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Genetic Evaluation of a Demographic Bottleneck in the Greater Prairie Chicken

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Abstract: *Although the theoretical relationship between population size, fitness, and genetic variation is well established, only a few studies have provided direct evidence that ties a decline in both genetic variation and fitness to a demographic bottleneck for a natural system. We report on a genetic comparison of four populations of the Greater Prairie Chicken (*Tympanuchus cupido*) with different demographic histories. Specifically, we compared a population from Illinois that has suffered an extreme demographic contraction and an associated decline in population fitness (measured in terms of hatchability rates) with populations from Kansas, Nebraska, and Minnesota with no known history of bottlenecks or associated declines in fitness. Using the polymerase chain reaction, we amplified six microsatellite loci from which levels of heterozygosity, allelic diversity, and geographic differentiation (F_{ST} and R_{ST}) of the studied populations were estimated. Results of this analysis showed that the Illinois Prairie Chicken had the lowest estimate of mean heterozygosity per locus and approximately two-thirds the allelic diversity, sharing 95–100% of all their alleles with each of the other populations. This finding suggests that the Illinois Prairie Chicken originally had higher levels of genetic diversity that were subsequently lost through an extreme demographic contraction. To our knowledge this is the first example of loss of genetic diversity being associated with a decrease in population fitness as a result of a known demographic bottleneck in a wild bird species.*

Evaluación Genética de un Cuello de Botella Demográfico en la Gran Gallineta de la Pradera

Resumen: *Aunque la relación teórica entre el tamaño poblacional, la aptitud biológica y la variabilidad genética ha sido bien establecida, son pocos los estudios que han proporcionado evidencia directa que asocie una disminución de la variación genética y de la aptitud biológica con un cuello de botella demográfico en sistemas naturales. Reportamos una comparación genética de cuatro poblaciones de la gran gallineta de la pradera (*Tympanuchus cupido*) que tuvieron historias demográficas diferentes. Específicamente comparamos una población de Illinois, la cual ha sufrido una contracción demográfica extrema asociada con decrementos en la aptitud biológica poblacional (medida en términos de tasas de eclosión), con poblaciones de Kansas, Nebraska y Minnesota, las cuales no presentan una historia de cuello de botella poblacional ni decrementos en la aptitud biológica. Mediante el uso de la reacción en cadena de la polimerasa (PCR) amplificamos seis marcadores de microsatélites con los cuales estimamos niveles de heterocigosis, diversidad alélica y diferenciación geográfica (F_{ST} y R_{ST}) en las poblaciones estudiadas. La gran gallineta de la pradera en Illinois presenta el menor valor de heterocigosidad promedio por locus y aproximadamente 2/3 de la diversidad alélica, compartiendo 95–100% de sus alelos con cada una de las otras poblaciones. Estos resultados sugieren que la población de Illinois presentaba originalmente niveles mayores de variabilidad genética, los cuales fueron perdidos consecuentemente a través de una contracción demográfica extrema. A nuestro entender, este estudio*

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representa el primer ejemplo en el que se asocia la pérdida de la diversidad genética en una especie de ave silvestre con la disminución en la aptitud poblacional y como resultado de un cuello de botella demográfico conocido.

Introduction

Within a population the number of individuals is a crucial determinant of the amount of genetic variability that can be maintained. Small, isolated populations tend to lose genetic variation over time, which may in turn increase the probability of population extinction or reduce opportunities for future adaptive change (Lande & Barrowclough 1987; Meffe & Carroll 1994).

Several studies have shown a positive association between population size and level of genetic variation in natural populations. For example, lower levels of genetic variability have been reported for geographically peripheral populations of the Sonoran topminnow (*Poeciliopsis occidentalis*; Vrijenhoek et al. 1985) and in fragmented populations of Red-cockaded Woodpeckers (*Picoides borealis*; Stangel et al. 1992). Billington (1991) has shown similar results by examining genetic variation in wild populations of different sizes of a dioecious conifer endemic to New Zealand. The effects of bottlenecks on genetic variation has also been addressed empirically. For example, Leberg (1992) has shown that experimentally bottlenecked populations of mosquitofish (*Gambusia holbrooki*) have low levels of allozyme genetic variation.

