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Temporal changes in allele frequencies and low effective population size in greater prairie-chickens

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Abstract

The number of greater prairie-chickens in Wisconsin has decreased by 91% since 1932. The current population of approximately 1500 birds exists primarily in four isolated management areas. In previous studies of the Wisconsin populations we documented low levels of genetic variation at microsatellite loci and the mitochondrial DNA control region. Here we investigate changes in genetic structure between the four management areas in Wisconsin over the last 50 years. We estimated the harmonic mean effective population size (N_e) over the last 50 years by comparing allele frequencies from the early 1950s with those from contemporary samples. Using a pseudo-likelihood approach that accounted for migration, estimates of N_e (15–32 prairie-chickens within each management area) were 10 times lower than census numbers from booming-ground counts. These low estimates of N_e are consistent with increased habitat fragmentation and an increase in genetic isolation between management areas over the last 50 years. The reduction of gene flow between areas has reduced N_e, increased genetic drift and, consequently, reduced genetic variation. These results have immediate consequences for the conservation of the prairie-chicken, and highlight the importance of how mating systems and limited dispersal may exacerbate the loss of genetic variation in fragmented populations.

Keywords: conservation, effective population size, fragmentation, gene flow, grouse, microsatellite, mtDNA control region, population genetic structure, *Tympanuchus*

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Introduction

Populations are expected to lose genetic variation at selectively neutral loci at a rate of $1/(2N_e)$ per generation, where N_e is the effective population size (Wright 1969). Habitat fragmentation leads to smaller, more isolated populations in which N_e is reduced. This increases the rate of loss of genetic variation and, ultimately, the risk of extinction (Gilpin & Soulé 1986; Soulé & Mill 1998; Frankham *et al*. 2002). Thus, the ability to estimate N_e has important implications for conservation, but it has been notoriously difficult to estimate N_e in wild populations (for reviews see Caballero 1994; Schwartz *et al*. 1998; Wang & Caballero 1999; Waples 2002) because estimates can fluctuate depending on demographic properties specific to the population (Ardren & Kapuscinski 2003; Palm *et al*. 2003; Kaeuffer *et al*. 2004). Since N_e influences genetic variation, a number of methods have been developed that estimate N_e through changes over time in estimates of genetic variation (see Williamson & Slatkin 1999). The majority of these 'temporal approaches' for estimating N_e assume that changes in allele frequency over time are primarily the result of genetic drift, and that the effects of selection, mutation and immigration (migration) are negligible.

Migration between sampled populations can have an important effect on accurate estimates of N_e, depending on the time between sampling periods (Wang & Whitlock 2003). For example, when migration occurs from a 'source' population into a 'focal' population, the allele frequencies of the focal population will be more similar to the source population (Gaggiotti 1996). If this migration is not taken into account and the sampling period is short, migration

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will cause the allele frequencies to change rapidly as if genetic drift were very strong, as occurs in a population with a small N_e. Thus, estimates of N_e will be too low relative to the true value (underestimates). In contrast, if the sampling period is much longer, the bias is reversed. Over the long-term, constant migration and drift should cause populations to approach an equilibrium level at which the rate of change is slowed relative to what would be expected under genetic drift alone. As a consequence of the slower rate of change, the estimate of N_e will be too high relative to the true value, and, hence, overestimated (Wang & Whitlock 2003). Indeed, the estimate of N_e will approach the N_e of the entire metapopulation contributing migrants to the focal population. Therefore, in the case of nearby populations that have recently become fragmented, methods that account for migration should provide more precise estimates of N_e than methods that assume that genetic drift is the only force changing allele frequencies over time.

In this study, we used estimates of genetic variation spaced approximately 50 years apart to estimate N_e in the greater prairie-chicken (*Tympanuchus cupido*). Prior to European settlement, the greater prairie-chicken occurred throughout the prairies of central North America. However, populations began to decrease with the disappearance of prairie grasslands (Johnsgard 2002), and, today, this species is forced to persist in an agricultural landscape. During the twentieth century, populations of greater prairie-chickens have become extinct in Iowa (1952), Michigan (1983) and the Canadian prairie provinces (1960–70s), and populations in Illinois, Missouri, North Dakota and Wisconsin have become small (< 2000) and isolated (Houston 2002; Johnsgard 2002). Theoretically, because of its lek breeding behaviour, this species should be vulnerable to the effects of drift when population size is significantly reduced. Previous comparative genetic studies have found qualitative evidence for drift within greater prairie-chicken populations that have witnessed a significant decline in abundance over the last century (Bouzat *et al*. 1998a,b; Westemeier *et al*. 1998; Bellinger *et al*. 2003; Johnson *et al*. 2003). Therefore, accurate estimates of N_e would be of importance to continuing efforts to prevent further local extinctions of this species.

Within Wisconsin, the size of prairie-chicken populations has decreased by 91% since 1932. Approximately 1500 prairie-chickens currently exist in the state (Anderson & Toepfer 1999) with the majority (> 90%) residing in four management areas near Stevens Point (Fig. 1). However, despite close proximity (10–60 km) among management areas, both radiotelemetry and genetic evidence suggest that barriers to dispersal exist between the northern and southern management areas (Halfmann 2002; Johnson *et al*. 2003). Over a 3-year period, only a single female out of 306 radio-marked juveniles moved between the northern and southern management areas (Halfmann 2002). Previously,

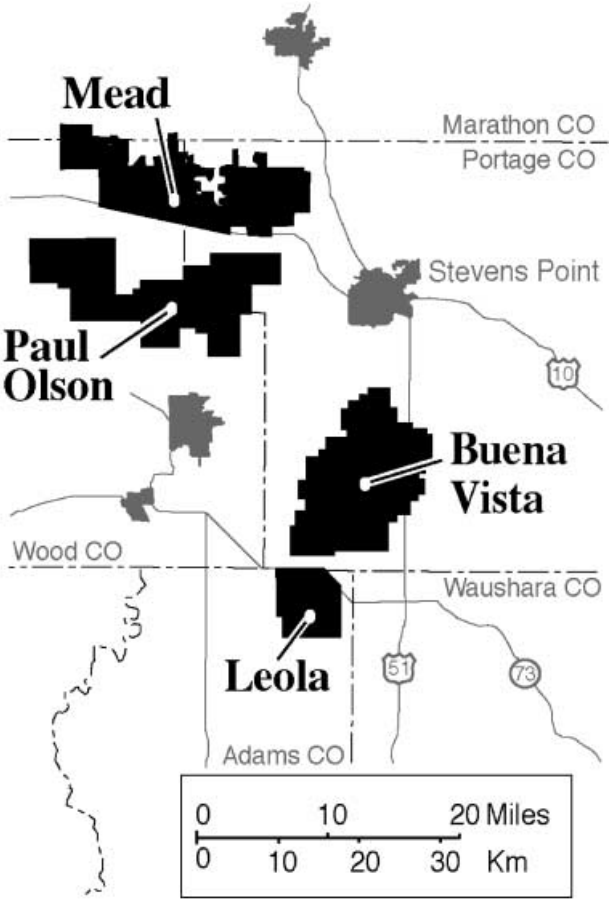

Fig. 1 Distribution of Wisconsin's greater prairie-chicken management areas (Mead, Paul Olson, Buena Vista, and Leola).

we found a significant reduction in microsatellite DNA variation over a 50-year period at one of the Wisconsin management areas (Buena Vista Marsh; Bellinger *et al*. 2003). Here, we extend that study by including historic and contemporary samples from the remaining three management areas in Wisconsin. By incorporating temporally spaced genetic samples from the entire range of prairie-chickens in Wisconsin, we can estimate N_e while accounting for migration and drift, and we can investigate how habitat fragmentation has influenced levels of genetic variation over the past 50 years.

