

Genetic evaluation of a proposed introduction: the case of the greater prairie chicken and the extinct heath hen

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Abstract

Population introduction is an important tool for ecosystem restoration. However, before introductions should be conducted, it is important to evaluate the genetic, phenotypic and ecological suitability of possible replacement populations. Careful genetic analysis is particularly important if it is suspected that the extirpated population was unique or genetically divergent. On the island of Martha's Vineyard, Massachusetts, the introduction of greater prairie chickens (*Tympanuchus cupido pinnatus*) to replace the extinct heath hen (*T. cupido cupido*) is being considered as part of an ecosystem restoration project. Martha's Vineyard was home to the last remaining heath hen population until its extinction in 1932. We conducted this study to aid in determining the suitability of greater prairie chickens as a possible replacement for the heath hen. We examined mitochondrial control region sequences from extant populations of all prairie grouse species (*Tympanuchus*) and from museum skin heath hen specimens. Our data suggest that the Martha's Vineyard heath hen population represents a divergent mitochondrial lineage. This result is attributable either to a long period of geographical isolation from other prairie grouse populations or to a population bottleneck resulting from human disturbance. The mtDNA diagnosability of the heath hen contrasts with the network of mtDNA haplotypes of other prairie grouse (*T. cupido attwateri*, *T. pallidicinctus* and *T. phasianellus*), which do not form distinguishable mtDNA groupings. Our findings suggest that the Martha's Vineyard heath hen was more genetically isolated than are current populations of prairie grouse and place the emphasis for future research on examining prairie grouse adaptations to different habitat types to assess ecological exchangeability between heath hens and greater prairie chickens.

Keywords: ancient DNA, conservation genetics, heath hen, mtDNA, population introduction, *Tympanuchus*

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Introduction

An emerging goal of conservation biology is to restore degraded ecosystems. When local extirpation has removed a species from an ecosystem, population introduction may be a useful tool for ecological restoration. Such a strategy would be applicable only after careful analysis revealing that the original causes of the extirpation could be

ameliorated and that natural recolonization is unlikely. The World Conservation Union (IUCN) states that, for introduction projects, individuals should be taken from the same subspecies as those extirpated (IUCN 1995); however, this may not be possible in cases where entire subspecies have become extinct. In such cases, efforts must be made to evaluate the suitability of possible replacement populations. Such efforts should consider the genetic relatedness, phenotypic and ecological similarity and conservation value of introduction candidates (Seddon & Soorae 1999). In this study, we use mitochondrial

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DNA (mtDNA) sequences to examine genetic divergence between the extinct heath hen (*Tympanuchus cupido cupido*) and extant populations of greater prairie chickens (*T. c. pinnatus*) to help determine whether the greater prairie chicken represents a suitable substitute for introduction.

The decline of the heath hen began when the first Europeans arrived on the East Coast of North America. Before European settlement, Native Americans used fire to maintain a mosaic of forests, shrublands, agricultural fields and grasslands (Pyne 1982). After European diseases decimated Native American populations, the formerly open habitats of the Northeast became largely forested, resulting in major changes to bird communities (Droge 1998) and probably reducing suitable heath hen habitat. Although the clearing of forests by European settlers probably once again increased heath hen habitat, hunting pressure was extreme, and by 1821 the formerly common bird was rare in New England (Forbush 1927). The last reports of heath hens in Pennsylvania and New Jersey are from 1869, and it is doubtful that the heath hen survived much after that on mainland North America (Forbush 1927). After that time, it was restricted to the island of Martha's Vineyard, Massachusetts. On this island, the heath hen's doomed struggle against extinction played out. After a fire in 1906 reduced the population to approximately 80 individuals, the Massachusetts Legislature created a refuge for the remaining birds. Extensive efforts to preserve the heath hen included a failed reintroduction to Long Island, attempted predator eradication, fire control and the planting of cultivated crops for dietary supplementation (Gross 1928). On Martha's Vineyard it appeared that these efforts were paying off, as E. H. Forebush counted 800 heath hens on the refuge in April 1916 and estimated that there were 2000 heath hens living on the island (Forbush 1927). However, on 12 May 1916 a massive fire broke out on Martha's Vineyard, burning nearly 20% of the island during nesting season and devastating the heath hen population. The fire was followed by heavy raptor predation the following year and the suspected eruption of a poultry disease, all of which contributed to the ultimate decline of the heath hen (Gross 1928). The world's last heath hen, affectionately nicknamed 'Booming Ben', died in 1932, having been the lone survivor of his kind for nearly 4 years (Cokinos 2000). Detailed descriptions of the events leading to the extinction of the heath hen are given in Gross (1928) and Cokinos (2000).

Because the heath hen did not gain the attention of ornithologists and museum collectors until after the mainland population had been extirpated, there is uncertainty regarding the extent of the heath hen's original geographical range and the scope of its morphological variation. The portion of the heath hen's range from Virginia north into New England (depicted in Fig. 1) is well documented. However, Brewster (1885) and Gross (1928) speculate that the heath

hen may once have roamed as far south as the Carolinas and as far west as western Pennsylvania, eastern Ohio and Kentucky, possibly contacting populations of Midwestern greater prairie chickens, although the information supporting this view is sparse.

Because no mainland heath hens appear to have been preserved in museum collections (Gross 1928), the scope of the morphological variation of the heath hen throughout its original range will never be known. W. Brewster's original description in 1885, based solely on birds from Martha's Vineyard, highlights the morphological differences between heath hens and Midwestern greater prairie chickens. Heath hens were smaller in size and had shorter tarsi than greater prairie chickens. Their neck tufts contained four to five rather than seven to 10 pinnae (rigid neck feathers erected during courtship displays), which were narrower and acutely rather than obtusely lance-pointed, and the heath hen's plumage was more red above and more white below than that of the Midwestern greater prairie chicken (Brewster 1885). However, in a subsequent study A. O. Gross noted his difficulty in telling the two subspecies apart based on external morphological differences (Gross 1928).