Considerable evidence supports the idea that genetic diversity enhances fitness-related characteristics. Higher levels of heterozygosity have been associated with decreased morphological variation, increases in individual performance, and developmental homeostasis (Mitton & Grant 1984; Allendorf & Leary 1986; Mitton 1993). Furthermore, the effects of inbreeding on relative fitness have been addressed experimentally by breeding individuals that are differentially related (Waser & Price 1989; Trame et al. 1995) or by breeding individuals from subpopulations with different sizes and demographic histories (Brewer et al. 1990; Heschel & Paige 1995; Holtsford 1996).

Although the theoretical relationship between population size, fitness, and genetic variation is well established, only a few studies have provided evidence for an association between genetic variability and reproductive performance resulting from bottlenecks in natural populations (Packer et al. 1991; Keller et al. 1994). Most studies, however, have *assumed* that depauperate levels of genetic variability and lowered fitness are the result of previous demographic bottlenecks (Bonnell & Selander 1974; O'Brien et al. 1983; Ellegren et al. 1993). There are two ways to gather strong supporting evidence for

this hypothesis. One is to measure genetic variability and fitness correlates before and after a given population bottleneck, currently a difficult task due to the lack of pre-bottleneck data. The second approach is to measure and relate genetic diversity and fitness estimates of current populations with different ecological histories in terms of demographic bottlenecks (Packer et al. 1991). Assuming there is no strong direct selection resulting from an environmental change during the bottleneck or among current disjunct populations, these two approaches would provide supportive evidence for the idea that bottlenecks can lead to a decline in genetic diversity and population fitness.

We used the second approach to evaluate the effects of a demographic bottleneck on the genetic variability of the Greater Prairie Chicken (*Tympanuchus cupido pinnatus*). Specifically, we compared a population from Illinois that has suffered an extreme demographic contraction and an associated decline in population fitness with three others from Kansas, Nebraska, and Minnesota in which there have been no documented history of bottlenecks or associated decline in fitness.

The Greater Prairie Chicken is a grassland-prairie species with limited dispersal and a lek mating system; it was originally distributed throughout the central plains of North America (Yeatter 1943). Since European settlement, populations have become increasingly affected by loss of natural habitat and the introduction of exotic species such as Ring-necked Pheasants (*Phasianus colchicus*; Westemeier & Edwards 1987). The negative effects of pheasants include competition for habitat and interspecific nest parasitism. By the early 1900s the Prairie Chicken population in the state of Illinois, originally estimated in the millions in the 1860s, began to decline dramatically from an estimated 25,000 birds in 1933 to less than 50 in 1993 (Westemeier et al. 1991; R. L. Westemeier, unpublished data). The species is now restricted to only 2 of the 74 Illinois counties in which they persisted in 1912 (Westemeier & Edwards 1987). In contrast, the Kansas, Minnesota, and Nebraska populations have remained comparatively large, with wide distributions and sizes ranging from 4000 in Minnesota (Wolfe 1995) to more than 100,000 in Kansas and Nebraska (Christisen 1969; Westemeier & Edwards 1987) (Fig. 1). In addition, population fitness in Illinois, estimated with hatchability and fertility rates, has decreased compared to the larger populations in Kansas, Minnesota, and Nebraska (R. L. Westemeier et al. unpublished data).



Figure 1. Map of the geographic distribution of the Greater Prairie Chicken showing the location of studied populations. Estimated population sizes are Illinois (IL), <50; Kansas (KS), >100,000; Minnesota (MN), >4000; Nebraska (NE), >100,000.

Methods

Demographic Data and DNA Analysis

Estimates of population sizes, based on censuses and harvest rates, were obtained from published studies of the Kansas, Minnesota, and Nebraska populations (Baker 1953; Silvy 1968; Sisson 1976; Svedarsky 1979). Demographic estimates for the Illinois population were obtained from a long-term study by R.L.W. Hatchability rates were estimated as the mean number of hatched eggs per total number of eggs in successful nests. Hatch rates for Kansas and Minnesota were calculated from published studies (Baker 1953; Silvy 1968; Svedarsky 1979). Data for the Nebraska population were kindly provided by L. L. McDaniel of the U.S. Fish and Wildlife Service, Valentine National Wildlife Refuge (personal communication).