Materials and methods

Tissue collection and DNA extractions

Tissue samples of adult greater prairie-chickens were collected during 1951–54 and 1998–2000 from four separate management areas (Mead, Paul Olson, Buena Vista and Leola) in central Wisconsin (Fig. 1). Samples in addition to those described previously (Bellinger *et al*. 2003; Johnson *et al*. 2003) include historic samples from Mead (Township

25–26N, Ranges 4–7E) and Paul Olson (T22–23N, R4–9E) collected between 1951 and 1954 and samples from Leola (T20, R7E) collected in 1951. Approximately equal proportions of males and females were sampled in each management area, with the exception of the contemporary Buena Vista where 94% of sampled birds were male.

DNA was extracted from blood and feather samples using methods described in Bellinger *et al*. (2003) and Johnson *et al*. (2003), whereas DNA was extracted from bone samples following methods described below, modified from Fleischer *et al*. (2000) and Lambert *et al*. (2002). To reduce the potential for contamination with contemporary prairie-chicken samples, historic extraction procedures were conducted in a new laboratory facility that had never been used for DNA research. Briefly, 0.5–1.0 g of bone from wing samples was pulverized using sterile equipment. Individual samples were then placed in 10–12 mL of 500 mM ethylenediaminetetraacetic acid pH 8.0 at room temperature for 3–4 days to decalcify the bone tissue. Samples were re-suspended in 5 mL extraction buffer (10 mM Tris–HCl pH 8.0, 1.0 mM NaCl, 0.9% sodium dodecyl sulphate) containing 1.0 μg/mL dithiothreitol and 0.45 μg/mL proteinase K, and incubated overnight at 50 °C. Samples were then washed twice with Tris-saturated phenol and once with chloroform : isoamyl alcohol (24 : 1), then concentrated to 200 μL on an Ultrafree-4 centrifugal filter membrane (Millipore), followed by a purification step to remove additional polymerase chain reaction (PCR) inhibitors by using a DNeasy Tissue Kit (Qiagen), and eluted using 100 μL supplied buffer. Samples were extracted in sets of 10 and blank controls were included in each group. No blank controls (n = 8) amplified prairie-chicken DNA when subjected to PCR.

Genotyping and sequencing

Six microsatellite loci (ADL44, ADL146, ADL230, LLST1, LLSD4, and LLSD9) were used in the microsatellite analyses (see Johnson *et al*. 2003 for description of loci), and 384 base pairs of the 5′ region of the mitochondrial DNA (mtDNA) control region were sequenced using primers 16775 L (Quinn 1992) and 521H (Quinn & Wilson 1993). Microsatellite procedures were carried out as described by Bellinger *et al*. (2003) and the mtDNA procedures were described by Johnson *et al*. (2003). However, the following exceptions were used with historic microsatellite and mtDNA samples: all individuals that were initially genotyped as homozygotes in the microsatellite analysis were amplified twice more and no change in genotype was observed, 1.0 M Betaine (Sigma) was used per reaction to improve mtDNA PCR efficiency, and QIAquick gel extraction kits (Qiagen) were used for gel purification steps. Mitochondrial DNA samples were sequenced with a CEQ 8000 capillary sequencer (Beckman Coulter) using a CEQ Dye Terminator Cycle Sequencing Quick Start Kit (Beckman Coulter). Additional unique sequences that were not reported in Johnson *et al*. (2003) were submitted to GenBank (accession numbers: AY608323–AY608334).

Statistical analyses

Microsatellite genotypes were tested for linkage equilibrium and departure from Hardy–Weinberg equilibrium within each population at each locus using the computer program ARLEQUIN vs. 2.0 (Schneider *et al*. 2000). Sequential Bonferroni corrections were applied to correct for multiple simultaneous comparisons (Rice 1989). Mean number of alleles per locus (allelic diversity) and mean heterozygosity were calculated using the program GDA (Lewis & Zaykin 2000). Measures of allelic richness were included to control for differences in the number of alleles among populations that differ in sample size (Leberg 2002) and were calculated using the program FSTAT vs. 2.9.3 (Goudet 1995). To investigate temporal changes in microsatellite DNA diversity, differences between populations in mean number of alleles, allelic richness and observed heterozygosity were tested using a Wilcoxon signed rank test, which pairs the data by locus. Estimates of F_{IS}, as 1 – (observed heterozygosity/expected heterozygosity) were also conducted, and its significance between populations was tested by permutation (10 000) using FSTAT. Temporal changes in mtDNA diversity were investigated by comparing population estimates of mitochondrial haplotype diversity (h), nucleotide diversity (π) and Tajima's D using the program DNASP vs. 3.52 (Rozas & Rozas 1999).

To investigate temporal changes in population genetic structure with both microsatellite and mitochondrial DNA, pairwise F_{ST} values were calculated following Weir & Cockerham (1984) and Tamura (1992), respectively, as implemented in ARLEQUIN vs. 2.0 (Schneider *et al*. 2000). Microsatellite pairwise R_{ST} (Slatkin 1995) values were also calculated using the program RSTCALC (Goodman 1997), however, our results with F_{ST} and R_{ST} were qualitatively similar and therefore we only report F_{ST} values here. Differences in population structure between sampling years and management areas were tested using permutations (10 000) among populations with Fisher's exact test.

We also investigated microsatellite DNA spatial genetic structure using the Bayesian method of Pritchard *et al*. (2000) and Falush *et al*. (2003), implemented in the program STRUCTURE version 2.0. This method identifies genetically distinct clusters (K) based on allele frequencies across loci. The most likely value of K is assessed by comparing the likelihood of the data for different values of K. Calculations were conducted with a burn-in period of 100 000, followed by 500 000 iterations. Each simulation was performed four times using an ancestry model incorporating admixture, along with a model of correlated allele frequencies that did

not include prior information on population origin (see Falush *et al*. 2003).

