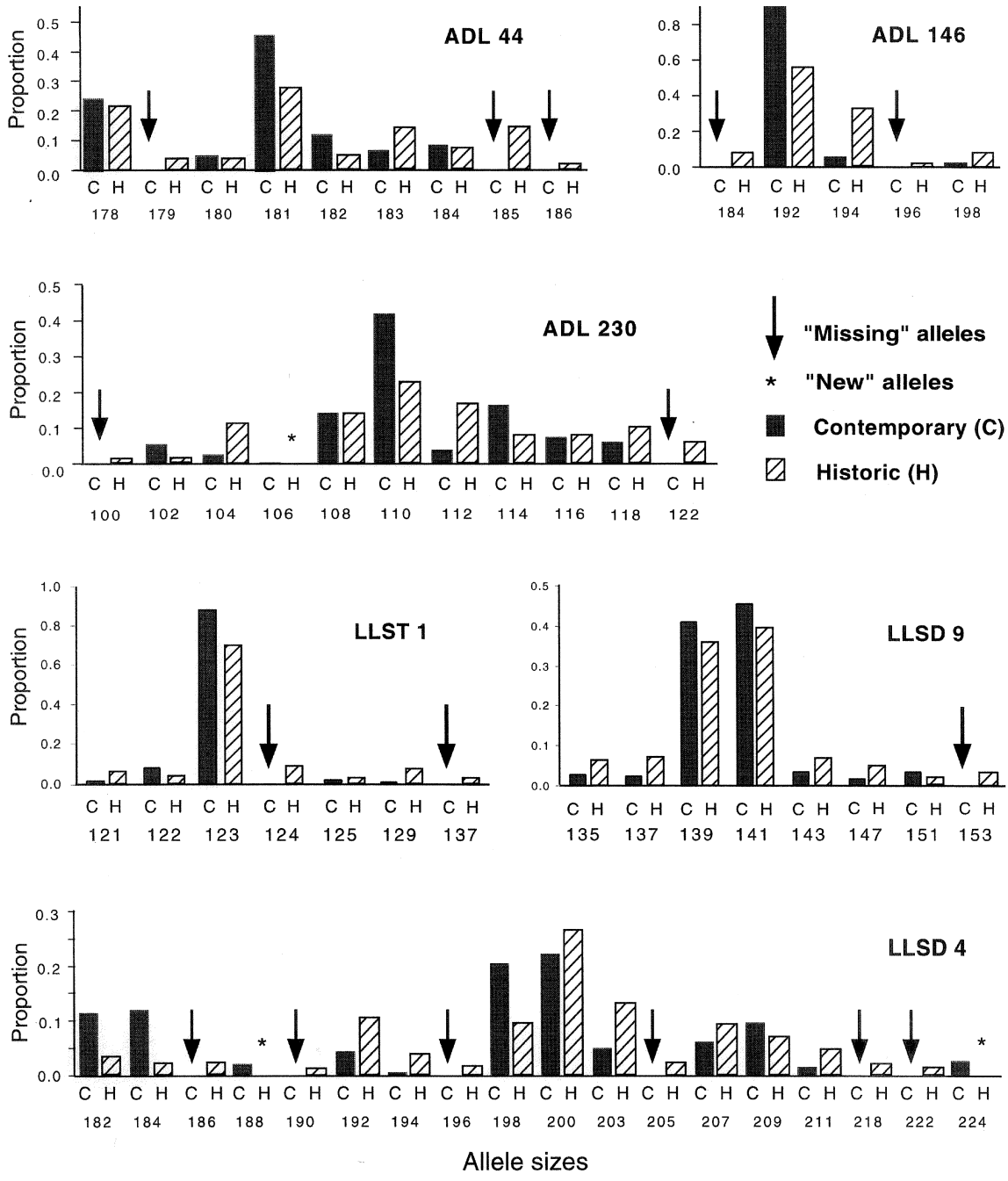

Figure 2. Proportion of prairie chicken samples with alleles of various sizes at six microsatellite loci, Buena Vista Marsh, Wisconsin. Contemporary samples are from 1996 to 1999 (n = 87), and historic samples are from 1951 (n = 40–45).

sin were documented during 1950–1969 (Hamerstrom & Hamerstrom 1973), but it is unlikely any immigrants have come from outside Wisconsin.

Population mean heterozygosity also decreased significantly during the past 50 years (Wilcoxon signed rank test, $Z = -1.992$, $p = 0.046$; Table 1). Observed heterozygosity ranged from 0.49 (ADL146) to 0.89 (ADL230) in the historic population and from 0.16 (ADL146) to 0.91 (LLSD4) in the contemporary population. Average population heterozygosity for five of the six loci was higher in the historic than the contemporary sample (Table 1). At LLSD4, heterozygosity increased from 0.84 to 0.91 over the past 50 years despite the loss of four alleles. The program Bottleneck (Piry et al. 1999) did not detect a heterozygote excess or deficiency with the two-phase (TPM) model of microsatellite mutation.

