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RESEARCH ARTICLE

Beyond the beneficial effects of translocations as an effective tool for the genetic restoration of isolated populations

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Abstract Translocations are becoming increasingly popular as appropriate management strategies for the genetic restoration of endangered species and populations. Although a few studies have shown that the introduction of novel alleles has reversed the detrimental effects of inbreeding over the short-term (i.e., genetic rescue), it is not clear how effective such translocations are for both maintaining neutral variation that may be adaptive in the future (i.e., genetic restoration) and increasing population viability over the long-term. In addition, scientists have expressed concerns regarding the potential genetic swamping of locally adapted populations, which may eliminate significant components of genetic diversity through the replacement of the target population by the source individuals used for translocations. Here we show that bird translocations into a wild population of greater prairie-chickens (*Tympanuchus cupido pinnatus*) in southeastern Illinois were effective in both removing detrimental variation associated with inbreeding depression as well as restoring neutral genetic variation to historical levels. Furthermore, we found that although translocations resulted in immediate increases in fitness, the demographic recovery and long-term viability of the population appears to be limited by the availability of suitable habitat. Our results demonstrate that although translocations can be effective management tools for the genetic restoration of wild populations on the verge of extinction, their long-term viability may not be guaranteed unless the initial conditions that led to most species declines (e.g., habitat loss) are reversed.

Keywords *Tympanuchus cupido* · Bottlenecks · Genetic rescue · Translocations

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Introduction

Over the last few years conservation geneticists have embraced the concept of *genetic restoration* (Tallmon et al. 2004; Hedrick 2004, 2005) as a comprehensive approach to cope with the detrimental effects of isolation and small population size on the genetic variation of endangered populations. This concept emphasizes three major goals: (1) removing the genetic load expressed through inbreeding depression in small populations (a process commonly known as genetic rescue), (2) retaining local adaptive variation (i.e., preventing genetic swamping with other populations), and (3) restoring historical levels of genetic diversity to maintain neutral variation that might be beneficial in future environments (i.e., increasing evolutionary potential).

Translocations are becoming increasingly popular as a management strategy for the genetic restoration of endangered species populations (Tallmon et al. 2004; Pimm et al. 2006; Wisely et al. 2007). The utility of translocations has been mainly based on evidence of their immediate beneficial effects on fitness. For example, a few case studies from natural populations have shown that gene flow from other populations reversed the detrimental effects of inbreeding over the short-term (Westemeier et al. 1998a; Madsen et al. 1999; Vilà et al. 2003; Arrenda et al. 2004; Hogg et al. 2006) by increasing both fitness and population size. However, other studies have also emphasized harmful effects of translocations, which may include outbreeding depression due to lack of adaptation of introduced individuals, and the potential elimination of the genetic distinctiveness of the population to be restored (i.e., genetic swamping) (Clewell 2000; Frankham et al. 2002; Hufford and Mazer 2003; Edmands 2007). Although both theoretical and experimental studies have evaluated the importance of population size and migration in maintaining fitness and adaptive genetic variation (e.g., Newman and Tallmon 2001; Swindell and Bouzat 2005, 2006), to our knowledge, no study has documented in nature the direct role of translocations in restoring historical levels of genetic variation, which may be indicative of adaptive genetic variation in natural populations.

The concept of genetic restoration has played a pivotal role in defining management actions aimed at decreasing the detrimental effects of genetic stochasticity, since reduced genetic diversity in small populations has been shown to decrease population growth and increase probability of extinction (e.g., Westemeier et al. 1998a; Saccheri et al. 1998). However, several authors have emphasized that the recovery and long-term persistence of small populations do not depend on genetics but mainly on demographic and environmental factors driving the extinction vortex (Caughley 1994; Lande 1998). An overall assessment of the potential effects of translocations as a tool for the genetic restoration and long-term viability of natural populations would, therefore, require: (1) Assessment of historical levels of genetic diversity prior to a major population decline; (2) Documentation that levels of genetic diversity have been effectively restored to historical levels as a result of managed translocations; and (3) Demographic trends indicating changes in fitness and population size following translocations in the absence of habitat changes.

In 1998, a long-term study on a remnant population of greater prairie-chickens (*Tympanuchus cupido pinnatus*) in southeastern Illinois documented concurrent declines in population size and fitness as well as an overall reduction in genetic diversity (Bouzat et al. 1998a; Westemeier et al. 1998a), suggesting that inbreeding depression was one of the major factors driving this population to extinction. Although translocation of birds into Illinois from larger and genetically diverse populations resulted in immediate increases in fitness (Westemeier et al. 1998a), concerns existed over both the long-term benefit of translocations on population viability and the potential genetic swamping of the Illinois population by the introduction of birds from other locations. Although this and a few other case studies (e.g., Madsen et al. 1999; Vilà et al. 2003; Arrenda et al. 2004; Hogg et al. 2006) have documented the beneficial effects of translocations in alleviating the detrimental effects of inbreeding depression in endangered wild populations, there is still a need for well-documented studies on the potential role of translocations in restoring overall genetic diversity to historical levels and increasing population viability (Pimm et al. 2006; Maher et al. 2006; Beier et al. 2006).