To visualize the genetic relationships among management areas, unrooted neighbour-joining phenograms were constructed for the microsatellite data using a pairwise chord distance matrix (D_{CE}; Cavalli-Sforza & Edwards 1967) and Nei's standard genetic distance (D_S; Nei 1972) calculated using the program POPULATIONS vs. 1.2.28 (http://www.cnrs-gif.fr/pge/bioinfo/populations/index.php), and unrooted neighbour-joining phenograms were constructed using haplotype distances (Tamura 1992) for the mtDNA data as described above. In addition, a multivariate ordination was conducted for the microsatellite data using the software PCAGEN (http://www.unil.ch/izea/softwares/pcgen.html) with 10 000 randomization steps.

Estimating N_e

The harmonic mean of the variance effective population size (N_e) over the past 50 years was calculated from the microsatellite temporal data sets using: (i) the standardized variance of change in allele frequencies, F (Waples 1989, 1990) and (ii) pseudo-likelihood estimates (Wang 2001; Wang & Whitlock 2003). Recent studies based on the 'temporal approach' have shown that likelihood methods provide more accurate estimates of N_e than methods using F-statistics (Williamson & Slatkin 1999; Wang 2001), especially when levels of genetic drift are strong (Berthier *et al*. 2002) and when calculations account for migration (Wang & Whitlock 2003). Nevertheless, we include the estimates from F-statistics for comparative purposes.

For the estimation of N_e from F, allele frequencies were calculated for all observed alleles for each sampling period within each management area. The standardized variance of allele frequencies (F_k) for each locus and a weighted mean standardized allele variance across all loci were estimated using methods described in Waples (equation 11; Waples 1989, 1990). Because prairie-chickens do not conform to the discrete generation model, we could not simply use the number of years between samples for the sampling interval between time periods. Prairie-chickens have a high variance in male mating success with older males (= 2 years) gaining the majority of copulations (Schroeder & Robb 1993). Therefore, we used a method that incorporates life history in terms of age structure within a population to estimate the number of generations (b) between sampling periods (Waples 1990; Tajima 1992). Our estimate of b was approximately 30 generations between our sampling periods, using data from Wisconsin on the proportion of breeding birds at each age class, annual mortality (approximately 50%) and an equal sex ratio (Hamerstrom & Hamerstrom 1973; Schroeder & Robb 1993; J. Toepfer, unpublished data). To account for error in our calculation of b, we also present estimates of N_e using 20 and 40 generations between the two sampling periods. The $1 - \alpha$ confidence limits for F were calculated following a χ^2 distribution as described in equation 16 of Waples (1989).

Calculations of N_e using a pseudo-likelihood approach were conducted using the program MLNE 1.0 (Wang & Whitlock 2003). This program allows the estimation of N_e with or without taking into account levels of immigration from a source population using both pseudo-likelihood and F-statistics. However, values for the F-statistic when migration is assumed are not reliable because when sampling intervals become large, values for N_e tend to approach levels for the whole species rather than the focal population (see Wang & Whitlock 2003). As such, a number of our estimates for N_e that used F-statistics and included migration were infinite in size and therefore those values are not reported.

We conducted our pseudo-likelihood analyses by setting the sampling interval to 30 ± 10 generations, similar to our F-statistics, and used either the temporally spaced individual management areas as our focal populations or the northern (Mead and Paul Olson) and southern areas (Buena Vista and Leola) as two separate focal populations. This allowed us to determine the effects on calculations of N_e based on different migration scenarios among various defined focal populations.

By combining all four management areas into one population, we are effectively assuming that there is no immigration because the contemporary Wisconsin population is completely isolated from populations in other states (the nearest populations are separated by > 600 km of inhospitable habitat). However, during the time of our historic samples (i.e. 1951–54), there were additional birds located outside the four management areas in surrounding counties (Westemeier 1971; Hamerstrom & Hamerstrom 1973), while today over 93% of prairie-chickens are restricted to these four areas (Anderson & Toepfer 1999). In addition, substructure among management areas has developed over the last 50 years that suggests a reduction in gene flow among areas (Halfmann 2002; Johnson *et al*. 2003; this study). As a consequence, there are several possible combinations of focal and source populations that can be used for analysis. We used the first method to analyse each of the four management areas individually, whereas we used the second method to analyse the populations within two regions (northern and southern) that are genetically distinct (Johnson *et al*. 2003). In the first method we examined each focal management area using the combined allele frequency data from the remaining three management areas to serve as one combined source population. In the second method a single management area within each region was used as the source population that provided immigrants to the focal population (e.g. within the northern region Mead was a source when calculating N_e for Paul Olson). We used a third method to analyse the northern or southern areas

using the populations in the other region as the source for the focal area. For each of the above simulations, we used the allele frequency data from the historic Wisconsin populations or pooled samples of both historic and contemporary populations as source populations. This was done to investigate how the allele frequency data from different source populations influenced estimates of N_e and migration.

Results

Estimates of genetic diversity

All six microsatellite loci were polymorphic in each population (Table 1), and all population/locus combinations were in Hardy–Weinberg equilibrium after adjusting the significance (α) level for the number of pairwise comparisons of populations and loci ($n = 60$; $\alpha = 0.001$). Likewise, estimates of F_{IS} (values not shown) for each management area were not significantly different from zero ($P > 0.05$), and no evidence of linkage disequilibrium was apparent after adjusting the significance level for multiple comparisons. A total of 73 alleles were detected across all loci, with the 1951–54 sampling period having more alleles per management area than the contemporary samples from each of the management areas (Table 1). Nine of the 11 alleles that were unique to a single management area were from the 1951–54 sampling period (Mead, one allele; Paul Olson, five alleles; Buena Vista, one allele; and Leola two alleles). The contemporary samples (1998–2000) from Buena Vista and Leola each had a single unique allele.

Levels of genetic diversity at microsatellite loci were lower in the four contemporary management areas than in the same areas 50 years ago (Table 1; see also Bellinger *et al*. 2003). Mean number of alleles and allelic richness were significantly lower in each of the contemporary management areas than in the same areas during 1951–54 (Wilcoxon signed rank tests; P-values < 0.027 and P-values < 0.027, respectively). However, levels of observed heterozygosity were not significantly different (Wilcoxon signed rank tests; $0.10 > P > 0.05$) between the two sampling periods, with the exception of Buena Vista ($P = 0.046$, see also Bellinger *et al*. 2003).