In addition to the heath hen, the other prairie grouse of the genus *Tympanuchus* have been negatively impacted by human activity, and their ranges have been greatly reduced since historical times (Fig. 1). The Attwater's prairie chicken (*T. c. attwateri*), which once occurred along the Gulf Coast of Texas and Louisiana, declined as a result of agriculture and urban development and is now restricted to two small populations in Texas. These populations are persisting largely as a result of supplementation from captive-bred birds and, at fewer than 70 individuals, face the pending threat of extinction (Silvy *et al.* 1999). The greater prairie chicken, which occupied the tallgrass prairies of the Midwestern United States, has declined substantially as cropland has gradually replaced native grasslands (Svedarsky *et al.* 2000). The lesser prairie chicken (*T. pallidicinctus*) of the southern Great Plains of the United States has declined more than 90% from historic levels due to the decline and fragmentation of native vegetative communities (Fuhlendorf *et al.* 2002). Similarly, agriculture has taken a toll on sharp-tailed grouse (*T. phasianellus*) populations in the United States (Johnsgard 1983).

Headed by the Nature Conservancy and local conservation groups, efforts to restore native vegetation and a natural fire regime on Martha's Vineyard are under way. These efforts carefully consider the appropriate vegetation to restore. Palaeoecological research indicates that prairie-like grasslands were not a major component of the Martha's Vineyard landscape at the time of European settlement (Foster & Motzkin 1999; Motzkin & Foster 2002). Instead, open oak shrub and ericaceous communities may have dominated. According to Gross (1928), this type of heathland, dominated by scrub oak (*Quercus ilicifolia*) and