In this study, we report demographic trends and levels of genetic variation at both nuclear (microsatellites) and mitochondrial (control region) DNA markers prior to and 10 years after the initiation of bird translocations into an isolated population of greater prairie-chickens in Jasper County, Illinois. We evaluated the effectiveness of translocations in recovering genetic diversity to historical levels and increasing population viability. Furthermore, the genetic analysis allowed an evaluation of the potential 'genetic swamping' following translocations into the focal population. Genetic and demographic data presented in this study demonstrate that although translocations may be an effective management strategy for the overall genetic restoration of endangered wild populations, their long-term viability cannot be guaranteed unless we take into consideration demographic and environmental factors that have contributed to the original species decline.

Methods

Population sampling and DNA extraction methods

Results from this study are based on the genetic analysis of 623 samples collected from 12 contemporary and six historical populations of greater (*T. cupido pinnatus*) and Attwater's (*T. cupido attwateri*) prairie-chicken (Table 1). Samples from the Illinois focal population included historical samples ($n = 22$; 1936–1970), pre-translocation samples ($n = 32$; 1971–1991), and post-translocation samples from Jasper County collected after 10 years from the initiation of bird translocations in 1992 ($n = 18$; 2003). Contemporary non-bottlenecked populations included those from Kansas (Wabaunsee County), Minnesota (Norman and Wilken Counties), Nebraska (Garfield County), and Missouri (Barton and Dade Counties). In

Table 1 Genetic diversity estimates (microsatellite and mtDNA control region) for prairie-chicken populations

Population	Sample size (micro/mtDNA)	Microsatellite DNA (6 loci)			mtDNA control region		
		Mean number of alleles/locus	Allelic richness (±SE)	$H_e \pm SE$	Number of haplotypes	Haplotype diversity (h ± SE)	Nucleotide diveristy ($\pi \pm SE$)
Illinois							
Historic (1936–1970)[a]	–/22	–	–	–	8	0.883 ± 0.007	0.007 ± 0.000
Pre-trans (1971–1991)	32/32	5.2	4.7 ± 0.9	0.654 ± 0.055	4	0.728 ± 0.007	0.005 ± 0.000
Post-trans (2003)	18/18	5.5	5.5 ± 0.6	0.676 ± 0.058	7	0.876 ± 0.009	0.013 ± 0.000
Kansas (1999)[b]	47/20	10.3	8.4 ± 1.7	0.763 ± 0.060	11	0.858 ± 0.015	0.010 ± 0.002
Nebraska (1997–98)[b]	48/20	10.5	8.0 ± 1.7	0.731 ± 0.081	15	0.968 ± 0.006	0.009 ± 0.001
Minnesota-North (1999)[b]	45/20	9.5	7.3 ± 1.7	0.729 ± 0.063	9	0.847 ± 0.014	0.009 ± 0.001
Minnesota-South (1999)[e]	35/20	9.0	7.7 ± 1.6	0.721 ± 0.083	8	0.889 ± 0.008	0.010 ± 0.000
Missouri (1999)[b]	20/20	7.7	7.5 ± 1.4	0.709 ± 0.081	8	0.842 ± 0.010	0.012 ± 0.000
Wisconsin historic							
Mead (1951–1954)[c]	29/18	8.7	7.6 ± 1.6	0.718 ± 0.078	11	0.941 ± 0.008	0.010 ± 0.001
Paul Olson (1951–1954)[c]	25/19	8.3	7.7 ± 1.6	0.703 ± 0.099	10	0.860 ± 0.016	0.008 ± 0.000
Buena Vista (1951)[c,d]	42/19	9.2	7.6 ± 1.2	0.725 ± 0.066	10	0.889 ± 0.013	0.012 ± 0.001
Leola (1951)[c]	29/17	8.5	7.6 ± 1.1	0.709 ± 0.086	9	0.890 ± 0.013	0.012 ± 0.000
Wisconsin bottlenecked							
Mead (1998–00)[b]	32/20	6.3	5.5 ± 0.9	0.598 ± 0.081	3	0.484 ± 0.025	0.010 ± 0.002
Paul Olson (1998–99)[b]	33/20	5.1	4.4 ± 0.8	0.597 ± 0.065	4	0.679 ± 0.017	0.016 ± 0.004
Buena Vista (1998–00)[b,d]	87/20	7.0	5.6 ± 1.1	0.560 ± 0.119	5	0.511 ± 0.029	0.013 ± 0.003
Leola (1998–00)[b]	29/20	6.2	5.4 ± 0.7	0.560 ± 0.105	6	0.784 ± 0.014	0.014 ± 0.003
Attwater's prairie-chicken							
Historic (1887–1948)[a,e]	–/19	–	–	–	12	0.912 ± 0.011	0.009 ± 0.000
Bottlenecked (1990–1994)	36/36	6.5	5.9 ± 1.0	0.723 ± 0.051	8	0.751 ± 0.011	0.008 ± 0.001

Population samples include historic (Illinois Historic), pre- (Illinois Pre-trans) and post-translocation (Illinois Post-trans) samples from the Illinois focal population, samples from large contemporary populations in Kansas, Nebraska, Minnesota and Missouri, and pre- and post-bottlenecked populations from Wisconsin and the Attwater's prairie chicken

[a] Microsatellite frequencies not evaluated due to small sample size and potential presence of null alleles

[b] Johnson et al. (2003)

[c] Johnson et al. (2004)

[d] Bellinger et al. (2003)

[e] Johnson and Dunn (2006)

H_e, Expected heterozygosity

addition, we performed a genetic analysis of historic and contemporary populations that had a documented history of demographic bottlenecks. These samples included pre- and post-bottleneck samples from four Wisconsin prairie-chicken populations and the Attwater's prairie-chicken (APC; Table 1).