Estimates of mtDNA variability were also lower in contemporary management areas than in the same areas surveyed 50 years ago. Using a modified t-test (Nei 1987), haplotype diversity (h) was significantly ($P < 0.001$) lower in all contemporary populations, ranging from 0.484 in Mead ($t_{17} = 17.4$) to 0.784 in Leola ($t_{16} = 5.5$), while the same management areas 50 years ago had levels of haplotype diversity from 0.860 in Paul Olson to 0.941 in Mead (Table 1). In contrast, nucleotide diversity (π) was similar between time periods and ranged from 0.008 in Paul Olson (1951–54) to 0.012 in Leola (1951) and 0.010 in Mead (1998–00) to 0.016 in Paul Olson (1998–99; Table 1).

Table 1 Measures of genetic diversity (microsatellite and mtDNA) for four greater prairie-chicken management areas in Wisconsin over two time periods (1951–54 to 1998–2000). Standard errors are given in parentheses

		Microsatellite DNA (6 loci)			MtDNA control region			
Population	Sample size (micro/mtDNA)	Mean alleles/locus	Allelic richness†	H_O	No. of haplotypes	Haplotype diversity	Nucleotide diversity	Tajima's D
Wisconsin (1951–54)	125/73	11.8 (2.3)	8.8 (1.7)	0.68 (0.09)	23	0.900 (0.003)	0.010 (0.000)	−0.969
Wisconsin (1998–2000)	181/80	8.5 (1.9)	6.2 (1.2)	0.59 (0.10)	7	0.641 (0.006)	0.013 (0.000)	2.015
WI management areas (1951–54)								
Mead	29/18	8.7 (1.8)	8.4 (1.7)	0.71 (0.11)	11	0.941 (0.008)	0.010 (0.001)	−0.669
Paul Olson	25/19	8.3 (1.6)	8.3 (1.6)	0.67 (0.11)	10	0.860 (0.016)	0.008 (0.000)	−0.041
Buena Vista*	42/19	9.2 (1.5)	8.2 (1.3)	0.71 (0.08)	10	0.889 (0.013)	0.012 (0.001)	−0.427
Leola	29/17	8.5 (1.2)	8.3 (1.2)	0.61 (0.09)	9	0.890 (0.013)	0.012 (0.000)	−0.046
(1998–2000)								
Mead	32/20	6.3 (1.1)	6.0 (1.1)	0.61 (0.10)	3	0.484 (0.113)	0.010 (0.002)	1.283
Paul Olson	33/20	5.1 (1.1)	4.8 (1.0)	0.64 (0.07)	4	0.679 (0.074)	0.016 (0.004)	2.744‡
Buena Vista	87/20	7.0 (1.3)	6.1 (1.1)	0.56 (0.13)	5	0.511 (0.128)	0.013 (0.003)	0.738
Leola	29/20	6.2 (0.8)	6.0 (0.7)	0.57 (0.10)	6	0.784 (0.067)	0.014 (0.003)	1.697

H_O, observed heterozygosity.
*Microsatellite results from Bellinger *et al.* (2003).
†Calculated based on minimum sample size of 25 birds.
‡Tajima's D statistic, $P < 0.05$.

Table 2 Distribution of 25 observed mtDNA control region haplotypes among four Wisconsin management areas over two sampling periods (1951–54 & 1998–2000)

	2 3	9 0	1 1 0 1	1 2 1 6	1 2 7	1 2 8	1 4 0	1 5 0 0	1 6 0	1 6 5	1 6 6	1 7 2	1 7 3	1 7 4	1 8 0	1 8 3	1 8 6	1 9 0	1 9 3	1 9 4	1 9 7	2 0	2 2 6	2 3 0	2 5 1	2 7 0	2 7 8	2 8 6	2 9 7	1998–00 Wisconsin Me	PO	BV	Le	1951–54 Wisconsin Me	PO	BV	Le
Hap1	G	T	A	A	G	G	G	G	T	A	G	G	G	G	G	A	A	G	G	A	A	A	T	G	A	G	T	T	A	14	10	14	8	1		2	2
Hap2	.	.	.	.	.	A	A	A	.	G	.	.	.	.	A	.	G	.	A	.	.	.	.	A	G	A	.	.	.			2	1			2	
Hap3	.	.	.	.	A	A	.	.	.	G	.	.	.	.	A	-	G	.	.	.	.	.	.	A	G	A	.	.	.			1	1				
Hap4	.	.	.	.	.	A	.	A	.	G	.	.	.	.	A	.	G	.	A	.	.	.	.	A	G	A	.	.	.	4	4	1	1	3	2	1	3
Hap5	.	.	G	.	.	A	.	A	.	G	A	A	.	.	A	.	G	.	A	.	.	.	.	A	G	A	.	.	.		5	2					
Hap7	.	.	.	.	.	A	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	G	.	A	G	A	.	.	.	2			5	2	7	6	5
Hap11	.	.	.	.	.	A	.	.	.	G	.	.	A	.	A	.	G	.	A	.	.	.	.	A	G	A	.	.	.						1		
Hap12	.	.	.	.	.	A	.	A	.	G	A	.	.	.	A	.	G	.	A	.	.	.	.	A	G	A	.	.	.							1	1
Hap14	.	.	.	.	.	A	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	A	G	A	C	.	G					1	1		
Hap15	.	.	G	.	.	A	.	A	.	G		.	.	.	A	.	G	.	A	.	.	.	.	A	G	A	.	.	.				4		2	1	
Hap21	.	.	.	.	.	A	.	A	.	.	.	.	.	.	A	.	.	.	A	.	.	G	.	A	G	A	.	.	.						2	1	1
Hap23	.	.	.	.	.	A	.	.	.	G	.	.	.	.	A	-	G	.	A	.	.	.	.	A	G	A	.	.	.					2	1		1
Hap37	A	.	.	.	.	A	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	A	G	A	.	.	.					1			
Hap41	.	.	.	.	.	A	.	.	.	G	.	.	.	.	A	.	.	.	A	G	.	.	.	A	G	A	.	.	G						2		
Hap42	.	.	.	.	.	A	.	A	.	G	.	.	.	.	A	.	G	A	A	G	.	.	.	A	G	A	.	.	.						1		
Hap43	.	.	.	.	.	A	.	.	.	.	.	A	.	.	A	G	.	.	A	.	.	G	.	A	G	A	.	.	.					1		2	
Hap44	.	.	.	.	.	A	.	.	.	G	.	.	.	C	A	.	G	.	A	.	.	.	.	A	G	A	.	.	.								2
Hap45	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.					1			1
Hap46	.	A	.	G	.	A	.	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	.	A	.	A	.	.	.								1
Hap47	.	.	.	.	.	A	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	A	A	G	A	.	.	.					2			
Hap48	.	.	.	.	.	A	.	.	.	G	.	.	.	.	A	.	G	.	A	.	.	.	.	A	G	A	.	.	.					3			
Hap49	.	.	.	.	.	A	.	A	.	G	.	A	.	.	A	.	G	.	A	.	.	.	.	A	G	A	.	.	.					1			
Hap50	.	.	.	.	.	A	.	.	.	.	.	.	.	.	A	.	.	.	A	.	G	.	.	A	G	A	.	.	.						1		
Hap51	.	.	.	.	.	A	.	.	.	G	.	.	A	.	A	.	G	.	A	.	.	.	.	A	G	A	.	C	.						1		
Hap52	.	.	G	.	.	A	.	.	.	G	.	.	.	.	A	-	G	.	A	.	.	.	.	A	G	A	.	.	.						1		

Me, Mead; PO, Paul Olson; BV, Buena Vista; Le, Leola.
The vertical numbers indicate the position of variable nucleotides within the 384-bp sequence. Dots indicate that the same nucleotide is present as in haplotype 1 and a dash (-; see position 183) indicates a deletion. Numbers under each management area indicate the number of individuals with that haplotype. Haplotypes 1–37 correspond to the same haplotypes reported in Johnson *et al*. (2003).

