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Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie-chickens

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Abstract

Greater prairie-chickens (*Tympanuchus cupido pinnatus*) were once found throughout the tallgrass prairie of midwestern North America but over the last century these prairies have been lost or fragmented by human land use. As a consequence, many current populations of prairie-chickens have become isolated and small. This fragmentation of populations is expected to lead to reductions in genetic variation as a result of random genetic drift and a decrease in gene flow. As expected, we found that genetic variation at both microsatellite DNA and mitochondrial DNA (mtDNA) markers was reduced in smaller populations, particularly in Wisconsin. There was relatively little range-wide geographical structure (F_{ST}) when we examined mtDNA haplotypes but there was a significant positive relationship between genetic (F_{ST}) and geographical distance (isolation by distance). In contrast, microsatellite DNA loci revealed significant geographical structure (F_{ST}) and a weak effect of isolation by distance throughout the range. These patterns were much stronger when populations with reduced levels of genetic variability (Wisconsin) were removed from the analyses. This suggests that the effects of genetic drift were stronger than gene flow at microsatellite loci, whereas these forces were in range-wide equilibrium at mtDNA markers. These differences between the two molecular markers may be explained by a larger effective population size (N_e) for mtDNA, which is expected in species such as prairie-chickens that have female-biased dispersal and high levels of polygyny. Our results suggest that historic populations of prairie-chickens were once interconnected by gene flow but current populations are now isolated. Thus, maintaining gene flow may be important for the long-term persistence of prairie-chicken populations.

Keywords: conservation, fragmentation, gene flow, grouse, isolation by distance, population genetic structure, sex-biased dispersal

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Introduction

Loss of suitable habitat and the subsequent fragmentation of populations are recognized as important factors in species extinction, as they may result in smaller and more isolated populations as well as reduced genetic diversity (Frankham 1996; Young & Clarke 2000; Frankham *et al*. 2002). The relative loss of genetic diversity through genetic drift will depend on the effective population size (N_e) and the level of gene flow (isolation). Few studies in the wild have examined multiple populations at various stages of fragmentation to investigate how levels of isolation affect gene flow and genetic drift (Saccheri *et al*. 1998; Hutchison & Templeton 1999; Segelbacher & Storch 2002).

Gene flow is expected to be greater when populations are closer, and, as a consequence, nearby populations should be more similar at neutral loci. This relationship is referred to as isolation by distance, and it assumes a stepping-stone model of gene flow and sufficient time for populations to reach equilibrium conditions (Wright 1943; Kimura & Weiss 1964). However, levels of gene flow are

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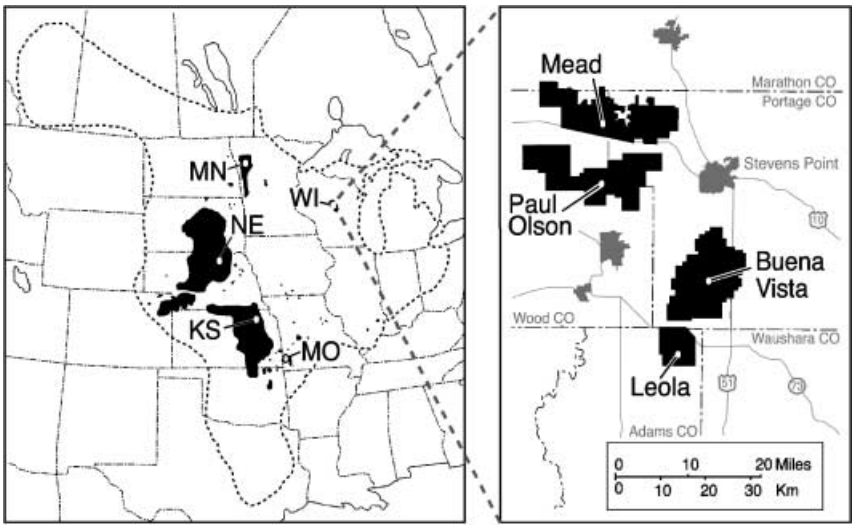

Fig. 1 Historic (dashed line & lower figure inset) and contemporary distribution of greater prairie-chickens in North America. The sample locations (Nebraska, NE; Kansas, KS; Minnesota, MN; Missouri, MO; and Wisconsin, WI) and Wisconsin's four management areas (Mead, Paul Olson, Buena Vista and Leola) are shown by white circles.

not only dependent on the distance between populations but also on the nature of the surrounding landscape between populations (Whitlock & Barton 1997; Gibbs 2001; Templeton *et al*. 2001; Brooker & Brooker 2002). Populations that have recently become fragmented are vulnerable to violating a number of assumptions related to population genetic analyses. In particular, measuring gene flow using *F*-statistics can sometimes be misleading because populations that have recently become fragmented are less likely to be in regional or migration/drift equilibrium (Bossart & Prowell 1998; Hutchison & Templeton 1999; Whitlock & McCauley 1999).

By incorporating analyses of isolation by distance, Hutchison & Templeton (1999) proposed a method to detect nonequilibrium conditions in sets of populations. Under equilibrium conditions, gene flow offsets the effects of genetic drift and, thus, pairwise F_{ST} estimates will increase with geographical distance. In contrast, when genetic drift is stronger than gene flow and populations are no longer in equilibrium, such as in cases of fragmentation, measures of F_{ST} should be more variable, resulting in a lack of relationship between F_{ST} estimates and geographical distances (Hutchison & Templeton 1999). Thus, small isolated populations should not conform to the isolation-by-distance model, because they are unlikely to be in equilibrium between genetic drift and gene flow. In contrast, when isolation by distance is observed among populations, genetic drift and gene flow have probably reached equilibrium conditions. It is important to recognize that the occurrence of isolation by distance may reflect historic rather than current levels of gene flow, especially if isolation is recent and N_e is large enough to reduce the effects of drift (Bossart & Prowell 1998).