DNA was extracted from blood of adult prairie-chickens collected from contemporary populations, and either toe pads or feather tissue of museum specimens or wings from historical populations. Methods for DNA extractions are described elsewhere (Bouzat et al. 1998a; Johnson et al. 2003, 2004, 2007; Johnson and Dunn 2006). All historic prairie-chicken samples were extracted in an independent laboratory facility that had not been exposed previously to prairie-chicken DNA, while using standard methodology for ancient DNA analysis to reduce the potential for contamination.

Microsatellite analysis

The microsatellite analysis was based on the amplification and typing of six microsatellite loci originally designed for the domestic chicken (ADL44, ADL146, ADL230) and the red grouse (*Lagopus lagopus*; LLST1, LLSD4, LLSD9). Amplification and genotyping procedures are described elsewhere (Bouzat et al. 1998a; Johnson et al. 2003, 2004, 2007). Genetic diversity estimates were calculated using the programs ARLEQUIN (Excoffier et al. 2005) and FSTAT

(Goudet 1995), which included mean number of alleles per locus, allelic richness, and observed (H_o) and expected (H_e) heterozygosities. Pre- and post-translocation levels of genetic diversity were compared to each other and to average levels of genetic diversity obtained from non-bottlenecked populations using the Wilcoxon signed rank test, which paired the data by locus. Non-bottlenecked populations included large contemporary populations as well as historic pre-bottlenecked populations from Wisconsin. Fisher exact tests for Hardy–Weinberg equilibrium frequencies were performed for each locus/population combination using the Bonferroni correction for multiple comparisons.

Indirect estimates of population inbreeding coefficients (F) were obtained from the ratio of observed and expected heterozygosities for each population sample (Hedrick 2000). In addition, we calculated the average or effective inbreeding coefficients (F_e) for the pre- and post-translocation samples as $1 - (H_{inbred}/H_{outbred})$ (Frankham et al. 2002), using the average heterozygosity of non-bottlenecked large populations as a reference outbred population.

In addition to the standard measures of genetic diversity, we performed a cluster analysis using the program STRUCTURE (Pritchard et al. 2000) to evaluate the effectiveness of the translocation in restoring historical levels of genetic diversity. STRUCTURE implements a model-based clustering method for inferring population structure, assigning individuals to populations, and identifying migrants and admixed individuals using unlinked multilocus genotype data. Runs were performed using the Admixture Model with correlated frequencies among populations because this model has better power to detect subtle population structure (Pritchard et al. 2000). After a burnin period of 100,000 replications, an additional 500,000 replications were used to compute the posterior probabilities for identifying the number of distinct populations (K) from the data. We performed at least four independent runs for each K to verify consistency in our estimates of posterior probabilities.

Mitochondrial DNA analysis

PCR was conducted to amplify and sequence a DNA fragment of 394 base pairs (bp) of the mitochondrial DNA control region (Domain I) from 623 samples collected throughout the historic and contemporary range of the species (see Table 1). PCR amplification conditions and DNA primers are described elsewhere (Johnson et al. 2003, 2004). Following PCR, samples were sequenced using either a Beckman Coulter TDCS kit (Beckman Coulter, Fullerton, CA) and run on a CEQ 8000 capillary sequencer or with ABI Big Dye Terminator chemistry (Applied Biosystems, Foster City, CA) run on an ABI 3730 automated sequencer. Estimates of mtDNA diversity (i.e., haplotype diversity and nucleotide diversity) and corresponding standard errors were obtained using the program DNASP (Rozas et al. 2003). DNA sequences from all populations but one (the contemporary WI-Paul Olson population) conformed to neutral expectations as determined by Tajima's *D* statistic (Tajima 1989). As with microsatellites, pre- and post-translocation levels of mtDNA genetic diversity were compared to each other and to average levels of genetic diversity obtained from non-bottlenecked populations using standard *t*-tests (Nei 1987).