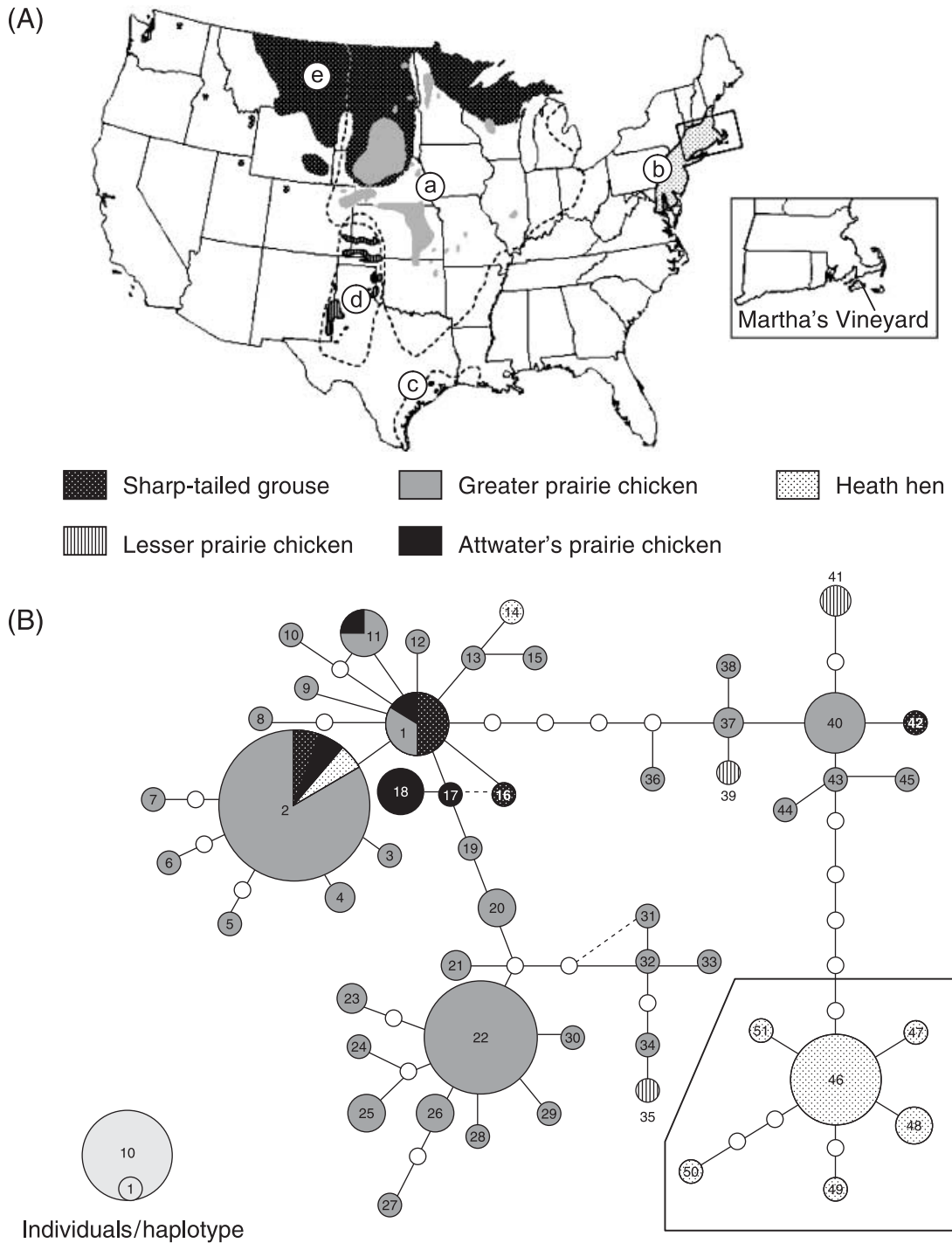


Fig. 1 (A) Map of current and historical ranges of *Tympanuchus* taxa. Ranges for (a) the greater prairie chicken, (b) the heath hen, (c) the Attwater's prairie chicken, (d) the lesser prairie chicken, and (e) the sharp-tailed grouse are depicted, with historical ranges outlined. The heath hen was extirpated from the mainland about 1870 and survived on the island of Martha's Vineyard until going extinct in 1932. (B) The haplotype network is derived from 480-bp of the mtDNA control region. The relative sizes of the circles represent the number of individuals contained within each haplotype (see legend, bottom left), and pie slices represent the fraction of each taxon contained within each haplotype. The shading in the network corresponds to that in the range map, with the greater prairie chicken in gray, the heath hen lightly stippled, the Attwater's prairie chicken in solid black, the lesser prairie chicken vertically striped, and the sharp-tailed grouse darkly stippled. Open circles represent missing haplotypes and dashed lines indicate that multiple connections between haplotypes are possible, but not favored. Haplotypes 46-51, contained within the bottom right polygon, are reliable heath hen haplotypes. Haplotypes 2 and 14 contain samples originally thought to be from heath hens, but which lacked collection information and may instead represent greater prairie chickens (see text).

huckleberry (*Gaylussacia baccata*), was typical of that used by the heath hens of Martha's Vineyard. Living in this semi-open habitat, the heath hen was a generalist forager on both native and non-native species. Gross (1928) reports of the heath hen opportunistically taking fresh shoots in the spring, insects, leaves (especially *Rumex acetosella*) and various fruits (*Myrica carolinensis*, *Arctostaphylos wa-ursi*, *Berberis vulgaris*, *Mitchella repens*, *Vaccinium* spp., *Rosa* spp.) during summer, and acorns year-round. Research by the Nature Conservancy and the Ecosystems Center of the Marine Biological Laboratory are ongoing to identify the structure, species composition and means of restoration of native heathland habitat.

This study was conducted to assess the genetic distinctness of the extinct heath hen and to provide insights on the biological options associated with reintroducing a population of greater prairie chickens as an ecological equivalent to Martha's Vineyard. As the heath hen was probably the most area-sensitive species on Martha's Vineyard, restoring sufficient habitat to support an introduced population of greater prairie chickens would serve the purpose of protecting many other populations of rare plants, avifauna and invertebrates of the heathlands.

Materials and methods

Our analysis includes a total of 117 *Tympanuchus* individuals, including 18 heath hens, eight Attwater's prairie chickens, 80 greater prairie chickens, four lesser prairie chickens and seven sharp-tailed grouse (Appendix I). No mainland heath hen specimens appear to exist in museum collections (Gross 1928). Therefore, all the heath hen specimens (with collection information) examined in this study were collected on Martha's Vineyard after the mainland population had been extirpated. Five specimens sampled from museum collections on Martha's Vineyard (MV1, MV2, MV3, MV4, MV5, see Appendix I) originated in private collections, and thus lacked detailed collection information. Total genomic DNA was extracted either from museum skin tissue samples (heath hens) or from blood samples. For heath hen museum skins, a small (1 mm²) piece of tissue was cut from the pad of one toe using a sterile scalpel blade. DNA was extracted using the DNEasy Tissue Kit and Tissue Protocol (Qiagen), slightly modified to reduce the likelihood of contamination and to increase yield (see Mundy *et al.* 1997). To further avoid contamination, all museum skin extractions were performed in a room separated from the main laboratory and designated for ancient DNA, and negative extraction controls were carried out. Extractions from modern blood samples were performed using the Easy DNA Kit (Invitrogen) and the standard manufacturer's protocol.

Primers for the polymerase chain reaction (PCR) of the entire mtDNA control region (CR) were designed initially

from the ND6 and tRNA-Phe genes of the domestic chicken (*Gallus gallus*) mitochondrial genome (Desjardins & Morais 1990). PCR amplification and sequencing of the entire 1148 base pair (bp) CR in a randomly chosen group of 20 greater and Attwater's prairie chickens revealed most of the variation to be in the first 500 bp of the CR, adjacent to the tRNA-Glu. This region corresponds to domain I and is typically highly variable in birds (Baker & Marshall 1997). Primers were designed to PCR and sequence this variable region. Primers EXT-DLFor (5'-GACTAATCCCAAGGACTACG-3') and INT-DL1Rev (5'-TGACTTCGTGAAAAGTGAGG-3') were used to amplify a 540 bp fragment from all modern samples. PCR amplification was performed in a Touchdown Thermocycler (Hybaid) using 50 µL reactions containing 1 µL genomic DNA, 10 mM Tris-HCL (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.8 mM DNTPs, 0.4 µM of each primer and 1.5 units of Amplitaq (Applied Biosystems). Each PCR run consisted of an initial 2-min denaturation step at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 56 °C and 1 min at 72 °C. PCR runs were concluded with a 5-min extension step at 72 °C. Negative PCR controls were run with each round of PCR.

Due to the degraded nature of DNA obtained from museum skin material (Cooper 1994; Landweber 1999), we were unable to amplify the entire 540 bp fragment from museum skin samples. Therefore, we designed additional primers to amplify shorter fragments: HH2For (5'-TGTAATCGGACATAAAACCT-3'), HH2Rev (5'-AGGTTTTATGTCCGATTACA-3'), HH4For (5'-CCCATTTGGTTATGCTCGAC-3') and HH4Rev (5'-GTTCGAGCATAACCAAATGGG-3'). Primer pairs EXT-DLFor and HH2Rev, HH2For and HH4Rev, and HH4For and INT-DL1Rev each amplify about 200 bp fragments, together spanning the entire region amplified in all modern samples. PCR reagents were as above with the addition of 0.01 mg BSA and the use of Amplitaq Gold (Applied Biosystems). Museum skin PCRs were run on a Robocycler Gradient 96 Temperature Cycler (Stratagene), with runs consisting of an initial 12 min denaturation step at 94 °C, followed by 40 cycles of 1 min at 94 °C, 1 min at 52 °C and 2 min at 72 °C, and concluded with a 5-min extension step at 72 °C. Negative PCR controls and negative extraction controls were run with each round of PCR. To help minimize the risk of contamination, we performed PCR reactions for a subset of museum skin samples in a previously unused laboratory facility in a different building from the main laboratory. Of 39 total museum skin extractions performed, 18 samples successfully yielded PCR products.