A number of ecological and demographic properties can also affect the relationship between genetic differentiation and distance (Bossart & Prowell 1998; Hedrick 1999; Hutchison & Templeton 1999; Whitlock & McCauley 1999). For example, demographic factors, such as sex-biased dispersal and high levels of polygyny, may affect estimates of population structure differently depending on the genetic marker used in the analysis (i.e. uniparental vs. diparental inheritance; Chesser & Baker 1996). Therefore, genetic analyses incorporating isolation by distance can be useful for identifying populations that are not under regional equilibrium, and, thereby, stimulate further investigation of how population structure has been affected by isolation, gene flow and genetic drift.

In this study, we examined the genetic effects of isolation and fragmentation on populations of greater prairie-chickens (*Tympanuchus cupido pinnatus*). Populations of greater prairie-chickens once existed in large blocks of open grassland throughout midwestern North America (Fig. 1; Aldrich 1963; Schroeder & Robb 1993; Johnsgard 2002). Today, these large open grasslands are one of the most endangered and fragmented habitats in North America because of loss of habitat and changes in agricultural practices (Westemeier 1971; Anderson & Toepfer 1999; Robbins *et al*. 2002). In addition, potential barriers to dispersal between isolated populations are increasing in the form of anthropogenic disturbance and the succession of hardwood stands (Hamerstrom *et al*. 1957; Westemeier 1971; Niemuth 2000). As a consequence, the greater prairie-chicken is threatened with extinction throughout much of its range and a number of isolated populations have either been extirpated or reduced to fewer than 2000 birds (Schroeder & Robb 1993; Svedarsky *et al*. 2000).

There is evidence that small populations of prairie-chickens have lost genetic variation (Bouzat *et al*. 1998a, 1998b; Bellinger *et al*. 2003), and this loss may be contributing to a decrease in reproductive success (Westemeier *et al*. 1998). This study extends previous research by examining a larger number of isolated greater prairie-chicken populations

Table 1 Census size, area sampled and genetic variation in populations of greater prairie-chickens

			Microsatellite DNA (six loci)					Mitochondrial DNA control region						
										Haplotype diversity		Nucleotide diversity		
Population	Census size (abundance)†	Area sampled (km)	*N*	Mean alleles /locus	Allelic richness	H_O	H_E	*N*	*N* haplotypes	*h*	SE	Π	SE	Tajima's *D*
Kansas	> 100 000	79.3	47	10.3	9.4	0.738	0.763	20	11	0.858	0.065	0.010	0.002	–0.941
Nebraska	> 100 000	54.1	48	10.5	9.2	0.701	0.731	20	15	0.968	0.028	0.009	0.002	–1.049
Minnesota	1 900	77.9	45	9.5	8.5	0.693	0.729	20	9	0.840	0.061	0.009	0.002	–1.387
Missouri	1 000	51.2	20	7.7	7.7	0.750	0.709	20	9	0.866	0.048	0.012	0.003	–0.218
Wisconsin (total)	1 200	60.0	181	8.5	6.5	0.586	0.595	80	7	0.641	0.054	0.013	0.003	2.015
WI populations														
Mead	120	30.0	32	6.3	6.2	0.614	0.598	20	3	0.484	0.025	0.010	0.002	1.283
Paul Olson	200	30.0	33	5.1	5.0	0.641	0.597	20	4	0.679	0.017	0.016	0.004	2.744*
Buena Vista	650	20.0	87	7.0	6.2	0.557	0.560	20	5	0.511	0.029	0.013	0.003	0.738
Leola	200	10.0	29	6.2	6.2	0.574	0.560	20	6	0.784	0.014	0.014	0.003	1.697

*$P < 0.05$.
†Anderson & Toepfer (1999), Svedarsky *et al*. (1999a).

with nuclear and mitochondrial markers [microsatellites and mitochondrial DNA (mtDNA) control region sequence data]. These analyses allowed us to examine how genetic variation is affected by isolation and drift in populations at various sizes and levels of isolation.

Materials and methods

Tissue collection and DNA extractions

Blood and feather samples of adult prairie-chickens were collected from one or two adjacent counties in Missouri (1999, Barton and Dade Counties), Kansas (1999, Wabaunsee County), Nebraska (1997–98, Garfield County), and Minnesota (1999, Norman County) to control for any effects of geographical variation within populations. In Wisconsin, samples were taken from all four of the management areas (1997–2000; Mead, Paul Olson, Buena Vista and Leola) that contain remnant populations of prairie-chickens. These areas occupy four adjacent counties (Fig. 1). For analysis, each location (including the four management units in Wisconsin) was initially considered to be a separate population ($n = 8$). Only adult birds were sampled to reduce the potential for sampling related individuals, and individuals were only sampled once when populations were sampled over multiple years (identified by leg bands). Approximately equal proportions of males and females were sampled in each population, with the exception of Missouri and Buena Vista in Wisconsin where 94% of sampled birds were male. Blood samples were stored in Queen's Lysis buffer (Seutin *et al*. 1991) at 4 °C. DNA was extracted from blood with a 5-M salt solution (Miller *et al*. 1988) and diluted to approximately 50 ng/μL prior to polymerase chain reaction (PCR). Feather samples were used in addition to blood samples from the Leola ($n = 11$) and Paul Olson ($n = 14$) areas in Wisconsin (see Bellinger *et al*. 2003 for details of DNA extraction from feathers).