Demographic trends

Abundance estimates from the Illinois prairie-chicken population from 1972 to 2006 were obtained from a long-term demographic study initiated in 1962, which involved cooperative efforts by the Illinois Department of Natural Resources and the Illinois Natural History Survey. This study represents one of the largest long-term datasets from a wild avian population, with over 40 years of demographic, fitness, and genetic diversity data. Abundance and fitness data from 1963 to 1997 were originally reported in Westemeier et al. (1998a). In the present study, we report post-translocation census data from 1998 to 2006. Abundance estimates are based on male prairie-chicken census using standard methods for prairie-chickens (Hamerstrom and Hamerstrom 1973). Observed hens are not used for abundance estimates because they represent a lesser and more variable proportion of their actual numbers than is typical for males. Changes in egg success (average number of eggs hatched per successful nest) and fertility rates (average number of fertile eggs per nest) between 1982 and 1997 were reported elsewhere (Westemeier et al. 1998a). In this study, we report additional historical rates of nest success (defined as the proportion of successful nests that hatched at least one egg) based on 312 nests recorded between 1963 and 1972, and pre-translocation rates for the period 1973–1987 (based on a total of 547 nests). For this estimate, we included only nests with known fate, excluding those nests that were known to be unsuccessful as a result of human disturbance. As expected, nest success is subjected to significant levels of variation given that this estimate is more likely to be affected by non-genetic factors (i.e., unsuccessful nests may result from abandonment, predation, flooding, etc.). Egg success, egg fertility, and nest success data reported in this study are based on a total count of 1,003 nests from the focal population in Jasper County, Illinois, detected between 1963 and 1991. Successful nests parasitized by ring-necked pheasants (*Phasianus colchicus*) were excluded for calculating egg success.

Results

Genetic analysis of six microsatellite loci revealed that, prior to the translocation of birds into the Illinois population, both heterozygosity and allelic richness were lower than those of contemporary large populations in Kansas, Nebraska, Missouri, and Minnesota, and historic pre-bottleneck populations in Wisconsin (Table 1). This trend was more evident for allelic richness, which showed, on average, 39% significant less diversity ($P < 0.027$) in the Illinois pre-translocation sample (Table 1 and Fig. 1). Although the expected heterozygosity (H_e) in the pre-translocation sample ($H_e = 0.654$) was $\sim$10% lower than the average value of large populations ($H_e = 0.723$), this difference was not significant. As expected, the pre-translocation estimates of heterozygosity and allelic richness of the Illinois population were not significantly different from those of other similarly bottlenecked populations from contemporary Wisconsin greater prairie-chickens and the federally endangered APC (*T. c. attwateri*) (Table 1 and Fig. 1). These results are consistent with those of a previous study (Bouzat et al. 1998a) that identified alleles in museum specimens from the historic Illinois population not present in the population prior to the translocations, therefore providing direct evidence that the demographic decline in the Illinois prairie-chicken population during the past century had resulted in a reduction in genetic diversity.

In contrast, the Illinois population 10 years after the initial translocation of birds maintained a 16% increase in microsatellite allelic richness and a 4% increase in H_e compared to pre-translocation levels (Table 1 and Fig. 1). Allelic richness increased from 4.728 (SE = 0.875) in the pre-translocated population to 5.500 (SE = 0.619) after the translocation, whereas H_e increased from 0.654 (SE = 0.055) to 0.676 (SE = 0.018). Although pre- and post-translocation levels of genetic diversity were not significantly different, probably due to low statistical power, comparisons with historical and non-bottlenecked populations were consistent with the idea that translocations effectively increased levels of genetic diversity at the microsatellite level. It is worth noting, however, that neither allelic richness nor expected heterozygosity in the 2003 sample showed higher levels of diversity than those observed in any of the contemporary large and historical populations (Table 1). This is not unexpected since levels of genetic variability in this population following the completion of translocations in 1996 have most likely declined due to genetic drift given its current census population size ($n = 51$ males in 2007). A multiple comparison of observed and expected heterozygosities per locus and population revealed Hardy–Weinberg equilibrium conditions for most loci/populations, with the exception of microsatellite locus LLSD9 in the Illinois pre-translocation and the Atwatter's prairie-chicken populations. This microsatellite locus showed a consistent deficiency of heterozygotes in both populations.

Indirect estimates of inbreeding coefficients revealed that the IL pre-translocated population had considerably higher levels of inbreeding (F = 0.200) than the average level estimated from large non-bottlenecked populations (F = 0.022; SE = 0.013). Following translocations, the inbreeding coefficient of the IL population decreased in half, resulting in an F = 0.099. Estimates of effective inbreeding coefficients were consistent with these results,