For all samples, electrophoresis of 5 µL of PCR products on 1% agarose gels stained with ethidium bromide was used to check for size and concentration. PCR fragments were cleaned using the GeneClean Kit (Bio101) and sequenced in both directions on an ABI Prism 377 automated DNA sequencer using BIGDYE TERMINATOR CYCLE SEQUENCING version 2.0 (Perkin Elmer). PCR products were sequenced

directly only if visualization on an agarose gel revealed high-intensity bands. Museum skin PCR products were sequenced directly and not cloned due to the potential risk of introducing artefacts through cloning.

Sequences were edited using SEQUENCHER 5.0 (Gene Codes Corp.) and aligned using CLUSTALX 1.18 (Thompson *et al.* 1997). At the 5' end of the alignment, sequences were trimmed to begin at the first nucleotide of the CR. At the 3' end of the alignment, sequences were trimmed to minimize the impact of missing data. Therefore, 480 bp of sequence data were analysed. Sequences were deposited in GenBank (Accession nos AY526753–AY526860). A haplotype network was constructed using TCS 1.13 (Clement *et al.* 2000). To be conservative about designating haplotypes, gaps were treated as missing data. When multiple connections to any haplotype were indicated, predictions from coalescent theory were used to choose the most probable alternative (Crandall & Templeton 1993; Posada & Crandall 2001). A haplotype tree was constructed using a starting tree obtained by the neighbour-joining (NJ) method (Saitou & Nei 1987) implemented in PAUP* 4.0b10 (Swofford 2000). MODELTEST 3.06 (Posada & Crandall 1998) was used to determine the most appropriate model of sequence evolution (HKY85 + Γ + I) and to estimate the gamma parameter (0.5604) and the proportion of invariable sites (0.5674). The NJ tree was subjected to branch swapping using the branch and bound algorithm, and trees were evaluated under the likelihood model described above. Statistical support for nodes was obtained from 1000 bootstrap replicates. In addition, a Bayesian phylogenetic analysis of haplotypes was performed using the likelihood model described above. MRBAYES 3 (Huelsenbeck & Ronquist 2001) was used to run 2 000 000 generations with a sampling frequency of 100 generations. The first 1000 trees were discarded to ensure that a stable likelihood had been reached. Bayesian support values were determined by calculating the percentage of trees found that contained that grouping. A phylogenetic analysis of grouse (Tetraoninae) by Drovetski (2002) provides evidence that *Dendragopus obscurus* is the sister group of *Tympanuchus*. Therefore, haplotype trees were rooted with two *D. obscurus* sequences. ARLEQUIN version 2 (Schneider *et al.* 2000) was used to estimate the haplotype diversity (h , the probability that two randomly chosen haplotypes are different), the mean number of pairwise differences between all pairs of haplotypes (π), and the nucleotide diversity (π_n , the probability that two randomly chosen homologous nucleotides are different) for each taxon and population. The Tamura & Nei (1993) distance correction was used to calculate π and π_n .

Results

The haplotype network based on 480 bp of the mtDNA control region is shown in Fig. 1. Haplotype 1 is the

ancestral haplotype in the sample. Little structuring by taxon is revealed by this network, with the exception of heath hen samples which, except for two samples, are all contained within the polygon in the bottom right corner of Fig. 1 (haplotypes 46–51). This group of heath hen haplotypes are all at least six mutational steps from the nearest greater prairie chicken haplotype (43). The greater prairie chicken haplotypes nearest to the heath hen group are all from Wisconsin (haplotypes 40, 43, 44, 45). However, Wisconsin samples are also contained within haplotypes scattered throughout the network (haplotypes 2, 4, 22, 26, 27). Attwater's prairie chicken samples are contained within multiple haplotypes (1, 2, 11, 17, 18) and do not form a distinct grouping. Similarly, lesser prairie chicken and sharp-tailed grouse samples are scattered throughout the network. In the phylogenetic reconstruction of haplotypes, NJ and Bayesian methods resulted in highly congruent topologies. No clades were well supported except the heath hen clade (haplotypes 46–51), which had a 96% bootstrap value and a 100% Bayesian support value (Fig. 2).

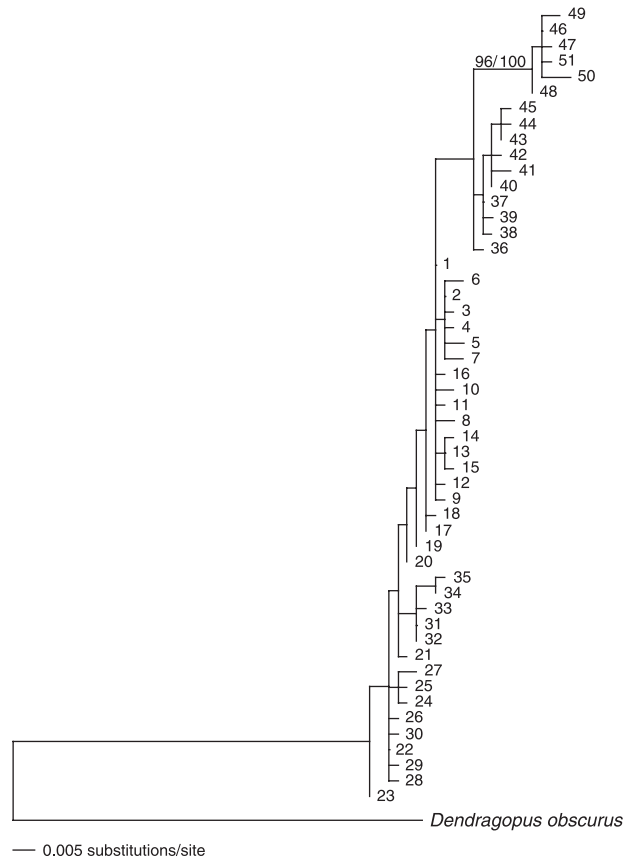


Fig. 2 Haplotype tree obtained by the NJ method and subjected to branch swapping and evaluation under the HKY85 + Γ + I likelihood model. The lengths of the branches are proportional to the amount of genetic change. The only well-supported node in the tree is the heath hen clade, with NJ bootstrap/Bayesian support values listed above the branch. Bootstrap and Bayesian support values for all other nodes were less than 50%.