Genomic DNA was extracted from blood samples from birds captured between 1992 and 1994 in Kansas ($n = 37$), Minnesota ($n = 38$), and Nebraska ($n = 20$). DNA from Illinois Prairie Chickens ($n = 32$) was extracted from tissue samples (muscle) of frozen birds from incidental mortalities occurring between 1974 and 1993. Fifty-eight microsatellite primers (Cheng & Crittenden 1994; Cheng et al. 1994) designed for the domestic chicken (*Gallus gallus*) were screened for amplification in the Greater Prairie Chicken. Six polymorphic microsatellite loci that yielded specific amplification products in the Greater Prairie Chicken were used for genotyping 127 birds. The PCR reactions were set up in 20- μ L volumes, each containing about 30 ng of DNA template, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 50 μ M concentrations of each dNTP, 0.25 μ M of each primer, and 0.5 U of Taq polymerase enzyme. The PCR reactions were performed in an MJ Research® thermocycler with a 3-minute denaturation step at 94° C, fol-

lowed by 34 cycles of 30 seconds denaturation at 94° C, 30 seconds annealing at 46–50° C, and 30 seconds of extension at 72° C, and then a final 5-minute extension step at 75° C. Samples were mixed with 0.5 volume of formamide dye solution, denatured for 5 minute at 95° C, and then run on a 6% acrylamide gel. Microsatellites ADL42, ADL23, and ADL44 were amplified by means of radioactively labeled dGTP (P³²) and were visualized by autoradiography of polyacrylamide gels. Genotypes were assigned by two independent scorers. Microsatellites ADL146, ADL162, and ADL230 were run in an ABI Model 373A Automated DNA Sequencer (Applied Biosystems, Inc.) and electropherograms were analyzed with Genotyper® software (Applied Biosystems, Inc.).

Genetic Variability and Gene Flow

Allele frequencies were determined by direct count. Mean heterozygosities and mean number of alleles per locus were calculated with Biosys-1 software (David Swofford, Illinois Natural History Survey, Champaign, IL.). Using a Monte Carlo technique, deviations from Hardy-Weinberg were tested by determining the exact probability of obtaining a χ^2 value as large or larger than the observed χ^2 (Roff & Bentzen 1989). Significant deviations from Hardy-Weinberg were not found, so we report observed heterozygosities. Genetic differentiation among populations was estimated by Wright's F_{ST} indice (Wright 1951) and the analogous R_{ST} designed for microsatellite data (Slatkin 1995). The hypothesis test for genetic differentiation among populations was made with a contingency table analysis of allele frequencies. Independence of rows (sites) and columns (allele frequencies) was evaluated with likelihood ratio statistics, and significance values were derived from exact tests using permutation procedures (Roff & Bentzen 1989; Metha & Patel 1992). Differences of mean heterozygosity and mean number of alleles per locus among populations were tested by a two-way analysis of variance with locus and population as main factors (Proc GLM, SAS Institute 1990). Interaction between locus and population was assessed by Tukey's non-additivity test. Model assumptions were evaluated by residual analysis. Normality of residuals was assessed with normal probability plots and the Shapiro-Wilk test (heterozygosity: $W = 0.97$, $p = 0.71$; allelic diversity: $W = 0.96$, $p = 0.46$). Heteroscedasticity was evaluated by plotting log of residuals versus log of predicted values. Spearman correlation coefficients indicated that residuals had homogeneous variances (heterozygosity: $Rho = -0.12$, $p = 0.60$; allelic diversity: $Rho = 0.23$, $p = 0.29$). To check for possible effects of different sample sizes we plotted the log of residuals versus sample size per population or locus. Pairwise comparisons of the mean number of alleles per locus between populations were performed by the Tukey's studentized range test (SAS Institute 1990).

Gene flow among populations, defined as the number of reproductively successful migrants per generation (Nm), was estimated by two methods based on the n island model of population structure. The first estimate was based on the relationship $F_{ST} = 1/(4Nm@ + 1)$, where N is the effective population size, m is the migration rate, $@ = [n/(n - 1)]^2$, and F_{ST} is the mean F_{ST} value calculated over all loci (Slatkin & Barton 1989).

The second estimate was calculated as

$$M_R = (ds - 1)/4ds [1/R_{ST} - 1],$$

where M_R is the estimate of Nm , ds is the number of populations, and R_{ST} is analogous to Wright's F_{ST} . The R_{ST} values were estimated as $R_{ST} = (S - Sw)/S$, where Sw is twice the average of the estimated variances of allele size within each population and S is twice the estimated variance in allele size in the collection of populations together (Slatkin 1995).