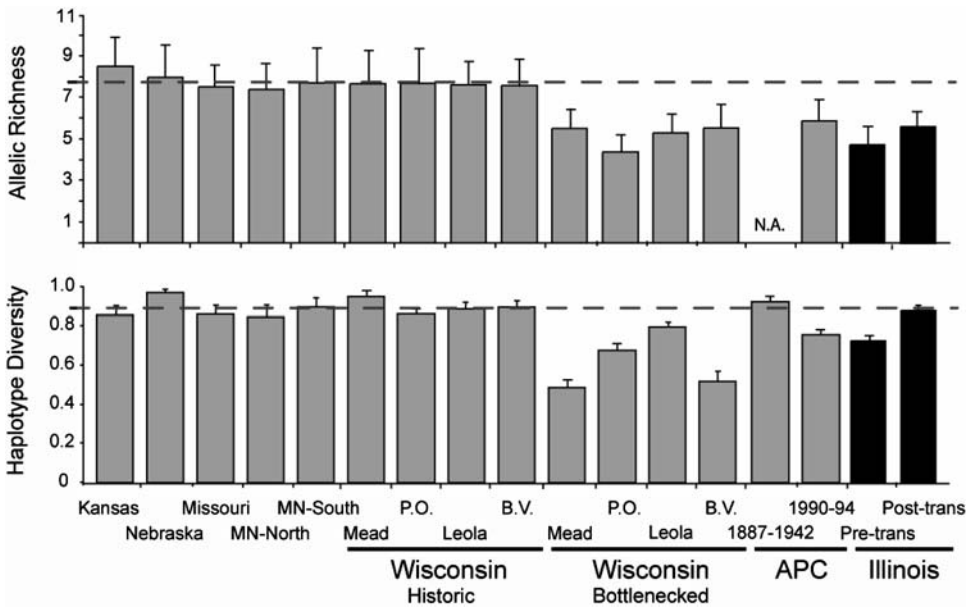

Fig. 1 Pre- (1974–1993) and post- (2003) translocation levels of genetic diversity (microsatellite allelic richness and mtDNA haplotype diversity) in the Illinois population (*black bars*) compared to five large contemporary populations (Kansas, Nebraska, Missouri and Minnesota-North and South), as well as five historical and contemporary populations from Wisconsin and the APC), which have gone through documented demographic bottlenecks. The dashed line represents the mean allelic richness/haplotype diversity value for non-bottlenecked populations

showing a decline in the level of inbreeding following translocations ($F_e = 0.095$ and 0.065 for the pre- and post-translocation samples, respectively).

Consistent with the microsatellite data, pre-translocation levels of mtDNA diversity in Illinois were also comparable to those of bottlenecked populations from Wisconsin as well as the contemporary population of the APC (Fig. 1 and Table 1). Following the translocation of birds into the Illinois population, mtDNA haplotype diversity increased significantly ($P < 0.001$) by ~22% from 0.728 (SE = 0.007) in the pre-translocated to 0.876 (SE = 0.009) in the post-translocated population (Fig. 1). Post-translocation levels of mtDNA diversity were not significantly different from the average level of haplotype diversity found in contemporary large populations as well as in the 1930–1960s historic Illinois greater prairie-chicken population (see Table 1). No consistent trends were observed with estimates of nucleotide diversity between historical and bottlenecked populations (i.e., Illinois and Wisconsin populations; Table 1). However, translocations into the Illinois population resulted in a significant increase in nucleotide diversity from 0.005 to 0.013 ($P < 0.01$).

To evaluate the effectiveness of the translocation program in recovering microsatellite levels of genetic diversity prior to the population bottleneck, we used a clustering method that allowed the identification of distinct subgroups based on multilocus genotype data, as implemented in the computer program STRUCTURE (Pritchard et al. 2000). This analysis, which included data from contemporary populations from Kansas, Nebraska, Missouri and Minnesota, historical pre-bottleneck populations from Wisconsin, and the pre- and post-translocation population samples from Illinois, revealed the highest posterior probability for clustering individuals into two major groups (K = 2; Fig. 2). These included one cluster consisting of the Illinois pre-translocated population and a second cluster grouping the Illinois post-translocated population with the contemporary populations from Kansas, Nebraska, Missouri and Minnesota, and the historical populations from Wisconsin. The estimated proportion of membership to each cluster revealed that translocations increased the proportion of Illinois individuals assigned to the cluster that included large contemporary and historical non-bottlenecked populations (Fig. 2). The proportion of membership in this cluster changed from 0.372 in the pre-translocated to 0.715 in the post-translocated sample, which indicate that translocations were effective in restoring the Illinois population as part of the common historical gene pool of the greater-prairie chicken. An analysis using all populations studied, including bottlenecked APC and contemporary Wisconsin greater prairie-chicken populations (see Table 1), provided similar results clustering the Illinois pre-translocation samples independently from the post-translocation samples, which again grouped together with samples from other non-bottlenecked populations throughout the distribution range of the greater prairie-chicken.