Table 1 Measures of molecular diversity for each population examined. For the greater prairie chicken, populations were both combined and considered separately. The sample size (n), the number of haplotypes (H), the haplotype diversity (h), the mean number of pairwise differences between all pairs of haplotypes (π) and the nucleotide diversity (π_n) are given

| Taxon or population | n | H | h | π | π_n |
|----------------------------|-----|-----|-------|-------|---------|
| Attwater's prairie chicken | 8 | 5 | 0.786 | 1.650 | 0.003 |
| Greater prairie chicken | 80 | 36 | 0.932 | 5.108 | 0.011 |
| Colorado | 1 | 1 | NA | NA | NA |
| Kansas | 13 | 9 | 0.910 | 3.658 | 0.008 |
| Minnesota | 19 | 8 | 0.807 | 2.926 | 0.006 |
| Missouri | 12 | 8 | 0.924 | 5.261 | 0.011 |
| North Dakota | 11 | 8 | 0.891 | 4.341 | 0.010 |
| Oklahoma | 3 | 3 | 1.000 | 7.920 | 0.016 |
| Wisconsin | 21 | 9 | 0.862 | 6.158 | 0.014 |
| Heath hen | 18 | 8 | 0.745 | 3.686 | 0.008 |
| Lesser prairie chicken | 4 | 3 | 0.833 | 7.036 | 0.015 |
| Sharp-tailed grouse | 7 | 5 | 0.857 | 3.322 | 0.007 |

Measures of molecular diversity shown in Table 1 reveal that the heath hen and Attwater's prairie chicken populations have lower values for h , π and π_n than the pooled sample of greater prairie chickens. In all cases, the sample of Attwater's prairie chickens had the lowest values for h , π and π_n .

Discussion

Of the prairie grouse species and subspecies examined, the heath hen appears to be the most genetically distinct based on mtDNA sequences. With the exception of two samples, all heath hens group into a cluster of six haplotypes that are at least five steps from the nearest greater prairie chicken haplotype. The two samples that lie outside this group (MV3 and MV4 with haplotypes 2 and 14, respectively) may not be true heath hens. These samples were from hunted birds that had been mounted together, held in a private collection, and donated to the Martha's Vineyard Museum without collection information. We initially assumed that these birds had been hunted on Martha's Vineyard, but it is possible that they were brought from the Midwest, either as mounted skins or as live birds collected after release. Due to the poor condition of these skins and the postures in which they were mounted, a morphological determination of whether they were heath hens or greater prairie chickens was not possible. If these two samples are not true heath hens, then the heath hens in this study form a diagnosable, well-supported monophyletic mtDNA clade. This contrasts with the other species and subspecies in the analysis, including the endangered Attwater's prairie chicken, which are scattered throughout the network and the haplotype tree.

Given the high degree of morphological similarity between heath hens and greater prairie chickens (Gross 1928), what could account for the large amount of genetic divergence? There are two possibilities. First, heath hens of the East might have been geographically isolated from greater prairie chickens of the Midwest for a relatively long period of time and diverged genetically while remaining morphologically similar. In this scenario, heath hens would possess diagnosable genetic characters and, according to proponents of the Phylogenetic Species Concept (e.g. Vogler & DeSalle 1994; Barrowclough & Flesness 1996; Amato *et al.* 1998; Cracraft *et al.* 1998; Goldstein *et al.* 2000), would represent a distinct evolutionarily significant unit (ESU). Thus, it would not generally be appropriate to consider greater prairie chickens as a taxonomic equivalent suitable for introduction. However, for proponents of ESU concepts that consider the importance of the adaptive process (e.g. Waples 1991; Crandall *et al.* 2000), whether greater prairie chickens might still be considered as an ecological equivalent to the heath hen is an open question in need of further study.

For the second possible explanation, it is important to highlight that all our heath hen samples possessing collection information were collected on Martha's Vineyard after the mainland population had been extirpated. The isolation of the Martha's Vineyard population and the extreme bottleneck experienced by this population may make it appear more genetically distinct than the mainland heath hen population truly was. There were fewer than 100 heath hens on Martha's Vineyard from 1896 to 1908 (Gross 1928). Therefore, the genetic distinctiveness of the heath hen revealed by this study might reflect the results of human disturbance and sampling bias rather than the effects of evolutionary history.

An example of how anthropogenic disturbance can influence phylogenetic diagnosability was uncovered by Goldstein & DeSalle (2003) in an examination of mtDNA diversity in extant vs. extinct populations of the threatened northeastern beach tiger beetle (*Cicindela dorsalis dorsalis*). From extant populations sampled, an isolated population from Martha's Vineyard was found to be diagnosable from all others by a single bp substitution (Vogler & DeSalle 1993). Strictly speaking, this population would therefore constitute a phylogenetic species. However, the subsequent examination of museum specimens from the Martha's Vineyard population and from now-extinct mainland populations revealed that the single diagnostic site was once widespread and that the Martha's Vineyard population was once polymorphic at this site (Goldstein & DeSalle 2003). Therefore, the effects of human disturbance — the extinction of many mainland populations and the reduced population size on Martha's Vineyard — made this population appear more genetically distinct than it once was.