Genotyping and sequencing

Six microsatellite loci originally developed for domestic chicken (ADL44, ADL146 and ADL230;Bouzat et al. 1998a) and red grouse (*Lagopus lagopus*; LLST1, LLSD4 and LLSD9; Piertney & Dallas 1997) were used for the microsatellite analysis. Microsatellite procedures were carried out as described in Bellinger et al. (2003). For the mtDNA analysis, 20 individuals were sequenced from each population that were also used in the microsatellite analysis ($n = 160$, Table 1). Individuals were sequenced at the highly variable 5′ region I of the control region (approx. 400 base pairs) using primers 16775L (Quinn 1992) and 521H (Quinn & Wilson 1993). Four individuals were sequenced using both blood and feather samples to confirm amplification of mtDNA and the absence of nuclear sequences of mitochondrial origin (Numt; Sorenson & Quinn 1998), and confirmed with mtDNA sequences from Lucchini *et al*. (2001) and Drovetski (2002). Control region amplification was performed in 50 μL reaction volumes using 50 ng total genomic DNA, containing 0.5 μM of each primer, 1.25 mM $MgCl_2$, 1× buffer II solution (Applied Biosystems), 0.4 mM dNTPs and 2.5 U Amplitaq Gold (Applied Biosystems). PCR was performed in an MJ Research thermal cycler under the following conditions: one denaturing cycle at 94 °C for 2 min, followed by 35 cycles at 94 °C for 40 s, 55 °C for

1 min and 72 °C for 1 min 40 s. This was then followed by an extension step at 72 °C for 7 min. PCR products were run on 2% low-melt Tris–acetate EDTA buffer (TAE) agarose gels containing ethidium bromide, excised and purified using a Wizard® PCR purification kit (Promega). Samples were sequenced with an Abi Prism™ 373 automated sequencer (Perkin Elmer) using a BigDye Terminator Cycle Sequencing Kit (Perkin Elmer), aligned using SEQUENCHER™ 4.1, and verified for accuracy. Unique sequences are in GenBank (accession numbers AY273829–AY273868).

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 version 2.0 (Schneider *et al*. 2000). Sequential Bonferroni corrections were applied to correct for multiple simultaneous comparisons (Rice 1989). Mean heterozygosity and mean number of alleles per locus (allelic diversity) were calculated using the program GDA (Lewis & Zaykin 2000). Measures of allelic richness were included to investigate differences in the number of alleles among populations independent of sample size (Leberg 2002). Allelic richness was calculated as described by Petit *et al*. (1998) using the program FSTAT version 2.9.3 (Goudet 1995). Differences between populations in mean number of alleles, allelic richness and observed heterozygosity were tested using a Friedman test (Lehmann 1975) with a Monte Carlo procedure (10 000 permutations) in the program STATXACT 4.0.1 (CYTEL Software Corp.). To examine the relationship between genetic diversity and census size, estimates of genetic diversity were regressed on the number of birds in each population, as estimated by state management agencies (Svedarsky *et al*. 1999a). These regression analyses included the maximum distance between sample locations (within a population) to account for differences in area sampled at each location (mean = 44.1 km; Table 1). Note that we intentionally sampled in a relatively small area in each state, even within large populations such as Kansas, to avoid biases from sampling potentially different subpopulations. Thus, these analyses test whether the genetic estimates measured at a particular location are influenced by the size of the surrounding population. We also estimated F_{IS}, as $1 - (H_O/H_E)$, where H_O and H_E are the observed and expected heterozygosities, respectively. The significance of F_{IS} between populations was tested by permutation (10 000) using FSTAT.

To investigate the effects of genetic drift on mtDNA diversity, mitochondrial haplotype diversity, nucleotide diversity and Tajima's *D* (Tajima 1989) were estimated for each population using ARLEQUIN version 2.0 (Schneider *et al*. 2000). Haplotype diversity (*h*) was the probability that two randomly chosen individuals have different haplotypes, and nucleotide diversity (π) was the average pairwise nucleotide difference between individuals within samples (Nei 1987). Tajima's *D* is expected to be zero when mating is random and populations have reached equilibrium; large significant values of *D* (negative or positive) indicate a deviation from neutrality (Tajima 1989). However, interpretation of observed departures from neutrality are only valid if the assumptions of neutral mutation-drift balance are satisfied (Wayne & Simonsen 1998; Gerber *et al*. 2001).

To investigate population genetic structure, both microsatellite pairwise F_{ST} (Weir & Cockerham 1984) and R_{ST} (Slatkin 1995) were calculated as implemented in ARLEQUIN version 2.0 (Schneider *et al*. 2000) and RSTCALC (Goodman 1997), respectively. Our results with F_{ST} and R_{ST} were qualitatively similar. We report only F_{ST} values because they appear to perform better than R_{ST} when populations are both weakly structured and the mutation rate is relatively low over short time frames (Gaggiotti *et al*. 1999; Lugon-Moulin *et al*. 1999; Bolloux & Goudet 2002). Mitochondrial DNA pairwise F_{ST} values were calculated using a distance matrix between haplotypes following Tamura's (1992) model as implemented in ARLEQUIN version 2.0 (Schneider *et al*. 2000). Pairwise F_{ST} calculations for mtDNA were conducted both with and without weighting the haplotype frequencies based on sequence information of evolutionary relationships among haplotypes as described below. Differences between populations were tested using permutations (10 000) among populations with Fisher's exact test (Fisher 1954). Additional hierarchical analyses of variation among locations were conducted using analysis of molecular variance (AMOVA) as described by Excoffier *et al*. (1992). Relationships between genetic differentiation and geographical distance separating populations (isolation by distance) were examined using ISOLDE in the program GENEPOP version 3.2a (Raymond & Rousset 1995). Isolation by distance was tested with a Mantel procedure (10 000 permutations; Mantel 1967) by correlating F_{ST} with the natural logarithm of the straight-line distance (km) between pairs of populations.

Genetic distances between sampled populations were estimated separately for microsatellite and mtDNA data. Neighbour-joining phenograms were constructed for the microsatellite data using a pairwise chord distance matrix (D_{CE}; Cavalli-Sforza & Edwards 1967). The D_{CE} matrix was calculated using the program PHYLIP version 3.57c (Felsenstein 1995). For the mtDNA population analysis, neighbour-joining phenograms were constructed using haplotype distances and frequencies among populations (Tamura 1992). Distance analyses among unique mtDNA sequences were calculated using neighbour-joining analyses in PAUP* 4.0b1 (Swofford 1998) with the substitution model HKY + Γ correction for rate variation among sites (Hasegawa *et al*. 1985). The neighbour-joining analyses

used a transition/transversion ratio of 55.662 and a gamma distribution of 0.0123, which were estimated from the sequence data using the program MODELTEST 3.06 (Posada & Crandall 1998).