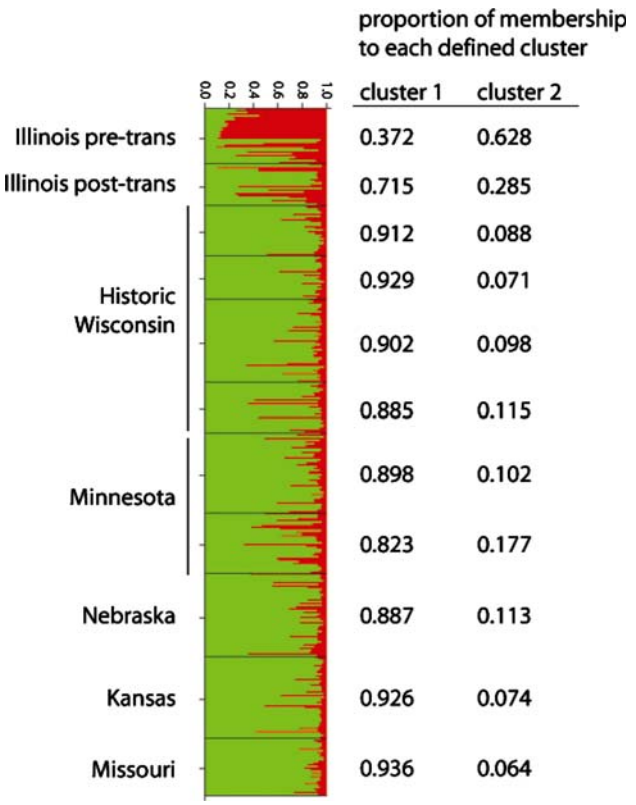

Fig. 2 Estimated population structure for contemporary populations from Kansas, Nebraska, Minnesota, and Missouri, historical populations from Wisconsin, and the pre- and post-translocation populations from Illinois. Each individual is represented by a horizontal line identifying the individual's estimated membership to a particular cluster (K = 2). Black lines separate sampling locations. The estimated proportions of membership to each cluster are also indicated. The figure shows a run for K = 2 where the Illinois population following translocation has clustered with other historical and contemporary populations as part of a common historical gene pool, while the pre-translocation Illinois population is diagnostically distinct from the other populations

Based on the mtDNA analysis, our results suggest that maternal lines historically present in the Illinois population were retained in the post-translocated population. The number of mtDNA haplotypes increased from 4 haplotypes in the pre-translocation sample to seven haplotypes following translocations (Table 2). Three of these seven haplotypes were present in Illinois prior to the translocations and, in fact, one of the retained haplotypes has only been documented in the Illinois population (based on a total of 572 samples sequenced throughout the species' range). This unique mtDNA haplotype was detected at intermediate frequencies in both pre- and post-translocation samples, as well as in the historic Illinois population represented by museum specimens collected prior to 1970

Table 2 Mitochondrial DNA haplotype distribution in the historic (Historical), pre- (Pre-trans) and post-translocation (Post-trans) samples from the Illinois population

Haplotype	Historical	Pre-trans	Post-trans
Hap4	4	0	1
Hap7	2	0	0
Hap12	1	0	0
Hap14	0	0	1
Hap36	5	0	0
Hap37	0	0	3
Hap48	2	13	3
Hap65	0	0	4
Hap83	0	0	2
Hap89	1	0	0
Hap122	3	9	4
Hap135	4	6	0
Hap136	0	4	0

Values indicate number of individuals with the specified haplotype. Haplotype Hap122 is unique to Illinois, while all other haplotypes have been sampled at least once either in historic or pre-translocation samples from Illinois, or elsewhere among sampled prairie-chicken populations

(Table 2). The remaining four haplotypes represented mtDNA lineages sampled throughout the distribution range of this species. The characterization of mtDNA haplotypes in the Illinois post-translocated population confirmed, therefore, the presence of Illinois historical as well as introduced maternal lines. The analysis of the 2003 post-translocation samples revealed that 10 of the 18 individuals (56%) sampled showed four mtDNA haplotypes that were not present in the pre-translocation nor in the historical samples from Illinois, which suggests that these haplotypes may have originated from the translocated birds.

Demographic trends on the abundance of the Illinois population estimated between 1972 and 2006, as well as prior estimates on fitness, allowed an indirect assessment of population viability (Fig. 3). The number of prairie-chickens in the Illinois population in Jasper County consistently decreased from 206 males counted on 13 leks in 1972 to six males remaining on a single ephemeral lek in 1994 (Westemeier et al. 1998a). Subsequently, following the translocation of 271 greater prairie-chickens (144 females and 127 males) between 1992 and 1996, numbers increased to 70 males counted on six leks (Fig. 3). As reported by Westemeier et al. (1998a), these translocations appeared to have rescued the population from local extinction by restoring hatching success and fertility to historical levels (94% and 99%, respectively, for the period 1963–1972; $n = 291$ nests; Westemeier et al. 1998a). Estimates of nest success showed similar trends, with a significant decline from 70% (95% CI = 62–78%)

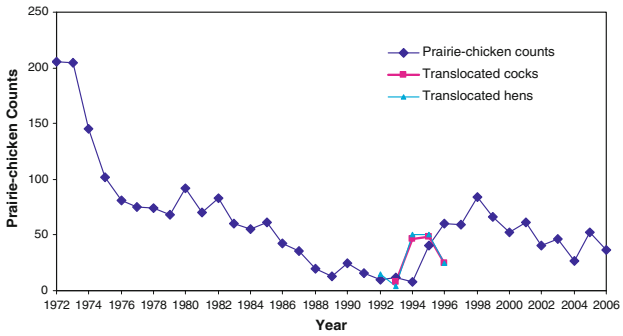

Fig. 3 Changes in greater prairie-chicken abundance between 1972 and 2005, as estimated by the number of prairie-chicken males (*diamonds*) on booming grounds in Jasper County, Illinois. Number of males (*squares*) and females (*triangles*) introduced between 1992 and 1996 are indicated in *dark* and *light grey*, respectively

estimated for the period 1963–1972 ($n = 312$ nests) to 51% (95% CI = 44–58%) between 1973 and 1987 ($n = 547$ nests). Following the 1992–1996 translocation period, a survey of 20 prairie-chicken nests from 1997 to 2000 reported an increase in nesting success to 55% ($n = 20$ nests; Walk 2004). Although these trends are consistent with the significant increases in hatchability and fertility rates previously documented (Westemeier et al. 1998a), confidence intervals prevented detecting statistical significance for the post-translocation increase in nest success. This is not unexpected since variation in the estimates of nesting success can be highly influenced by multiple factors resulting in unsuccessful nests (e.g., nest abandonment, predation, flooding, etc.).