While the genetic differences we have uncovered between heath hens and greater prairie chickens (at least six mutational

differences per 480 bp) are more substantial than those found by Vogler & DeSalle (1993) for the Martha's Vineyard tiger beetle population (one mutational difference per 656 bp), it is possible that our results reflect, at least in part, the impact of human disturbance. The low values of molecular diversity shown by the heath hen compared to the greater prairie chicken (Table 1) suggest that a demographic bottleneck probably reduced genetic diversity in this population. In addition, if samples MV3 and MV4 are indeed true heath hens and not imported greater prairie chickens, it would indicate that the heath hen was not monophyletic.

Another possibility that should be considered is that, due to a greater degree of isolation, the island population of heath hens was always more distinct from Midwestern greater prairie chickens than was the mainland population. Unfortunately, the lack of mainland heath hens in museum collections prevents us from knowing the true extent of the heath hen's original genetic diversity.

Our study confirms the findings of previous mtDNA analyses (Ellsworth *et al.* 1994, 1995, 1996) that *T. cupido*, *T. pallidicinctus* and *T. phasianellus*, while possessing significant morphological and behavioural differences, do not represent monophyletic mitochondrial lineages. This result either points to a recent divergence among these species or to the signature of historical and/or ongoing hybridization. This scenario is even more intriguing given that the heath hen, which showed minimal morphological and behavioural differentiation from the Midwestern greater prairie chicken and was probably geographically isolated more recently than the initial divergence of *T. cupido*, *T. pallidicinctus* and *T. phasianellus*, does appear to represent a monophyletic clade based on our data. In this context, either the heath hen appears monophyletic due to bias in our sampling but truly was not (as described above), or geneflow between *T. cupido*, *T. pallidicinctus* and *T. phasianellus* has resulted in mtDNA introgression among these species in areas of historical and current sympatry, and the heath hen, having been geographically isolated from all other prairie grouse populations, did not participate in this exchange of genes.

Although hybridization between *T. cupido* and both *T. pallidicinctus* (Bain & Farley 2002) and *T. phasianellus* (Evans 1966; Johnsgard & Wood 1968) are known to occur, Ellsworth *et al.* (1994) reject this explanation to account for the lack of mtDNA coalescence in these species in favour of a scenario of recent divergence, possibly during the late Pleistocene, and incomplete lineage sorting. They argue that the strong morphological and behavioural differences observable between these species are probably due to rapid evolution as a result of sexual selection and adaptation to different regional environments. If incomplete lineage sorting is the cause of mtDNA polyphyly among *Tympanuchus* species, then we must seriously consider the effects of sampling on the results described here, because morphological and behavioural data indicate that heath

hens diverged from greater prairie chickens *after* the divergence of *T. cupido*, *T. pallidicinctus* and *T. phasianellus*. Alternatively, the results of this study provide a reason to consider the possibility that hybridization and introgression have occurred with sufficient frequency to leave their mark on the mtDNA genealogy of Midwestern and Western *Tympanuchus* populations, while leaving the mtDNA of the geographically isolated heath hen alone to coalesce, uninterrupted, to monophyly.

It is also important to note that the Attwater's prairie chicken, a critically endangered subspecies that is currently being managed as an ESU, does not represent a phylogenetically distinguishable group based on mtDNA haplotypes. The small size of the Attwater's population is reflected in its low levels of genetic diversity (Table 1). Low genetic diversity has been associated with decreased population fitness in a small, isolated population of greater prairie chickens in Illinois (Bouzat *et al.* 1998; Westemeier *et al.* 1998). Therefore, if substantial habitat can be restored to support a viable population of prairie chickens on the Texas Gulf Coast, introducing greater prairie chickens from a genetically diverse source might serve to improve this population's prospects for survival, provided that relocated individuals are able to survive and reproduce successfully in their new environment. Similarly, small, fragmented greater prairie chicken populations, such as those in Wisconsin (see Bellinger *et al.* 2003; Johnson *et al.* 2003), might benefit from supplementation efforts focused on increasing numbers and genetic diversity. If a plan to introduce greater prairie chickens to Martha's Vineyard is to be formulated, it too must consider the ability of relocated birds to thrive in the available habitat and attempt to maximize genetic diversity, as any introduced population will begin small.

Conclusions

This study highlights the many complications that may be encountered when attempting to assess evolutionary distinctiveness, especially for extinct populations. Based on phylogenetic criteria and the mtDNA sequence data presented here, one might conclude that the Martha's Vineyard population of heath hens is an ESU because it has unique mtDNA character states not shared by other *Tympanuchus* populations. Does this mean that importing greater prairie chickens to Martha's Vineyard should be discounted as a possible conservation strategy? While the degree of differentiation we have uncovered suggests that historical isolation probably played some role in the genetic divergence of the heath hen from the greater prairie chicken, it is important to consider that island populations are often genetically distinct from their mainland counterparts and that human disturbance has probably impacted the genealogy of the heath hen. Therefore, our ability to sample only the isolated

Martha's Vineyard population may make the heath hen appear more genetically distinct than the pre-European population, as a whole, truly was. In addition, any management action depends on a clear articulation of the conservation goals for the Martha's Vineyard environment. The phylogenetic guidelines for designating ESUs are important, but by themselves are inadequate, for situations where a population is extinct and ecological restoration is the conservation goal to be achieved. Criteria that consider adaptation and ecological exchangeability are important to consider in such situations. A better understanding of the ecological role of prairie grouse, focusing on ecological adaptations to different habitat types, should help provide the additional information necessary to inform managers if introduced greater prairie chickens are likely to survive and reproduce in the restored heathland habitat of Martha's Vineyard. If so, they might serve as a valuable indicator of ecosystem integrity for this unique environment.