The above relationships between F_{ST} , R_{ST} , and gene flow assume that individuals disperse among subpopulations independently of geographic distance and that population sizes have been constant long enough for migration-drift equilibrium to have been reached (Slatkin & Barton 1989; Slatkin 1995). Because gene flow is a process that leads to genetic homogenization, significant levels of genetic differentiation among subpopulations (indicated by F_{ST} and R_{ST} indices) would suggest limited gene flow. Our estimates of gene flow are presented as relative measures of isolation among populations, not absolute numbers of successful migrants per generation. Because the studied populations have been recently reduced and fragmented, the present Nm would probably be smaller than that estimated here.

We performed a cluster analysis using unweighted pair-group method with arithmetic averaging (UPGMA)

on Nei's unbiased genetic identity coefficients using Biosys-1 software (Swofford & Selander 1981) to illustrate the genetic relationships among populations, not to infer phylogeny. Goodness of fit was estimated by the cophenetic correlation.

Results and Discussion

Measures of genetic variability among the four populations were based on six polymorphic microsatellite loci (Fig. 2). In all four populations, none of the six loci analyzed showed significant deviations from the genotype frequencies expected according to Hardy-Weinberg equilibrium. Results from the analysis of variance indicated no significant differences in the mean heterozygosity per locus among populations ($p = 0.638$). The Illinois population, however, showed the lowest value of heterozygosity for three of the six loci analyzed, with the lowest mean heterozygosity per locus (Table 1). The mean number of alleles per locus was significantly different among populations ($p < 0.0001$). Tukey's studentized range test indicated that the mean number of alleles per locus was significantly lower in the Illinois population ($p < 0.05$), showing about two-thirds the allelic diversity observed in all other populations (Table 1). These results show that allelic diversity is more drastically affected than heterozygosity, as is expected following population bottlenecks (Nei et al. 1975).

At each locus most alleles present in the Illinois population were shared with each of the other three populations, representing a subset of the total alleles found (Table 2). In addition, few alleles were specific to Kansas or Nebraska. These results suggest that all populations were originally part of an ancestral population with lev-

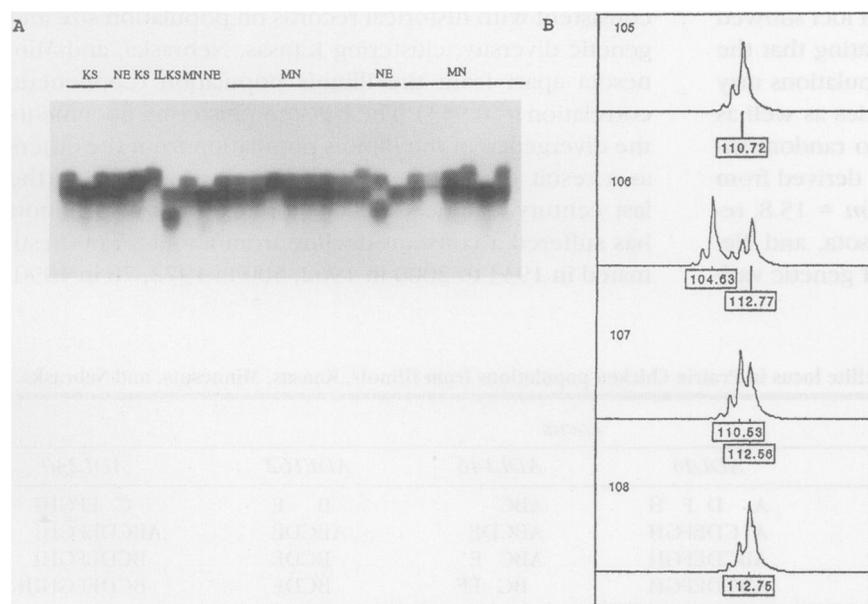


Figure 2. Example of an autoradiograph showing the amplification of microsatellite ADL42 of Prairie Chickens from Kansas (KS), Minnesota (MN), Nebraska (NE) and Illinois (IL). Four alleles were detected in this system (a). Example of electropherograms obtained by the amplification and detection of microsatellite ADL230 by an automated DNA sequencer (b). Values assigned to each peak indicate allele size in number of base pairs.

Table 1. Number of alleles (*n*), observed heterozygosities (*H*), F_{ST} and R_{ST} values with corresponding χ^2 exact probabilities for each microsatellite locus.