In spite of the rapid rebound in fitness and population size following translocations, abundance estimates have remained relatively low for the past 10 years, with an average of 53.18 (SE = 4.68) booming males counted in 6.36 (SE = 0.61) leks between 1996 and 2006 (Fig. 3). This low level of abundance has remained unchanged despite an increase in available habitat as part of the prairie-chicken sanctuaries of Jasper County. Prior to 1991, protected prairie-chicken habitat included 470 ha owned or leased by the Illinois Department of Natural Resources (229 ha) and The Nature Conservancy (241 ha). During the period 1991–2006, the Illinois Department of Natural Resources and the Audubon Society incorporated an additional 622 ha of land to the greater prairie-chicken sanctuaries, which represented a 132% increase in the area of protected habitat that existed prior to 1991. Although prairie-chickens may not be using all available habitat, we have documented the development of leks on new land acquisitions adjacent to existing habitat after habitat restoration. Notwithstanding, the Jasper population reached one of the lowest abundances in 2006, with 37 males surveyed in four leks.

Discussion

We have documented that bird translocations can be used as an effective tool for the transient genetic restoration of small isolated populations. The genetic analysis of both microsatellites and mtDNA markers indicated that prior to the translocation of prairie-chickens to Illinois, this population had experienced a significant decline in genetic diversity compared to that estimated from historical and contemporary non-bottlenecked prairie-chicken populations. Translocation of birds from other populations has effectively increased levels of genetic diversity at both the nuclear and mitochondrial DNA levels, which, after ten years, have remained higher than pre-translocation levels (16% and 22% higher for allelic richness and haplotype diversity, respectively). As expected with recently bottlenecked populations, changes in genetic diversity at the nuclear level were more apparent with measures of allelic diversity as opposed to heterozygosity, which showed a positive but non-significant trend (4%) following translocations. Similarly, haplotype diversity revealed more consistent trends than nucleotide diversity when considering the effects of past demographic bottlenecks on mtDNA variation (see also Johnson et al. 2007). Allelic richness and haplotype diversity proved, therefore, to be useful measures for monitoring the genetic effects of translocations at the nuclear and mtDNA levels, respectively.

Changes in genetic variation following translocations were also reflected in the estimates of inbreeding coefficients. Prior to the translocations, the IL greater prairie-chicken population revealed 10 times higher levels of inbreeding ($F = 0.20$) than the average large non-bottlenecked population ($F = 0.02$; $SE = 0.01$). Ten years after the initiation of the translocations, the inbreeding coefficient decreased in half ($F = 0.10$), but it was still considerably higher than that estimated from large populations. This is not unexpected, since the Illinois population remained considerably small after translocations (~ 50 males) and, thus, likely to be subjected to further inbreeding.

The STRUCTURE analysis of pre- and post-translocation samples indicated that translocations were effective in restoring the genetic composition of the Illinois population to the historical gene pool of the greater prairie-chicken. Post-translocation samples consistently clustered together with other populations throughout the range of the species, including contemporary large populations from Kansas, Nebraska, Missouri and Minnesota as well as historic pre-bottlenecked populations from Wisconsin. This is consistent with previous studies demonstrating that greater prairie-chicken populations from the central prairies were historically interconnected throughout their range, with populations recently becoming differentiated largely as a result of increased habitat fragmentation, isolation and small population size (Johnson et al. 2003, 2004).

In spite of obvious benefits, it is also known that translocations may have detrimental effects associated with the potential loss of local adaptation in the focal population (Tallmon et al. 2004; Clewell 2000; Frankham et al. 2002; Hufford and Mazer 2003; Edmands 2007). Therefore, concerns are warranted about maintaining the genetic integrity of remnant populations (i.e., retaining as much of the original gene pool as possible) and avoiding the replacement of the entire original population by translocated birds (i.e., genetic swamping). In our study, the analysis of the historical distribution of mtDNA haplotypes throughout the range of the species as well as the temporal characterization of historical, pre-translocation and post-translocation samples from the Illinois population allowed an assessment of the genetic introgression that resulted from translocations into the Illinois population. The increase in the number of mtDNA haplotypes in the post-translocated sample suggests that some independent maternal lines have been successfully introduced into Illinois from other states, increasing the number of mtDNA lineages from 4 to 7. Although most of the mtDNA haplotypes from the post-translocated Illinois population have been observed throughout the geographic range of the species, three of these were detected in Illinois prior to the translocations, with one being unique to this population (Table 2). The retention of mtDNA haplotypes historically present in the Illinois population confirms, therefore, that the overall increase in genetic diversity resulted from the genetic admixture between individuals from the focal and source populations, and not entirely from the genetic swamping of the Illinois population by translocated birds. The analysis of the 2003 post-translocation sample revealed, however, that the current Illinois population may have 56% female ancestry from translocated birds (i.e., assuming all haplotypes have been sampled). In the case of the greater prairie-chicken, this significant level of population mixing may not be a major concern since the STRUCTURE analysis and previous studies (Bouzat et al. 1998b; Johnson et al. 2003, 2004, 2007; Johnson and Dunn 2006) have demonstrated that historical populations from the central prairies (i.e., the source of translocated birds) represented a common gene pool. It is important to mention that these studies focused on neutral genetic markers (both at the nuclear and mitochondrial DNA levels), which may not have direct effects on functional genes. Although arguments warning against the potential genetic swamping of neutral variation are important, particularly on the long-term, managers should not disregard the potential swamping of functional genetic diversity, which could still occur if populations are locally adapted (Crandall et al. 2000).