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Appendix I

Samples included in this analysis

| Sample ID | Taxon | Population origin | Haplotype | Sample origin |
|-----------|----------------------------|-------------------|-----------|-----------------------------------|
| A451 | Attwater's prairie chicken | Texas (TX) | 1 | Fossil Rim Wildlife Center, Texas |
| AF532432 | Attwater's prairie chicken | Texas (TX) | 2 | Drovetski (2002) |
| 99-86 | Attwater's prairie chicken | Texas (TX) | 11 | Houston Zoo, Texas |
| A391 | Attwater's prairie chicken | Texas (TX) | 17 | Fossil Rim Wildlife Center, Texas |
| A182 | Attwater's prairie chicken | Texas (TX) | 18 | Fossil Rim Wildlife Center, Texas |
| A393 | Attwater's prairie chicken | Texas (TX) | 18 | Fossil Rim Wildlife Center, Texas |
| A409 | Attwater's prairie chicken | Texas (TX) | 18 | Fossil Rim Wildlife Center, Texas |
| A427 | Attwater's prairie chicken | Texas (TX) | 18 | Fossil Rim Wildlife Center, Texas |
| AF532430 | Greater prairie chicken | Colorado (CO) | 1 | Drovetski (2002) |
| AN1 | Greater prairie chicken | Kansas (KS) | 2 | Wild |
| WAB123 | Greater prairie chicken | Kansas (KS) | 8 | Wild |
| C3143 | Greater prairie chicken | Kansas (KS) | 11 | Wild |
| PL | Greater prairie chicken | Kansas (KS) | 11 | Wild |
| CH2 | Greater prairie chicken | Kansas (KS) | 13 | Wild |
| C3115 | Greater prairie chicken | Kansas (KS) | 15 | Wild |
| AN2 | Greater prairie chicken | Kansas (KS) | 19 | Wild |
| VALMORT | Greater prairie chicken | Kansas (KS) | 22 | Wild |
| WAB119 | Greater prairie chicken | Kansas (KS) | 22 | Wild |
| CH3 | Greater prairie chicken | Kansas (KS) | 22 | Wild |
| K2MORT | Greater prairie chicken | Kansas (KS) | 22 | Wild |
| C3117 | Greater prairie chicken | Kansas (KS) | 30 | Wild |
| AF532431 | Greater prairie chicken | Kansas (KS) | 34 | Drovetski (2002) |
| E9495 | Greater prairie chicken | Minnesota (MN) | 2 | Wild |
| MN264328 | Greater prairie chicken | Minnesota (MN) | 2 | Wild |
| MN343 | Greater prairie chicken | Minnesota (MN) | 6 | Wild |
| MN H348 | Greater prairie chicken | Minnesota (MN) | 7 | Wild |
| C341 | Greater prairie chicken | Minnesota (MN) | 12 | Wild |
| MN284751 | Greater prairie chicken | Minnesota (MN) | 20 | Wild |
| MN284 | Greater prairie chicken | Minnesota (MN) | 20 | Wild |
| MN284168 | Greater prairie chicken | Minnesota (MN) | 20 | Wild |
| MN284420 | Greater prairie chicken | Minnesota (MN) | 21 | Wild |
| MN356 | Greater prairie chicken | Minnesota (MN) | 21 | Wild |
| C148162 | Greater prairie chicken | Minnesota (MN) | 22 | Wild |
| MN284419 | Greater prairie chicken | Minnesota (MN) | 22 | Wild |
| MN284703 | Greater prairie chicken | Minnesota (MN) | 22 | Wild |
| MN284704 | Greater prairie chicken | Minnesota (MN) | 22 | Wild |
| MN284798 | Greater prairie chicken | Minnesota (MN) | 22 | Wild |
| MN284799 | Greater prairie chicken | Minnesota (MN) | 22 | Wild |
| MN283169 | Greater prairie chicken | Minnesota (MN) | 22 | Wild |
| MN284346 | Greater prairie chicken | Minnesota (MN) | 22 | Wild |
| MN355 | Greater prairie chicken | Minnesota (MN) | 28 | Wild |
| M25 | Greater prairie chicken | Missouri (MO) | 2 | Wild |
| M26 | Greater prairie chicken | Missouri (MO) | 2 | Wild |
| M28 | Greater prairie chicken | Missouri (MO) | 2 | Wild |
| M33 | Greater prairie chicken | Missouri (MO) | 3 | Wild |
| M10 | Greater prairie chicken | Missouri (MO) | 23 | Wild |
| M27 | Greater prairie chicken | Missouri (MO) | 23 | Wild |
| M4 | Greater prairie chicken | Missouri (MO) | 24 | Wild |
| M11 | Greater prairie chicken | Missouri (MO) | 25 | Wild |
| M30 | Greater prairie chicken | Missouri (MO) | 25 | Wild |
| M32 | Greater prairie chicken | Missouri (MO) | 31 | Wild |
| M24 | Greater prairie chicken | Missouri (MO) | 33 | Wild |
| M22 | Greater prairie chicken | Missouri (MO) | 38 | Wild |
| ND283607 | Greater prairie chicken | North Dakota (ND) | 1 | Wild |
| ND149253 | Greater prairie chicken | North Dakota (ND) | 2 | Wild |
| ND284706 | Greater prairie chicken | North Dakota (ND) | 2 | Wild |
| ND293669 | Greater prairie chicken | North Dakota (ND) | 2 | Wild |
| ND283669 | Greater prairie chicken | North Dakota (ND) | 2 | Wild |
| ND283249 | Greater prairie chicken | North Dakota (ND) | 5 | Wild |