Results

Estimates of genetic diversity

All six microsatellite loci in the eight populations were polymorphic (Table 1), and all of the 48 population/locus combinations were in Hardy–Weinberg equilibrium after adjusting the significance (alpha) level for the number of pairwise comparisons of populations and loci ($n = 48$; alpha = 0.001). There was also no evidence of linkage disequilibrium after adjusting the significance level for multiple comparisons. A total of 83 alleles were detected across all loci, ranging from a maximum of 63 alleles detected in Nebraska, to a minimum of 31 alleles detected in the Paul Olson population in Wisconsin. Twelve alleles were unique to a single population (Kansas, three alleles; Nebraska, three alleles; Missouri, three alleles; Minnesota, one allele and Leola, two alleles). Estimates of F_{IS} (values not shown) for each population were not significantly different from zero ($P > 0.05$), which suggests that heterozygosity was not reduced relative to a random-mating population with the same allele frequencies (Hartl & Clark 1997).

Genetic diversity at microsatellite loci was lower in the Wisconsin samples than in samples from Minnesota, Kansas, Nebraska and Missouri (Table 1). Mean number of alleles, allelic richness and H_O were lower in the Wisconsin subpopulations compared to the other populations surveyed (10 000 permutations; $P < 0.001$, $P = 0.031$ and $P = 0.033$, respectively; Table 1). Allelic diversity was related positively to census estimates of population size (Table 1). Mean number of alleles was related positively to population size ($F_{1,5} = 11.97$, $P = 0.018$) in a multiple regression that also included area sampled ($F_{1,5} = 3.27$, $P = 0.13$) as a predictor (overall model $R^2 = 0.908$), and allelic richness was also related positively to population size ($F_{1,5} = 7.86$, $P = 0.038$) in a multiple regression that included area sampled ($F_{1,5} = 3.72$, $P = 0.11$) as a predictor (overall model $R^2 = 0.889$). However, the relationship between observed heterozygosity and population size was not significant ($F_{1,5} = 0.05$, $P = 0.84$) when area sampled ($F_{1,5} = 5.65$, $P = 0.063$) was included as a predictor (overall model $R^2 = 0.73$). Given that 73% of the variation was explained by this relationship, these results suggest that estimates of observed heterozygosity increase with the size of the area sampled.

Estimates of mtDNA variability were also lower in Wisconsin prairie-chicken populations than in populations surveyed in Minnesota, Kansas, Nebraska and Missouri. Forty unique haplotypes were observed among 160 birds in eight populations. Of 384 nucleotides scored from the mtDNA control region, 40 nucleotides were variable among individuals: 38 sites were transition substitutions, one of which was also a transversion, and two sites had a single nucleotide deletion (Table 2). Polymorphism within each population was consistent with neutral expectations (Tajima's $D = -1.387$–1.697; $P > 0.05$; Table 1), except for Paul Olson in Wisconsin (2.744) which was significantly positive ($P = 0.002$). Haplotype diversity (h) was high among populations outside Wisconsin, ranging from 0.847 in Minnesota to 0.968 in Nebraska, whereas within Wisconsin subpopulations, haplotype diversity was lower, ranging from 0.484 in Mead to 0.784 in Leola (Table 1). Nucleotide diversity (π) ranged from 0.009 in Nebraska and Minnesota to 0.016 in Paul Olson (Table 1).

Mitochondrial DNA haplotype diversity was related positively to population size estimates (ln-transformed) for each population ($F_{1,5} = 11.98$, $P = 0.018$) in a multiple regression that also included area sampled ($F_{1,5} = 0.01$, $P = 0.94$) as a predictor (overall model $R^2 = 0.835$). A large number of haplotypes (87.5%; Table 2) were found in only one population (three of which were only in Wisconsin: haplotypes 2, 3 and 5). A single haplotype (1) was observed in high frequency (58%) within Wisconsin, and this haplotype was only observed once outside Wisconsin (Table 2).

Analyses of population structure

Microsatellites. There was significant population subdivision at microsatellite loci (Table 3). Within Wisconsin, only Buena Vista and Leola did not show significant population subdivision ($F_{ST} = 0.017$, $P = 0.0013$) after adjusting for multiple comparisons ($P < 0.001$ indicates statistical significance). When Buena Vista and Leola were combined as one population significant differences in allele frequency distributions were still observed when compared with the Mead and Paul Olson populations ($F_{ST} = 0.048$ and 0.080, $P < 0.001$, respectively). All pairwise population comparisons between states were also significant, except those between Nebraska and Minnesota and between Nebraska and Kansas (Table 3). Within Wisconsin, the phenogram also suggests that there were two northern (Mead and Paul Olson) and one southern (Buena Vista and Leola) population (Fig. 2a). In fact, some of the genetic differences (F_{ST}) between populations within Wisconsin were larger than differences between populations from different states (Table 3, Fig. 3a). This suggests that at microsatellite loci there were genetic differences between five of our sample locations: Missouri, Mead, Paul Olson, Buena Vista/Leola and one large population composed of Kansas, Nebraska and Minnesota (Table 3). These population groupings are indicated on the neighbour-joining phenogram (Fig. 2a).