Locus	Populations								F_{ST}	R_{ST}	$p(\chi^2)$
	Illinois n = 32		Kansas n = 37		Minnesota n = 38		Nebraska n = 20				
	H	n	H	n	H	n	H	n			
ADL42	0.375	3	0.432	4	0.658	4	0.400	4	0.082	0.100	0.0000
ADL23	0.781	4	0.649	5	0.763	4	0.600	5	0.033	0.000	0.0000
ADL44	0.429	4	0.667	7	0.735	8	0.778	8	0.034	0.000	0.0033
ADL146	0.594	3	0.500	5	0.684	4	0.600	4	0.029	0.011	0.0060
ADL162	0.500	2	0.444	5	0.243	4	0.579	4	0.078	0.084	0.0000
ADL230	0.750	6	0.889	9	0.842	8	0.800	10	0.030	0.000	0.0000
Mean <i>H</i> per locus*	0.571 (0.068)		0.597 (0.071)		0.654 (0.086)		0.626 (0.060)		0.044	0.012	
Mean number of alleles per locus*	3.67 (0.56)A		5.83 (0.75)B		5.33 (0.84)B		5.83 (1.05)B				

*Standard errors of mean heterozygosities (direct-count estimate) and mean number of alleles per locus are indicated in parentheses. Different letters indicate significant differences at $p < 0.05$.

els of genetic variability similar to levels currently found in the larger populations, with the Illinois population losing considerable diversity as the result of a demographic bottleneck.

The genetic structure of natural populations is the result of a balance between mutation, genetic drift, and natural selection promoting differentiation of local populations and gene flow opposing that differentiation (Slatkin 1987). Historical accidents, however, may also play an important role in determining the amount of genetic variability preserved within each population. Population sizes and rates of migrational exchange will determine the amount of geographic variation expected due to genetic drift among populations such as those of Kansas, Minnesota, and Nebraska. In addition, historical events such as the drastic demographic contraction suffered by the Illinois population, mainly as a result of habitat loss, may reflect the importance of effective population size in maintaining genetic diversity. All loci showed highly significant exact probabilities, indicating that the degree of genetic differentiation among populations may be the result of differences in the frequencies as well as in the presence of alternative alleles due to random genetic drift (Table 1). Estimates of gene flow derived from F_{ST} and R_{ST} values showed $Nm = 2.9$ and $Nm = 15.8$, respectively. In addition, the Kansas, Minnesota, and Nebraska populations, all with similar levels of genetic vari-

ability, showed an $F_{ST} = 0.024$ and $R_{ST} = 0.005$ with estimated $Nm = 4.5$ and $Nm = 32.1$, respectively, when calculated independently from the Illinois population.

Our estimates of genetic differentiation among the studied populations indicate relatively low levels of gene flow. Although the value $Nm < 1$ has been proposed as the theoretical level for the fixation of alternative alleles due to random genetic drift (Wright 1943; Slatkin 1987), it does not represent an absolute limit above which genetic differentiation may or may not occur. The estimated levels of Nm have not prevented genetic differentiation among the studied populations and, therefore, might be considered relatively low given the recent fragmentation of the studied populations, the low levels of dispersal reported for the Greater Prairie Chicken (Schroeder & Braun 1993), and the species lek mating system, which drastically reduces the effective population size.

A phenogram generated by UPGMA analysis (Fig. 3) is consistent with historical records on population size and genetic diversity, clustering Kansas, Nebraska, and Minnesota apart from the Illinois population (cophenetic correlation = 0.953). The UPGMA clustering documents the divergence of the Illinois population from the others as a result of its demographic contraction. During the last century, Prairie Chicken population size in Illinois has suffered a constant decline from about 25,000 estimated in 1933 to 2000 in 1962, 500 in 1972, 76 in 1990,

Table 2. Individual alleles found at each microsatellite locus in Prairie Chicken populations from Illinois, Kansas, Minnesota, and Nebraska.