Twenty-five unique mtDNA haplotypes were observed among 153 birds. Of the 384 nucleotides sequenced from the mtDNA control region, 29 nucleotides were variable among individuals: 27 sites were transitions, two sites were transversions, and one site had a single nucleotide deletion (Table 2). Polymorphism within each management area, including the four areas combined for each sampling period, was consistent with neutral expectations (Tajima's D, $P > 0.05$; Table 1), with the exception of the contemporary Paul Olson management area (Tajima's $D = 2.744$, $P = 0.002$). All contemporary Tajima's D estimates were positive values, whereas the estimates for the same management areas from 50 years ago were all negative (Table 1). Positive Tajima D-values indicate that haplotypes at low frequency are absent from the population, while a significant deviation is likely caused by a lack of mutation-drift equilibrium or a violation of neutral assumptions (Wayne & Simonsen 1998).

Analyses of population structure

Microsatellite DNA. There were different patterns of genetic structure between the historic and contemporary periods. During the historic period (1951–54) there was relatively little genetic subdivision (F_{ST}) among the Wisconsin management areas (after adjusting for multiple comparisons). In contrast, there was significant genetic subdivision among the four management areas using contemporary samples (Johnson *et al*. 2003; Table 3). The neighbour-joining phenograms using D_S and D_{CE} show similar topology and both indicate divergence among the four management areas and clustering of the two sampling periods (data not shown), as expected, given the difference in levels of genetic diversity between the two sampling periods. In addition, among contemporary management areas, the microsatellite phenograms suggest divergence between northern (Mead and Paul Olson) and southern (Buena Vista

Table 3 F_{ST} values of microsatellite DNA (below the diagonal) and mtDNA (above the diagonal) population pairwise comparisons

Time period and location	1951–54				1998–2000			
	Mead	Paul Olson	Buena Vista	Leola	Mead	Paul Olson	Buena Vista	Leola
1951–54								
Mead	—	0.018	0.001	−0.017	**0.361**	0.166	0.331	0.092
Paul Olson	0.004	—	−0.007	0.025	0.470	**0.271**	0.443	0.172
Buena Vista	0.008	0.015	—	−0.018	0.347	0.174	**0.326**	0.082
Leola	0.017	0.009	**0.026**	—	0.270	0.114	0.252	0.023
1998–2000								
Mead	**0.047**	0.060	0.038	0.054	—	0.091	−0.033	0.081
Paul Olson	0.068	**0.086**	0.061	0.089	**0.027**	—	0.042	0.009
Buena Vista	0.056	0.064	**0.054**	0.095	**0.053**	**0.087**	—	0.071
Leola	0.070	0.083	0.055	**0.119**	**0.046**	**0.071**	**0.017**	—

A sequential Bonferroni adjustment (Rice 1989) was made to the significance level based on 16 comparisons of interest (i.e. comparisons between contemporary and historic samples from different locations were not tested for significance). Significant values are in bold type.

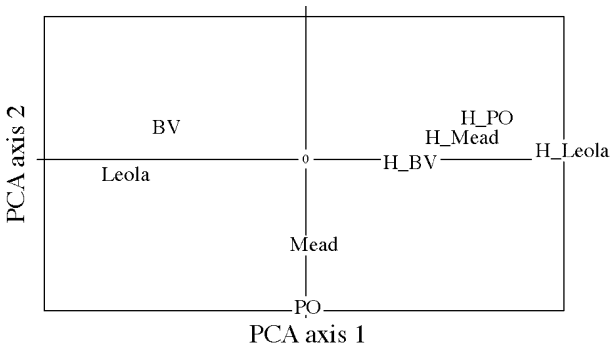

Fig. 2 Principal component analysis (PCA) showing the genetic relationships between the four management areas sampled during two time periods, 1951–54 and 1998–2000 ('H' denotes historic samples, 1951–54; PO, Paul Olson; BV, Buena Vista). PCA axis 1 explains 46.0% ($P = 0.001$) of the variance and PCA axis 2 explains 28.2% ($P = 0.002$). PCA axes 3 and 4 (not shown) explain 9.0% ($P > 0.05$) and 7.2% ($P > 0.05$) of the variance, respectively.

Table 4 Proportion of membership of each predefined management area in each cluster (K)

Management area	Proportion of membership (for $K = 2$)			
	Cluster 1	Cluster 2	Ln likelihood	
(1951–54)				
Mead	0.498	0.502	$K = 1$	−2677.3
Paul Olson	0.501	0.499	$K = 2$	−2711.9
Buena Vista	0.500	0.500	$K = 3$	−2980.8
Leola	0.501	0.499	$K = 4$	−3412.2
(1998–2000)				
Mead	0.382	0.620	$K = 1$	−2865.3
Paul Olson	0.136	0.860	$K = 2$	−2816.9
Buena Vista	0.721	0.280	$K = 3$	−2841.0
Leola	0.646	0.350	$K = 4$	−2867.8

Values presented are from both temporal data sets (1951–54 and 1998–2000) run separately for $K = 2$. Average ln likelihood values from four simulations for $K = 1$ to 4 with both temporal data sets are listed.

and Leola) management areas, while 1951–54 samples do not show this distribution. A principal components analysis revealed two clusters, one the 1951–54 samples and the other the contemporary samples (Fig. 2).

The Bayesian method of analysing population structure also revealed contrasting patterns between the two sampling periods. As above, the (1951–54) samples did not reveal any structure among management areas (Table 4). Note that in Table 4 individuals from each of the management areas in 1951–54 where equally likely to be assigned to cluster 1 or cluster 2 when there were two clusters. In contrast, there was stronger evidence of population structure among contemporary management areas, but it was not as strong as that revealed with conventional F_{ST} analyses. Using data from contemporary management areas, the largest likelihood ratio occurred with two population clusters, rather than one. Most individuals in the south (Buena Vista and Leola) were assigned to cluster 1, while most individuals in the north (Paul Olson and Mead) were assigned to cluster 2 (Table 4).