Geneloss (England & Osler 2001) predicted changes in allelic diversity and loss of heterozygosity that were consistent with the observed changes in genetic variation (Fig. 2). During the population bottleneck, the maximum effective population size of Greater Prairie Chickens at Buena Vista marsh was 416 birds (based on census counts and assuming random mating). A simulated bottleneck of 416 birds for 25 generations produced estimates of allelic diversity (8.7 ± 1.5 simulation, 7.0 ± 1.3 observed; one-tailed $t_5 = 1.2$, $p = 0.3$; Fig. 1) and heterozygosity (0.705 ± 0.06 simulation, 0.558 ± 0.13 observed; one-tailed $t_5 = 2.3$, $p = 0.07$; Fig. 1) that were not significantly different from the observed values. The estimate of N_e that took account of reproductive skew in males ($N_e = 77$) brought these estimates even closer to the observed values at 25 generations ($p = 0.45$ for allelic diversity; $p = 0.15$ for heterozygosity; Fig. 1).

In contrast to the low hatching success reported in Illinois (Westemeier et al. 1998), hatching success in Wisconsin remained high after the bottleneck. The percentage of all eggs that hatched in successful nests (nests with at least one hatched egg) was 91% in 1972 and 1974 ($n = 14$ successful nests; Toepfer 1988) and 89% in the late 1990s ($n = 15$ nests; J.T., unpublished data). Prior to the bottleneck, estimates of hatching success ranged from 90% (22 successful nests in 1929–1930, Gross 1930) to 95% (11 successful nests in 1936–1937; Hamerstrom 1939).

Discussion

Greater Prairie Chickens in Wisconsin went through a population bottleneck in the 1950s and have remained at a small population size (1500) for the past 40 years. This change in population size was associated with a significant decrease in allelic diversity and mean heterozygosity. Alleles that were apparently lost during the population bottleneck were formerly rare in the population (≤0.15), as predicted by theories of genetic drift (Nei et al. 1975). These results are consistent with those of several studies of other endangered populations of birds, including Greater Prairie Chickens in Illinois (Bouzat et al. 1998*b*). Nonetheless, the consequences of population bottlenecks for fitness and population viability appear to differ among studies. Indeed, our study shows that the fitness consequences of bottlenecks vary greatly between different populations of the Greater Prairie Chicken. To our knowledge, this is the first time different bottlenecks of the same species have been examined in the wild.

Populations that have recently undergone a bottleneck are expected to show a heterozygote excess at the majority of microsatellite loci. We found no such excess (or deficiency) using a two-phase model of microsatellite mutation. With an effective population size of 77 to 416 individuals over 46 years, the bottleneck in Wisconsin Greater Prairie Chickens should have shown a heterozygote excess for a minimum of 15–83 years (0.2–4.0 N_e generations; Cornuet & Luikart 1996; Luikart & Cornuet 1998). However, the power of our test may have been limited by a relatively low number of loci (Cornuet & Luikart 1996).

Bottlenecks of 77 and 416 birds that we simulated using GeneLoss were consistent with our observed loss of genetic diversity (Fig. 1). However, neither the large (416) nor small (77) simulated populations (Fig. 1) differed significantly from the contemporary population in terms of allelic diversity or heterozygosity. Thus, it is not possible to determine which estimate of effective population size (416 or 77) is most consistent with the observed reduction in genetic variation in the Greater Prairie Chicken. Nevertheless, the simulation results support the conclusion that prairie chickens in Wisconsin have lost significant genetic variation following a population bottleneck.

In Illinois the loss of allelic diversity was associated with a reduction in hatching success (<80% of eggs hatched; Westemeier et al. 1998), which, along with brood survival, is one of the most important factors affecting population growth (Wisdom & Mills 1997). In contrast, we did not see a similar reduction in fitness in Wisconsin prairie chickens. In fact, their hatching success in Wisconsin has remained relatively high since the early 1970s (89–91%). Hatching success throughout the range averages 89% (Table 4 of Peterson & Silvy 1996). There are several possible explanations for the apparent discrepancy, including (1) selection for individuals with relatively high hatching success (Fowler & Whitlock 1999); (2) more stressful environmental conditions in Illinois than Wisconsin, leading to greater loss of fitness in Illinois (Keller et al. 1994; Meagher et al. 2000); and (3) low levels of immigration into the Buena Vista management area (possibly from the other three management areas in Wisconsin), which prevented loss of fitness (Hedrick & Gilpin 1997). It is also likely that the population bottlenecks in Wisconsin and Illinois varied in intensity. There were 1500–2500 birds in both states by 1960, but the populations in Illinois were more fragmented and possibly more isolated than populations in Wisconsin. From 1960 to 1994 the number of males in Illinois declined to a low of 46, despite an increase in local habitat (Westemeier et al. 1998), while in Wisconsin the population contracted to four small management areas (13–50 km apart) and has remained at around 1500 individuals in total since 1960.

Although small populations (<1500) of Greater Prairie Chickens have persisted for several decades in Wisconsin, Missouri, and North Dakota, the pattern across most of the range for the past 50 years has been of fragmentation of habitat, decline of the species, and, eventu-

ally, its local extinction (Johnsgard 1983; Toepfer et al. 1990). The effective population size on the Buena Vista Marsh (77–416 birds) is below the theoretical minimum size (500–5000) necessary to ensure long-term viability in the face of environmental and genetic stochasticity (Lande 1999). Based on historic patterns and the loss of genetic diversity found in Wisconsin and Illinois, it seems likely that, without some management intervention, small fragmented populations of Greater Prairie Chickens will continue to lose genetic variation and eventually will go extinct.

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