Although overall bird translocations were effective in restoring genetic diversity to historic levels, demographic trends show that the potential recovery and population viability of the species still remains uncertain. As previously demonstrated, the translocation of birds from larger populations into Illinois resulted in a rapid increase in population size and fitness as measured by fertility and egg hatching rates (Westemeier et al. 1998a). However, despite these improvements in fitness, abundance estimates for this population have stabilized at relatively low numbers (53 males; SE = 4.7; $n = 10$) following the translocation period (1997–2006; Fig. 3). This suggests that current increases in population size and viability may be limited by demographic and/or environmental factors such as juvenile mortality, predation, competition with exotic species, and availability of suitable habitat, rather than genetic factors associated with inbreeding depression and overall reduced levels of genetic variation.

Sensitivity and life-stage simulation analyses in greater prairie-chickens revealed that nest success and brood survival represent vital rates with the greatest effect on population growth (Wisdom and Mills 1997; Wisdom et al. 2000). For example, first-year survivorship for this species accounted for >85% of the variation in population growth. Although nest success in established greater prairie-chicken populations has been reported at 46–50%, survival of fertile eggs can be as low as 20%, and early brood mortality can range between 45% and 85% (Wisdom and Mills 1997; Johnsgard 1983). In the Illinois population, Westemeier et al. (1998a) documented significant increases in both fertility and hatchability rates following translocations. Our estimates of nest success showed similar trends, with a considerable decline from 70% (95% CI = 62–78%; $n = 312$ nests) for the period 1963–1972 to 51% (95% CI = 44–58%; $n = 547$ nests) between 1973 and 1987, and a reported rebound to 55% ($n = 20$ nests) following the 1992–1996 translocation period (Walk 2004). Predation by mammals, birds and snakes, and nest parasitism by ring-necked pheasants (*Phasianus colchicus*) have shown to have direct impacts on nest abandonment and egg hatching success (Berger et al. 1962; Hamerstrom et al. 1964; Vance and Westemeier 1979; Westemeier et al. 1998b). The implementation of management activities aimed at decreasing levels of predation and nest parasitism did not result, however, in significant increases in population size (Walk 2004). In spite of the documented increases in the Illinois population's vital rates following translocations, the lack of consistent increases in abundance suggests that both habitat availability and habitat quality represent major limiting factors for the long-term persistence of this population. Although the area of protected habitat prior to the translocations (470 ha) has increased to a total of 1,092 ha in 2006 and management strategies for habitat restoration are currently being implemented (Svedarsky et al. 1999), the amount of suitable habitat for the establishment of successful leks may still be well below the threshold for population recovery. In fact, based on demographic trends of the greater-prairie chicken in Wisconsin between 1950 and 2006, Toepfer (2007) has estimated that over 225,000 acres (i.e., over 91,000 ha) of biologically interconnected grassland reserves would be required to sustain a viable population of 2,500 individuals. It is therefore clear that, as with many other species of conservation concern, habitat availability remains the most important factor determining the long-term viability of natural populations.

Although habitat quality and quantity may be affecting the Illinois greater prairie-chicken population, genetic deterioration may still represent a significant problem. The low effective size of the Illinois population suggests that additional inbreeding may accumulate rapidly even amongst the new alleles introduced into the population. This is apparent from the inbreeding coefficient estimates, which showed that, compared to average estimates from large populations (F = 0.022; SE = 0.013), there were considerable levels of inbreeding (F = 0.099) ten years after the translocations. It is therefore likely that more recent estimates of reproductive success might indicate decreased productivity associated with additional inbreeding.

This study demonstrated that controlled translocations can be effective management strategies for the genetic restoration of critically endangered populations, not only by removing detrimental variation associated with inbreeding depression (i.e., genetic rescue) but also by restoring neutral variation to historical levels. Further, this study emphasizes that genetics and demography cannot be considered independently when assessing the recovery of small populations on the verge of extinction (Soulé and Mills 1998). Although conservation programs may require genetic interventions through supplementation from wild and captive populations, the demographic recovery and long-term viability of endangered populations will not be effective unless these are complemented with management strategies aimed at adequate habitat conservation. As in the case of the Illinois prairie-chicken population, it is likely that current levels of genetic diversity are in decline due to small population size; therefore, this population will require additional genetic monitoring and possibly future supplementation. However, the long-term persistence of the Illinois prairie-chicken will not be guaranteed unless adequate habitat is secured. As Hamerstrom et al. stated back in (1957): "Grassland is of vital importance to prairie-chickens, the keystone in prairie chicken ecology … no grass, no chickens."

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