Mitochondrial DNA. In contrast to the microsatellite results, analysis of mtDNA sequences revealed no significant population subdivision among management areas within either of the two sampling periods (Table 3). However, the range of F_{ST} values was larger for the contemporary samples ($F_{ST} = -0.033–0.091$) than the values from the historic

samples (F_{ST} = −0.007–0.025). The neighbour-joining phenograms using Tamura (1992) distance measures revealed a tighter cluster for the 1951–54 management areas than what was observed with the contemporary samples (data not shown).

Estimates of effective population size

Estimates of N_e assuming migration from a source population were significantly lower than *F*-statistics assuming no migration (Tables 5 and 6). For example, in Paul Olson (assuming 30 generations), N_e was 100 with no migration for the pseudo-likelihood method and 15–32 with migration included, depending on the defined source population (Tables 5 and 6). Both the *F*-statistic and pseudo-likelihood methods produced similar estimates of N_e when migration was not included, and both methods produced larger estimates of N_e when we assumed a larger generation time. In contrast, most estimates of N_e did not change when we changed the generation time in the pseudo-likelihood analyses with migration (Tables 5 and 6).

The use of different defined source populations (i.e. using only historic data or combining historic and contemporary allele frequencies) did not significantly affect the estimate of N_e as there was overlap in the 95% credibility intervals (CI). However, in two cases, the choice of source population produced estimates of N_e that were much larger than the majority of calculations using the pseudo-likelihood approach (Tables 5 and 6). The estimated migration rates (m) between populations varied depending on the defined source population and, in some cases, the 95% CI was quite large (Tables 5 and 6).

Discussion

Using historic (1951–54) and contemporary samples, we documented a significant decline in genetic variability of greater prairie-chicken populations in Wisconsin. The harmonic mean effective population size (N_e) of prairie-chickens required to account for the change in allele frequencies was as low as 15–32 birds per management area, depending on the migration scenario used in the calculations. Using methods that ignore migration, estimates of N_e for each area ranged from 100 to 222 prairie-chickens per area. Despite different absolute values, both the *F*-statistic (no migration) and pseudo-likelihood (with and without migration) methods produced values that are extraordinarily low for all four management areas, and, thus, they raise concerns for the long-term viability of prairie-chicken populations in Wisconsin. These values are not unrealistic given the fragmentation of the Wisconsin populations and a lek mating system that probably reduces the effective number of breeding males. The genetic estimates of N_e from the pseudo-likelihood approach are just 5% (Buena Vista) to 16% (Leola) of the annual census estimates (Table 5).

Few studies have investigated temporal changes in the genetic composition of avian populations. Tarr *et al*. (1998) reported a significant decline in microsatellite variability and an increase in population structure (F_{ST}) following a founder event in the Laysan finch (*Telespiza cantans*). They documented a very low effective population size (N_e = 30) in the contemporary populations using the *F*-statistic methods of Waples (1989); however, they used the allele frequencies in the contemporary Laysan population as the source population, which assumes that the historic allele frequencies have not changed for approximately 20 years since the founding event. This assumption may be valid given that the Laysan population has remained relatively large (5000–20 000 birds) since the founding event (Tarr *et al*. 1998). In other cases such an assumption may not be valid. Furthermore, to our knowledge, this is the first genetically based estimate of N_e in a species with a lek mating system. In prairie-chickens only about 10% of males on a booming ground are thought to breed (Robel 1970), which should produce a lower N_e than that based on census numbers. Unfortunately, in this case we cannot separate the effects on N_e of the lek mating system from the effects caused by the fragmentation of populations.

Effective population size estimates

Given the proximity of management areas (10–60 km) and the observation that dispersal among management areas was a common event at least 30 years ago (Hamerstrom & Hamerstrom 1973), the use of temporal genetic methods that incorporate factors related to migration are justified and should produce more realistic values of N_e than methods that ignore migration (Wang & Whitlock 2003). Furthermore, the methods implemented in the program MLNE do not make any assumptions about mating system or require populations to be at equilibrium, and Wang & Whitlock (2003) have shown through simulation that these methods appear robust and produce more accurate estimates of N_e than methods ignoring migration. In calculating N_e, their model performs particularly well despite having to define an infinitely large source population (Wang & Whitlock 2003). With the exception of two cases (Table 6), we found little variation in estimates of N_e with different combinations of source population (e.g. 1951–54 data only or 1951–54 and 1998–2000 combined data) or different generation times. It is unclear why the two estimates of N_e for Mead and Buena Vista were much larger when we used the 1951–54 Paul Olson (HPO) and Leola (HLeola) data, respectively, as source populations for immigrants. The estimated levels of migration (m) for these two scenarios are quite low (< 0.002–0.085) and the values of N_e are similar to those values obtained assuming no migration.

Table 5 Estimates of effective population size (N_e) and migration rate (m) for each management area in Wisconsin using both *F*-statistic and pseudo-likelihood methods with and without assuming a single source population. Source populations were formed by pooling all samples from the 1951–54 (HWI) or both temporal data sets (HWI+WI), except for the focal population

			Without migration				With migration (source HWI)				With migration (source HWI+WI)			
			F-statistic		Likelihood		Likelihood				Likelihood			
Management area	Census estimate*	No. of generations†	N_e	(95% CI)	N_e	(95% CI)	N_e	(95% CI)	m	(95% CI)	N_e	(95% CI)	m	(95% CI)
Mead	174	20	126	(81.1–180.9)	145	(91.9–242.5)	19	(12.2–35.4)	0.190	(0.077–0.549)	17	(12.7–31.0)	0.991	(0.228–>1.00)
		30	189	(121.6–271.3)	214	(135.0–358.4)	19	(12.5–39.5)	0.194	(0.069–0.547)	17	(12.7–31.1)	0.992	(0.223–>1.00)
		40	252	(162.1–361.8)	282	(178.1–475.1)	19	(12.4–41.7)	0.187	(0.065–0.546)	17	(12.5–31.3)	0.986	(0.222–>1.00)
Paul Olson	181	20	72	(46.7–103.5)	69	(46.1–101.8)	15	(10.2–24.9)	0.120	(0.050–0.249)	15	(10.0–23.0)	0.223	(0.106–0.548)
		30	108	(70.0–155.2)	100	(66.8–149.1)	15	(10.3–28.3)	0.106	(0.043–0.232)	15	(10.0–26.3)	0.201	(0.087–0.529)
		40	145	(93.3–207.0)	131	(87.3–196.8)	17	(10.6–30.7)	0.095	(0.040–0.225)	15	(10.2–28.6)	0.215	(0.077–0.522)
Buena Vista	432	20	137	(89.0–195.3)	150	(103.0–222.9)	25	(17.7–46.7)	0.110	(0.044–0.242)	22	(16.6–36.0)	0.489	(0.175–>1.00)
		30	205	(133.5–293.0)	222	(150.4–329.6)	26	(17.7–49.7)	0.109	(0.043–0.246)	22	(16.2–37.1)	0.485	(0.164–>1.00)
		40	274	(177.9–390.6)	294	(199.2–36.8)	26	(17.5–50.1)	0.113	(0.044–0.246)	23	(16.6–37.3)	0.464	(0.161–>1.00)
Leola	99	20	87	(56.2–124.1)	95	(63.7–144.3)	16	(10.9–25.7)	0.170	(0.079–0.398)	15	(10.6–25.5)	0.621	(0.191–>1.00)
		30	130	(84.3–186.1)	140	(93.1–213.4)	16	(11.1–29.0)	0.157	(0.068–0.381)	15	(11.0–26.1)	0.602	(0.178–>1.00)
		40	174	(112.4–248.1)	184	(122.7–282.1)	16	(11.3–30.7)	0.155	(0.063–0.375)	15	(10.8–26.3)	0.620	(0.175–>1.00)