Mitochondrial DNA. In contrast, analysis of mtDNA sequences revealed no significant population subdivision, except

Table 2 Distribution of 40 observed mtDNA control region haplotypes from a sample of 160 greater prairie-chickens from eight populations (n = 20 for each population). The vertical numbers indicate the positions of variable nucleotides within the 384 bp sequence. Dots indicate the same nucleotide is present as in haplotype 1 and a dash (–; see position 183) indicates a deletion. Numbers under each population indicate the number of individuals with that haplotype

	1111111 2381112224 3490166780	1111111111 5566677777 0105602345	1111111122 8888899901 0345613692	2222222223 2235577896 0201508371	Populations							
					KS	MN	NE	MO	Mead	Paul Olson	Buena Vista	Leola
H1	GGGAAAAGGG	GGTAGAGGGA	GAGGAAGGAG	AGGATGTTAT	1				14	10	14	8
H2	.....AA	A..G.....	A...G.A...	..AG.A....							2	1
H3	.....AA.	...G.....	A-..G.....	..AG.A....							1	1
H4	.....A.	A..G.....	A...G.A...	..AG.A....	7	4	3	2	4	4	1	1
H5	....G...A.	A..GA.A...	A...G.A...	..AG.A....						5	2	
H6	.....A.	A..G..A...	A.-.G.A...	..AG.A....	1							
H7	.....A.	.....	A.....A...	G.AG.A....	4	7	3	7	2			5
H8	.....G..A.	A..G.....	A...G.A...	..AG.A....	1							
H9	.....A.	A..GA.....	A...G.A.G.	..AG.A....	1			1				
H10	.....	.....	.....	...G.A....	1							
H11	.....A.	...G...A..	A...G.A...	..AG.A....	1							
H12	.....A.	A..GA.....	A...G.A...	..AG.A....	1							
H13	.....A.	.....	A.....A...	..AG.....C	1							
H14	.....A.	.....	A.....A...	..AG.AC.G.	1							
H15	....G...A.	A..G.....	A...G.A...	..AG.A....			1			1		4
H16	.....A.	.....A.	A.....A...	..AG.A..G.		1						
H17	.....A.	.A.....	A.....A...	..AG.A....		2						
H18	.....A.	...G.G....	A...G.A...	..AG.A....		1						
H19	.....G.	..C....A..	.....	.....		1						
H20	.....G.A.	.....	A.....A.G.	G.AG.A....		2						
H21	.....A.	A.....	A.....A...	G.AG.A....		1						
H22	.A.G....A.	.....	A.....A...	..AG.A....		1						
H23	.....A.	...G.....	A-..G.A...	..AGCA....				1				
H24	.....A.	...G.....	A-..G.AA..	..AGCA....				1				
H25	.....A.	...G.....	.....	.....				1				
H26	.....A.	A..G..T...	A...G.A...	.AAG.A....				4				
H27	.....	.....	.....A	.....A....				1				
H28	.....A.	A..GA.....	A...G.A...	..AG.A...C				2				
H29	.....A.	.....	A.....A...	..AG.A..G.			1					
H30	.....A.	.....A...	A.....A...	G.AG.A....			1					
H31	.....A.	...G.....	A.....A...	G.AG.A....			1					
H32	.....A.	.....	A.....A...	G.AG.ACC..			1					
H33	.....A.	..C.....	A....GA...	..AG.A..G.			1					
H34	...G....A.	.....	A.....A...	..AG.A....			1					
H35	.....A.	A.CG.....	A...G.A...	..AG.A....			1					
H36	.....A.	.....	A.....A...	..AG.A....			1					
H37	A.....A.	.....	A.....A...	..AG.A....			2					
H38	..A.....A.	A..G.....G	A...G.A...	..AG.A....			1					
H39	.....A.	.....	A..A..A...	G.AG..A...			1					
H40	.....A.	A.....	A.....A...	..A...A...			1					

between three of the Wisconsin populations and other states (Table 3). The fourth population in Wisconsin (Leola) did not differ from other states (F_{ST} = 0.123–0.160). These results did not change when transition/transversion ratios and the calculated gamma distribution were incorporated.

The distinction between Wisconsin and other populations was also indicated by a neighbour-joining phenogram of mtDNA genetic distances (Fig. 2b). Population structure within Wisconsin did not correspond with geographical distance or with the neighbour-joining phenogram based on microsatellite distances (Fig. 2a). A neighbour-joining analysis using unique haplotypes among all individual birds, as opposed to populations, produced a phenogram without any geographical resolution. Different haplotypes unique to populations were scattered throughout the phenogram and a number of large polytomies were

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

	Wisconsin subpopulations							
	Mead	Paul Olson	Buena Vista	Leola	Nebraska	Minnesota	Missouri	Kansas
Mead	—	0.091	−0.033	0.081	**0.441***	**0.409***	**0.405***	**0.398***
Paul Olson	**0.027***	—	0.042	0.009	**0.256***	**0.258***	**0.186***	**0.167***
Buena Vista	**0.053***	**0.087***	—	0.071	**0.419***	**0.396***	**0.372***	**0.362***
Leola	**0.046***	**0.071***	0.017	—	0.160	0.139	0.153	0.123
Nebraska	**0.057***	**0.081***	**0.064***	**0.080***	—	−0.012	0.064	0.038
Minnesota	**0.078***	**0.088***	**0.090***	**0.092***	0.009	—	0.090	0.077
Missouri	**0.080***	**0.081***	**0.093***	**0.099***	**0.033***	**0.050***	—	−0.014
Kansas	**0.073***	**0.091***	**0.084***	**0.095***	0.008	**0.019***	**0.032***	—

Significant values (alpha < 0.001) are in bold and indicated by an asterisk.