Population	Locus					
	ADL42	ADL23	ADL44	ADL146	ADL162	ADL230
Illinois	ABC	ABCD	A D F H	ABC	B E	C EFGHI
Kansas	ABCD	BCDEF	A CDEFGH	ABCDE	ABCDE	ABCDEFGH
Minnesota	ABCD	ABCD	ABCDEF	ABC E	BCDE	BCDEF
Nebraska	ABCD	ABCDE	ABCDEF	BC EF	BCDE	BCDEF

	IL	KS	MN	NE
IL	1.000			
KS	.885	1.000		
MN	.885	.970	1.000	
NE	.885	.974	.970	1.000

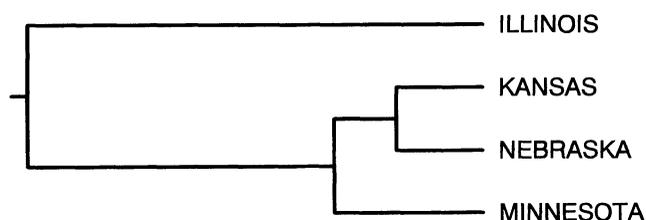


Figure 3. Cophenetic matrix and UPGMA phenogram based on *Nei* unbiased genetic identity coefficients. The tree was generated by Biosys-1 software. Cophenetic correlation = 0.953.

and less than 50 birds in 1993 (Westemeier et al. 1991; R. L. Westemeier, unpublished data). This reduction in population size is associated with a decrease in genetic diversity compared to that of the other populations that currently maintain population sizes larger than 4000 individuals. In addition, the Illinois population is the only one that has shown a drastic decrease in population fitness over the last 20 years. Hatchability rates of 93% reported in 1935–1936 (Yeatter 1943) have plummeted to an estimated 56% in 1990 (Westemeier et al. 1991). In contrast, Kansas, Minnesota, and Nebraska populations, all relatively large in number, show hatch rates of 83–100% per successful nest (Baker 1953; Silvy 1968; Sisson 1976; Svedarsky 1979). Data from 10 museum specimens collected in the 1930s from the Illinois population further substantiate our conclusions. Genotypes from this small sample showed six alleles not present in the current Illinois population but present in all three of the other populations (Bouzat et al. 1998).

Because the studied populations are geographically disjunct, observed differences in reported hatchabilities might be attributed either to the Illinois demographic contraction or to environmental differences among sites. But the fact that none of the three independent “control” populations showed declines in reproductive success while the Illinois population did lends support to the idea that the observed fitness reduction is the result of the demographic bottleneck, not environmentally induced differences. Furthermore, an experimental introduction of birds from the three genetically diverse populations to Illinois has led to an increase in the hatch rate to pre-bottleneck levels, suggesting a causal link between the reduction in genetic variation and the decline in fitness. A full analysis of demographic data and fitness

traits of the Illinois population is forthcoming (R. L. Westemeier et al., unpublished data).

Implications for Conservation

Although theoretical and experimental studies suggest an association among population size, genetic variability, and fitness, only limited evidence exists for natural populations. Even in cases for which such evidence exists one of the main problems is that the studies often lack control populations for comparison (Meffe & Carroll 1994). In this study we circumvent this problem. We have shown that Greater Prairie Chicken populations that have undergone different demographic histories exhibit different levels of genetic diversity associated with different levels of population fitness. The Illinois population, which has passed through a severe demographic contraction during the last century, has suffered a decrease in genetic diversity associated with a reduction in population fitness. In contrast, three other independent populations larger in number show no fitness decay and higher degrees of genetic variability.

We also provide a clear example of the importance of evaluating population genetic information for designing management strategies for the recovery of endangered species. The association between the absence of genetic variability and the reduction in reproductive parameters in the endangered Illinois Prairie Chicken suggests that the demographic contraction may have led to inbreeding depression. If that is the case, translocation of birds from other populations that are genetically more variable may alleviate the detrimental effects of inbreeding, leading to a decrease in the probability of extinction of the Illinois population due to genetic factors. Although a recent introduction has been followed by an increase in the reproductive parameters of the Illinois population, further studies—(both demographic and genetic)—will be necessary to evaluate translocations as a management strategy for the preservation of the Illinois Prairie Chicken.

During the last few years there has been considerable debate on the importance of genetic versus demographic factors in determining long-term population viability (Schonewald-Cox et al. 1985; Soulé 1987; Lande 1988, 1994). The adaptiveness of genetic diversity has been questioned because of a lack of direct evidence tying genetic diversity to fitness and because permanent inbreeding depression might be overcome through the selective purging of deleterious recessive alleles. We agree that demography (i.e., habitat destruction) plays a major role in the short-term persistence of most species affected by human activities. The importance of genetics cannot be disregarded, however, particularly in managing populations on the verge of extinction in which lack of genetic diversity due to low effective population size is likely to be associated with reduced fitness.

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