*Census estimates based on harmonic mean annual counts of males on leks (Anderson & Toepfer 1999) multiplied by two (assuming an equal sex ratio; Schroeder & Robb 1993).
†Number of generations between sampling periods used in the calculations of N_e and m.

Table 6 Estimates of effective population size (N_e) and migration rate (m) for each management area and the combination of areas based on results from Table 4. The choice of source populations are based on additional migration scenarios than those presented in **Table 5**

Management area	Number of generations*	Source population†	Likelihood				Source population†	Likelihood			
			N_e	(95% CI)	m	(95% CI)		N_e	(95% CI)	m	(95% CI)
Mead	20	HPO	64	(22.6–137.9)	0.017	(0.002–0.085)	HPO+PO	22	(13.0–52.1)	0.322	(0.090–>1.00)
	30	HPO	100	(25.6–212.3)	0.010	(<0.001–0.073)	HPO+PO	23	(13.0–60.6)	0.299	(0.076–>1.00)
	40	HPO	137	(28.2–288.0)	0.007	(<0.001–0.063)	HPO+PO	24	(12.9–66.8)	0.278	(0.068–>1.00)
Paul Olson	20	HMead	18	(11.6–37.6)	0.065	(0.017–0.159)	HMead+Mead	17	(11.2–29.2)	0.151	(0.067–0.376)
	30	HMead	20	(11.9–47.0)	0.058	(0.015–0.154)	HMead+Mead	17	(11.3–35.2)	0.142	(0.053–0.349)
	40	HMead	21	(12.1–55.2)	0.056	(0.014–0.153)	HMead+Mead	18	(11.4–40.5)	0.133	(0.045–0.341)
Buena Vista	20	HLeola	89	(40.4–171.4)	0.012	(<0.001–0.044)	HLeola+Leola	31	(19.5–62.4)	0.178	(0.063–0.560)
	30	HLeola	137	(55.2–260.4)	0.007	(<0.001–0.030)	HLeola+Leola	32	(19.8–75.9)	0.168	(0.051–0.549)
	40	HLeola	193	(71.1–352.6)	0.004	(<0.001–0.023)	HLeola+Leola	32	(19.8–86.4)	0.164	(0.045–0.546)
Leola	20	HBV	21	(13.1–39.3)	0.146	(0.056–0.405)	HBV+BV	21	(13.0–46.7)	0.436	(0.142–>1.00)
	30	HBV	25	(13.4–49.3)	0.116	(0.044–0.379)	HBV+BV	21	(12.9–55.5)	0.458	(0.111–>1.00)
	40	HBV	24	(13.7–57.5)	0.115	(0.038–0.367)	HBV+BV	21	(13.1–62.0)	0.488	(0.095–>1.00)
Mead + PO	20	HBV+HLeola	41	(26.7–71.9)	0.057	(0.021–0.127)	HBV+HLeola+BV+Leola	39	(25.6–63.4)	0.106	(0.051–0.212)
	30	HBV+HLeola	46	(28.2–91.0)	0.050	(0.017–0.113)	HBV+HLeola+BV+Leola	43	(26.9–79.3)	0.088	(0.040–0.189)
	40	HBV+HLeola	50	(28.9–108.8)	0.045	(0.015–0.107)	HBV+HLeola+BV+Leola	47	(27.9–94.4)	0.079	(0.033–0.178)
BV + Leola	20	HMead+HPO	64	(37.8–127.6)	0.031	(0.008–0.075)	HMead+HPO+Mead+PO	47	(31.0–84.3)	0.089	(0.036–0.193)
	30	HMead+HPO	87	(39.8–184.2)	0.023	(0.005–0.070)	HMead+HPO+Mead+PO	48	(31.9–103.8)	0.089	(0.030–0.185)
	40	HMead+HPO	102	(40.3–246.3)	0.019	(0.004–0.070)	HMead+HPO+Mead+PO	55	(31.9–118.5)	0.073	(0.026–0.182)

*Number of generations between sampling periods used in the calculations of N_e and m.
†Source populations used for simulations (H--, 1951–54 data; PO, Paul Olson; BV, Buena Vista).

Our estimates of migration (m) also changed significantly as different source populations were used in the simulations (Tables 5 and 6). Wang & Whitlock (2003) specified that the migration sources must be known a *priori* for their method to estimate N_e accurately. However, defining a source population is problematic in cases such as ours where the frequency of immigrants from possible source populations has changed during the sampling period.

In previous studies, allele frequencies from historic and contemporary populations have been combined to serve as the source population (Østergaard *et al*. 2003; Wang & Whitlock 2003). In this study, when we pooled the allele frequencies from historic and contemporary samples for the source populations, our estimates of m varied considerably among management areas. Furthermore, they were higher than when the 1951–54 data were used as the source populations and the 95% CI were quite large. Prairie-chickens are known to migrate between management areas in Wisconsin; although, the rate of migration has decreased significantly over the last 30 years (Hamerstrom & Hamerstrom 1973; Halfmann 2002; J. Toepfer, unpublished data). As a consequence, our estimates of m based on pooling the two time periods may not be valid, and the estimates based on the 1951–54 data may be more accurate. In addition, when the calculations were conducted with the management areas as two groups (i.e. Mead/Paul Olson and Buena Vista/Leola) and each group was the source population for the other group (Table 6), the values of N_e were consistent with the values obtained using each management area separately. Furthermore, estimates of m seemed realistic given current dispersal patterns in this population.