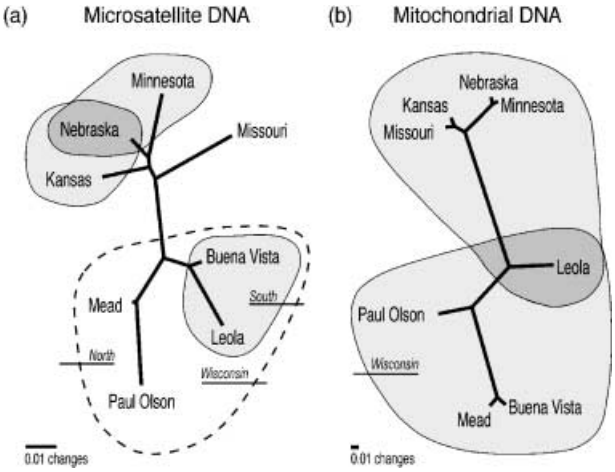

Fig. 2 Unrooted neighbour-joining phenograms of genetic distances using chord distances for six microsatellite loci (a) and Tamura (1992) distances for mtDNA control region sequences (b). Shading indicates populations that do not differ significantly in pairwise F_{ST} comparisons (see Table 3) and the dashed line indicates Wisconsin populations.

observed in the consensus phenogram (data not shown). Similar trees with no phylogeographic structure were produced with maximum parsimony and maximum likelihood analyses.

AMOVA analyses. Differences in population structure between microsatellite and mtDNA markers were also revealed with a hierarchical AMOVA. The percentage of genetic variation explained by grouping populations into two different sets was compared. One set of groups was based on the microsatellite analyses, which suggested that our samples came from five populations (Missouri, Mead, Paul Olson, Buena Vista/Leola and one large population composed of Kansas, Nebraska and Minnesota). The second set of groups was based on the mtDNA analysis, which suggested that our samples came from two populations (Wisconsin and all other states). Using microsatellite data, 4.8% and 5.9% of the variation was explained when the populations were analysed as two and five groups, respectively. In contrast, using mtDNA data, 26.4% and 19.3% of the variation was explained when the populations were analysed as two and five groups, respectively. Thus, 7% more variation was explained by two than five groups of populations with mtDNA data, but 1% less variation was explained by two than five groups of populations with microsatellite data. Similar results were found when a set of five populations was used that consisted of different states (Wisconsin, Minnesota, Nebraska, Kansas, Missouri). In this case, 2.5% of the microsatellite and 19.0% of the mtDNA variation was explained when states were used to group the data. Regardless of marker and grouping method, the largest proportion of the total variance occurred within populations (70–93%).

Isolation-by-distance analyses

The effect of geographical distance on genetic differentiation (F_{ST}) also differed between the nuclear and mitochondrial markers. Using data from all populations, there was a marginally nonsignificant relationship between F_{ST} and geographical distance (ln-transformed) with microsatellites ($r^2 = 0.12$, $P = 0.075$; Fig. 3a) and a significant positive relationship with the mtDNA data ($r^2 = 0.26$, $P = 0.019$; Fig. 3b). This difference became much stronger when the Wisconsin populations were removed from the analyses. In this case, there was no relationship between F_{ST} and geographical distance with microsatellites ($r^2 = 0.04$, $P = 0.285$; Fig. 3c), yet a strong positive relationship with the mtDNA data ($r^2 = 0.51$, $P = 0.041$; Fig. 3d). When the isolation-by-distance analyses were restricted to the four Wisconsin populations, neither the microsatellite ($r^2 = 0.35$, $P = 0.339$; Fig. 3e), nor the mtDNA data ($r^2 = 0.27$, $P = 0.792$; Fig. 3f) showed a relationship between F_{ST} and geographical distance.

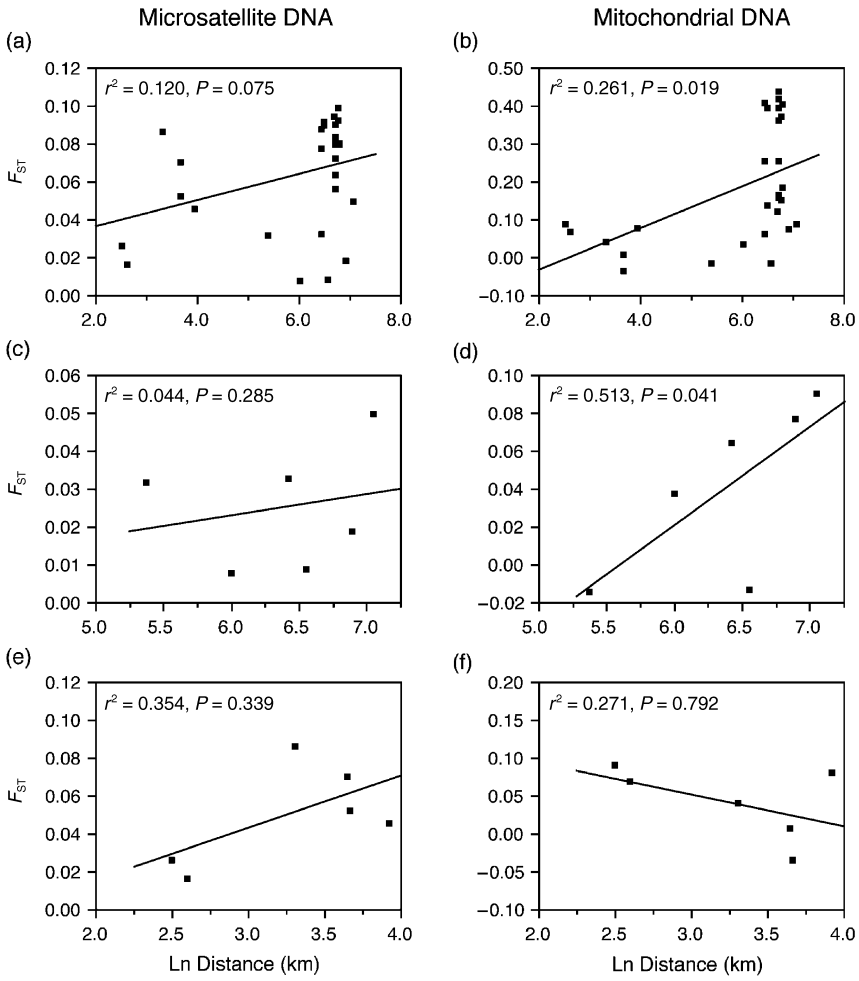

Fig. 3 Analysis of isolation by distance for microsatellite loci (left panels) and mtDNA control region sequences (right panels). F_{ST} values are plotted against ln-transformed straight line geographical distances (km) for pairwise comparisons of all eight populations (a, b), for all populations except Wisconsin (i.e. Nebraska, Minnesota, Kansas and Missouri) (c, d), and for the four subpopulations in Wisconsin (e, f). *P*-values represent significance of isolation by distance using Mantel's test (10 000 permutations).