The results of this analysis could potentially be influenced by sex-biased dispersal and the sex ratio of the sampled individuals. Female prairie-chickens disperse further than males and the majority of females mate in their first year (Schroeder & Robb 1993; Halfmann 2002) while only 10% of males appear to breed (Robel 1970). Thus, a sample with mostly females could lead to greater estimates of N_e and m between the two sampling periods. However, with the exception of the contemporary Buena Vista, we had approximately equal numbers of males and females in our samples and, thus, such bias is unlikely to have affected our results. On the other hand, it may influence comparisons among different studies, especially those based on migration rate (m). It would be worthwhile to conduct additional simulations with varying migration rates (i.e. subdivision and sex-biased dispersal), population sizes, and generation times to investigate how this method performs in conditions similar to our study. Currently, calculating N_e and m based on a finite demes model as discussed in Wang & Whitlock (2003) is not available (J. Wang, personal communication).

Population bottlenecks could also produce biases in our estimates of N_e, especially those obtained from F-statistics. A number of studies using F-statistics have documented an overestimation of N_e when alleles are lost as the bottleneck duration increases (Richards & Leberg 1996; Luikart *et al*. 1998) and their 95% confidence intervals tend to be wider and biased upward (Luikart *et al*. 1999; Berthier *et al*. 2002). Likelihood methods appear to perform better than F-statistics when there has been a population bottleneck during the sampling period (Berthier *et al*. 2002); unfortunately the pseudo-likelihood method of Wang & Whitlock (2003), which we used in this study, has not been specifically tested with a simulated bottleneck. More research is needed to investigate potential biases in estimates of N_e; however, from a management perspective, the bias caused by bottlenecks is likely to result in overestimates, and these estimates are already low enough to cause concern about the viability of Wisconsin populations.

Increased population subdivision and reduced genetic variability

Populations of prairie-chickens in Wisconsin have lost genetic variation as a consequence of genetic drift (Bellinger *et al*. 2003; Johnson *et al*. 2003). The lek mating system and long-term isolation of the management areas presumably decreased N_e leading to genetic drift (Whitlock & Barton 1997; Nunney 1999; Wang & Caballero 1999). The isolation of management areas is shown in the microsatellite neighbour-joining phenogram and the principal components analysis using both temporal data sets. The two methods suggests a northern and southern division within contemporary Wisconsin that was not present 50 years ago. Furthermore, the management areas cluster within the same sampling period, which suggests that the loss of genetic variation has been relatively similar in all four areas.

Recent studies have documented artificial inflation of values of population differentiation when genetic drift was strong (i.e. after a bottleneck). Consequently, significant F_{ST} and related measures may not accurately represent population differentiation (Chakraborty & Nei 1977; Hedrick 1999; Goodman *et al*. 2001). Using a model-based clustering algorithm implemented in the program STRUCTURE, we were able to document an increase in assignment probability to more than one cluster using the two temporal data sets. The contemporary data set suggests the presence of two clusters, whereas the 1951–54 samples suggest that there was one cluster (i.e. one panmictic population). Thus, the change in genetic structure has occurred in Wisconsin within the last 50 years. In contrast, no structure was observed between different states (i.e. Kansas, Missouri, Nebraska, and Minnesota) that are geographically isolated over much greater distances than the management areas within Wisconsin (data from Johnson *et al*. 2003). When contemporary Wisconsin was included in this data set, the Wisconsin samples clustered with high frequency (> 85%)

separate from the other states (data not shown). However, this cluster was not observed when we replaced the contemporary Wisconsin samples with the 1951–54 samples from Wisconsin.

In contrast to the microsatellite results, the mitochondrial data did not show significant subdivision (F_{ST}) between management areas, and the neighbour-joining phenograms do not indicate geographical structuring. This difference between genetic markers may be a result of factors associated with the effective size of the marker in relation to demographic processes (i.e. mating behaviour or dispersal bias; Piertney *et al*. 2000) and its power to detect population structure relative to multiple microsatellite loci. These issues are described in more detail in Johnson *et al*. (2003) using the contemporary samples from this study and additional populations from other states. Regardless of this, the mitochondrial data show a significant reduction in haplotype diversity (h) over the last 50 years, and similar to the microsatellite results, the mtDNA neighbour-joining phenograms also show temporal clustering of management areas. These results, in combination with positive Tajima's D-values, further suggest that the N_e of prairie-chicken populations in Wisconsin has been reduced to low enough levels where the effects of genetic drift are significant.

Maintenance of viable populations

Our results clearly demonstrate a change in the genetic structure of prairie-chicken populations within a relatively short period of time (i.e. < 50 years), and these changes coincide with changes in the surrounding landscape. The rate at which genetic drift can influence changes in allele frequencies depends on the N_e, which is strongly affected by population structure (Whitlock & Barton 1997; Wang & Caballero 1999). A reduction in gene flow between northern and southern populations of prairie-chickens in Wisconsin has reduced the N_e in each management area and led to genetic drift. By incorporating methods that include both migration and drift and their associated influences on allele frequency change over time, we were able to document low levels of N_e that are much lower than levels currently associated with viable populations (N_e = 500–5000; Franklin 1980; Lande 1995; Franklin & Frankham 1998; Lynch & Lande 1998).

Dispersal appears to be an important demographic factor in maintaining genetically viable populations of this species, and the minimal levels of migration needed to maintain a viable population may be much larger than the one-migrant-per-generation rule (Mills & Allendorf 1996; Vucetich & Waite 2001). In fact, in spite of extensive fragmentation and isolation of populations throughout their current range, a number of larger populations (> 2000 birds) in Kansas, Nebraska and Minnesota still possess evidence of historical connections through isolation-by-distance (Johnson *et al*. 2003). Through anthropogenic habitat deterioration and fragmentation, a significant proportion of the historic distribution of greater prairie-chickens has been reduced by local extinctions. The remaining isolated populations vary in size, but this study shows that internal fragmentation through the loss or reduction of dispersal capabilities among subpopulations may have dramatic impacts on levels of genetic variability and, thus, the evolutionary potential of surviving populations (Templeton *et al*. 2001; Caizergues *et al*. 2003; Reed & Frankham 2003; Stockwell *et al*. 2003). Therefore, maintaining connections between local populations and preserving habitat throughout the range appears to be the primary challenge for conserving this species (Reed 1999; Gibbs 2001; Doherty *et al*. 2003).

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The authors are studying the population genetics of greater prairie-chickens throughout their range. This study formed part of the PhD work of Jeff Johnson, who is currently working on various projects concerning the conservation of diurnal raptors. Renee Bellinger is interested in conservation genetics and is currently working as a Research Assistant at Oregon State University. Dr John Toepfer heads the research program of the Society of *Tympanuchus cupido pinnatus*, a nonprofit society dedicated to the preservation of greater prairie-chickens. Peter Dunn is an Assistant Professor interested in avian mating systems and conservation genetics.