Some additional analyses of microsatellite isolation by distance were conducted to explain why inclusion of the Wisconsin samples resulted in a stronger isolation-by-distance effect (compare Fig. 3a and c). The effect appeared to be a consequence of including the pairwise comparisons of Wisconsin populations with populations in other states, rather than the comparisons between populations within Wisconsin. When only the population comparisons between Wisconsin and other states were added to the analysis of all other populations (i.e. Figure 3c) the slope of the regression line increased from 0.006 (in Fig. 3c) to 0.03. In contrast, the slope of the regression line became negative (−0.005) when only the population comparisons within Wisconsin were included. Thus, the positive slope of the line in Fig. 3(a) was mainly the result of the large geographical and genetic distances between Wisconsin and other populations. Overall, there was stronger isolation by distance at mitochondrial than microsatellite DNA markers, and much of this difference was the result of differences in microsatellite structure between Wisconsin and other states.

Discussion

Prior to the late 1800s, greater prairie-chickens numbered in the millions and existed throughout the prairie grasslands in North America (Aldrich 1963; Johnsgard 2002). Today, greater prairie-chickens occupy a large part of Nebraska and Kansas, but populations in Minnesota, Missouri and Wisconsin have decreased in size and have become increasingly isolated over the past 100 years. Genetic diversity at both microsatellite and mtDNA markers was found to be correlated positively with population size estimates; thus, small populations showed reduced genetic variation. Nevertheless, there were differences among populations that may be the result of the unique history of each population. Our analyses of mtDNA haplotypes revealed relatively little range-wide geographical structure, yet there was a significant positive relationship between genetic (F_{ST}) and geographical distance (isolation by distance), suggesting that populations were in equilibrium between genetic drift and gene flow (Hutchison & Templeton 1999). In contrast, microsatellites revealed stronger geographical

structure and a weaker effect of isolation by distance. Given the geographical isolation of current populations, the weaker isolation by distance suggests that the effects of genetic drift were stronger than gene flow at microsatellite loci.

Differences between nuclear and mitochondrial markers

Several other studies have also found greater population differentiation using nuclear than mitochondrial markers (Kim *et al*. 1998; Wilmer *et al*. 1999; Piertney *et al*. 2000; Wirth & Bernatchez 2001) but others have found the reverse (Paetkau *et al*. 1998; Castella *et al*. 2001; Pardini *et al*. 2001; Petit *et al*. 2001; Scribner *et al*. 2001; Kerth *et al*. 2002). A number of explanations can account for such differences between nuclear and mtDNA markers. One explanation is that there are different intensities of selection on each marker; however, in all but one case, both markers in this study were in mutation/drift equilibrium within each population, consistent with unselected (neutral) markers. Mutation rates are higher for microsatellite than mitochondrial DNA (Frankham *et al*. 2002), which could also lead to greater population differentiation, but it would probably take thousands of generations for such differences to accumulate in geographically isolated populations (Whitlock & McCauley 1999), and prairie-chicken populations probably became fragmented relatively recently (< 150 years). A more likely explanation for the difference in genetic structure is that the N_e of maternally inherited markers, such as mtDNA, differ from those of biparentally inherited markers (e.g. microsatellites).

It is usually assumed that the N_e, for uniparentally inherited genes is one-half that of diparentally inherited genes (Birky *et al*. 1983) and differences between these markers are the result of the slower rate at which diploid, nuclear markers reach equilibrium (Wilson *et al*. 1985; Birky *et al*. 1989). However, differences in genetic structure are also influenced by patterns of mating, sex-biased dispersal and other demographic parameters. Chesser & Baker (1996) used simulation models to show that the N_e of uniparentally inherited genes can be over three times larger than the level observed with biparentally inherited genes under certain conditions, such as a polygynous mating system and female-biased dispersal. Greater prairie-chickens fit these conditions as they have high levels of polygyny (a lek mating system) and greater female than male dispersal (Hamerstrom & Hamerstrom 1973; Halfmann 2002). In red grouse, Piertney *et al*. (2000) employed the Chesser & Baker (1996) model and demonstrated that under realistic ecological parameters and observed levels of female-biased dispersal, population structure may be stronger with microsatellite than mitochondrial DNA markers.

Microsatellite and mtDNA markers also revealed different effects of geographical isolation on genetic variation in prairie-chickens. There was a stronger pattern of isolation by distance with mitochondrial than microsatellite DNA markers, particularly after excluding the small, isolated populations in Wisconsin (Fig. 3c,d). In small, isolated populations allele frequencies will drift independently without regard to geographical isolation, producing a wide scatter of pairwise population comparisons and, consequently, a lack of isolation by distance (Hutchison & Templeton 1999). These populations may also diverge quickly from other populations, which produces large genetic distances (Hedrick 1999). These large distance estimates appear to explain the stronger positive relationship between genetic and geographical distance when Wisconsin populations were included (compare Fig. 3a and c). Populations of greater prairie-chickens have been isolated for less than 150 years. Our results suggest that this has been sufficient time for drift to influence microsatellite DNA in most populations (Fig. 3c), but not enough time for drift to influence mtDNA in relatively large populations (> 2000 birds; Fig. 3d). The isolation-by-distance relationship for mtDNA was positive throughout the range (Fig. 3b,d), but not significant when only the Wisconsin populations were examined (Fig. 3f). This suggests that mtDNA isolation by distance occurs outside, but not within, Wisconsin. Based on the range-wide patterns, it is suggested that the mtDNA variation reflects prefragmentation relationships, which would suggest that an equilibrium between gene flow and drift used to exist throughout most of the historic range. The AMOVA results support this hypothesis, as genetic variation at mtDNA was explained better by two large groups (Wisconsin vs. all other populations) than by five smaller ones. In contrast, the AMOVA using microsatellites found the opposite pattern. Thus, it appears that recent fragmentation and isolation of greater prairie-chicken populations has had a stronger effect on microsatellite than mtDNA population structure. Indeed, a number of phylogenetic studies using mitochondrial markers have had difficulty in resolving the *Tympanuchus* complex, which includes the lesser prairie-chicken (*T. pallidicinctus*) and sharp-tailed grouse (*T. phasianellus*) (Ellsworth *et al*. 1994; Lucchini *et al*. 2001; Dimcheff *et al*. 2002; Drovetski 2002).

Effects of isolation on genetic diversity

Isolation and fragmentation of greater prairie-chicken populations during the past 100 years has been extensive (Aldrich 1963; Johnsgard 2002). However, the relative degree of habitat fragmentation varies among the remaining greater prairie-chicken populations. Greater prairie-chickens occupy a large proportion of Nebraska and Kansas and estimates of genetic diversity for both mtDNA control region and microsatellite DNA are high compared to other populations surveyed in this study. In contrast, prairie-chicken populations in Minnesota, Missouri and Wisconsin have

decreased in size and have become increasingly isolated over the past 100 years. Each of these populations has different histories of habitat fragmentation and isolation, which may explain the differences in population structure.

Almost the entire population of greater prairie-chickens in Missouri (~1000 birds) resides in the southwest portion of the state near Kansas, which has a large population (Mechlin *et al*. 1999). Gene flow from Kansas may have helped to maintain relatively high genetic diversity in Missouri; however, it is not known if birds currently disperse from Kansas to Missouri, so the high genetic diversity could indicate either historic or contemporary gene flow. Although some birds have been translocated to northern Missouri over the last 20 years, it should be noted that the population sampled in this study was in the southwest and isolated from any translocated birds (L. Mechlin, personal communication).

In Minnesota, the population has remained around 2000 birds for the last 25 years (Svedarsky *et al*. 1999b; Svedarsky *et al*. 2000). The Minnesota samples were collected in the northwest corner of the state where habitat for prairie-chickens is contiguous throughout five counties, and birds have been observed dispersing throughout this area (J. Toepfer, unpublished data). Although the Minnesota population is 700–900 km from populations sampled in Nebraska and Kansas, no significant genetic differences were found between these three populations and there were high levels of genetic variation. The maintenance of habitat connections within Minnesota may facilitate gene flow and increase N_e, despite its small size and complete isolation from larger populations (Gibbs 2001; Mech & Hallett 2001).

The effect of genetic drift on microsatellite and mtDNA variation was most apparent in Wisconsin. We showed previously that microsatellite diversity in the contemporary Buena Vista population was lower than in samples collected from the same location in 1951 (Bellinger *et al*. 2003). In this study, genetic diversity was lower in all four Wisconsin populations than in all other populations surveyed. Interestingly, Wisconsin, Minnesota and Missouri have approximately similar numbers of birds in about the same overall area of habitat. However, birds in Wisconsin have become restricted to four separate management areas (Anderson & Toepfer 1999), and recent evidence suggests that there are barriers to dispersal between these areas. In the 1960s, the two nearest booming grounds between the southern (Buena Vista) and northern (Paul Olson) management areas were separated by 8 km (Westemeier 1971). Today, the distance between the two nearest booming grounds has increased to 22 km as a consequence of habitat change (Halfmann 2002), primarily encroachment of trees and residential property. During a 5-year radio-telemetry study, Halfmann (2002) documented no female or chick dispersal between the northern and southern management areas, yet dispersal between these areas was common 30 years earlier (Westemeier 1971; Hamerstrom & Hamerstrom 1973). Thus, our microsatellite analysis corroborates the lack of dispersal found with radio-telemetry. The reduction in gene flow within Wisconsin probably produces a smaller N_e (Whitlock & Barton 1997; Gibbs 2001) and, consequently, a greater chance for genetic drift. This relationship is further supported by positive Tajima *D*-values in all four Wisconsin subpopulations (Table 1) and the lack of mtDNA geographical structure within Wisconsin (in both the neighbour-joining and isolation-by-distance analyses).

Conservation implications

Our results suggest that genetic variation at neutral markers is associated with population size, and genetic variation is reduced significantly within isolated populations of < 2000 greater prairie-chickens. The presence of mtDNA isolation by distance suggests that, historically, populations of greater prairie-chickens were interconnected as one large metapopulation and female-biased dispersal was a probable mechanism helping to connect populations and maintain genetic variability. In contrast, population genetic differentiation at microsatellites is probably a consequence of recent habitat fragmentation and the interaction of genetic drift and gene flow at various stages of isolation.

Given that genetic variation was associated positively with population size, managers should attempt to maintain large populations of more than 2000 birds, as in Kansas and Nebraska, or smaller connected metapopulations (Gibbs 2001; Brooker & Brooker 2002). In Wisconsin, reconnecting the four existing populations may increase the overall N_e by allowing an increase in gene flow among populations, similar to what may be occurring in Minnesota (J. Toepfer, unpublished data). In fact, 30 years ago, Hamerstrom & Hamerstrom (1973) emphasized that the connection among management areas was vital to maintaining the prairie-chicken in Wisconsin. The loss of metapopulation dynamics within Wisconsin appears to be fairly recent, and the ability of birds to disperse may be an important factor in the extirpation of small populations of prairie-chickens over the last century.

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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. 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.