Appendix I *Continued*

| Sample ID | Taxon | Population origin | Haplotype | Sample origin |
|------------|-----------------------------|---------------------------|-----------|-------------------------------|
| ND284229 | Greater prairie chicken | North Dakota (ND) | 9 | Wild |
| ND284705 | Greater prairie chicken | North Dakota (ND) | 29 | Wild |
| ND284052 | Greater prairie chicken | North Dakota (ND) | 32 | Wild |
| ND284297 | Greater prairie chicken | North Dakota (ND) | 36 | Wild |
| ND283244 | Greater prairie chicken | North Dakota (ND) | 37 | Wild |
| OK125 | Greater prairie chicken | Oklahoma (OK) | 11 | Wild |
| OK72 | Greater prairie chicken | Oklahoma (OK) | 25 | Wild |
| OK153 | Greater prairie chicken | Oklahoma (OK) | 37 | Wild |
| WS284321 | Greater prairie chicken | Wisconsin (WI) | 2 | Wild |
| WS284323 | Greater prairie chicken | Wisconsin (WI) | 2 | Wild |
| WS284806 | Greater prairie chicken | Wisconsin (WI) | 2 | Wild |
| WS283285 | Greater prairie chicken | Wisconsin (WI) | 2 | Wild |
| WS284105 | Greater prairie chicken | Wisconsin (WI) | 2 | Wild |
| WS284822 | Greater prairie chicken | Wisconsin (WI) | 4 | Wild |
| WSOR351P | Greater prairie chicken | Wisconsin (WI) | 4 | Wild |
| WSOR357P | Greater prairie chicken | Wisconsin (WI) | 22 | Wild |
| WS283278 | Greater prairie chicken | Wisconsin (WI) | 26 | Wild |
| WS283279 | Greater prairie chicken | Wisconsin (WI) | 26 | Wild |
| WS284136 | Greater prairie chicken | Wisconsin (WI) | 26 | Wild |
| WS284215 | Greater prairie chicken | Wisconsin (WI) | 27 | Wild |
| WS284750 | Greater prairie chicken | Wisconsin (WI) | 40 | Wild |
| WS284776 | Greater prairie chicken | Wisconsin (WI) | 40 | Wild |
| WS284819 | Greater prairie chicken | Wisconsin (WI) | 40 | Wild |
| WS284820 | Greater prairie chicken | Wisconsin (WI) | 40 | Wild |
| WS284131 | Greater prairie chicken | Wisconsin (WI) | 40 | Wild |
| WS284192 | Greater prairie chicken | Wisconsin (WI) | 40 | Wild |
| WS284207 | Greater prairie chicken | Wisconsin (WI) | 43 | Wild |
| WS289137 | Greater prairie chicken | Wisconsin (WI) | 44 | Wild |
| WS284287 | Greater prairie chicken | Wisconsin (WI) | 45 | Wild |
| MV3 | Heath hen | No collection information | 2 | Martha's Vineyard Museum |
| MV4 | Heath hen | No collection information | 14 | Martha's Vineyard Museum |
| MV1 | Heath hen | No collection information | 46 | Martha's Vineyard Museum |
| MV2 | Heath hen | No collection information | 46 | Martha's Vineyard Museum |
| MV5 | Heath hen | No collection information | 46 | Felix Neck, Martha's Vineyard |
| YPM 69503 | Heath hen | Martha's Vineyard | 47 | Yale Peabody Museum |
| YPM 69502 | Heath hen | Martha's Vineyard | 50 | Yale Peabody Museum |
| MCZ 297274 | Heath hen | Martha's Vineyard (1889) | 48 | Museum of Comparative Zoology |
| MCZ 246053 | Heath hen | Martha's Vineyard (1890) | 46 | Museum of Comparative Zoology |
| MCZ 181418 | Heath hen | Martha's Vineyard (1896) | 46 | Museum of Comparative Zoology |
| MCZ 181417 | Heath hen | Martha's Vineyard (1896) | 46 | Museum of Comparative Zoology |
| MCZ 181423 | Heath hen | Martha's Vineyard (1896) | 46 | Museum of Comparative Zoology |
| MCZ 181419 | Heath hen | Martha's Vineyard (1896) | 48 | Museum of Comparative Zoology |
| MCZ 181425 | Heath hen | Martha's Vineyard (1896) | 48 | Museum of Comparative Zoology |
| MCZ 301010 | Heath hen | Martha's Vineyard (1899) | 51 | Museum of Comparative Zoology |
| MCZ 187180 | Heath hen | Martha's Vineyard (1903) | 49 | Museum of Comparative Zoology |
| MCZ 297275 | Heath hen | Martha's Vineyard (1909) | 46 | Museum of Comparative Zoology |
| MCZ 297272 | Heath hen | Martha's Vineyard (1912) | 46 | Museum of Comparative Zoology |
| AJ297175 | Lesser prairie chicken | Unknown | 41 | Lucchini <i>et al.</i> (2001) |
| AJ297174 | Lesser prairie chicken | Unknown | 41 | Lucchini <i>et al.</i> (2001) |
| AF532433 | Lesser prairie chicken | Kansas (KS) | 35 | Drovetski (2002) |
| AF532434 | Lesser prairie chicken | Kansas (KS) | 39 | Drovetski (2002) |
| AF532435 | Sharp-tailed grouse | Idaho (ID) | 1 | Drovetski (2002) |
| ND284339ST | Sharp-tailed grouse | North Dakota (ND) | 1 | Wild |
| ND327ST | Sharp-tailed grouse | North Dakota (ND) | 1 | Wild |
| ND368ST | Sharp-tailed grouse | North Dakota (ND) | 2 | Wild |
| NDYW85ST | Sharp-tailed grouse | North Dakota (ND) | 16 | Wild |
| ND284334ST | Sharp-tailed grouse | North Dakota (ND) | 42 | Wild |
| AF532436 | Sharp-tailed grouse | Washington (WA) | 10 | Drovetski (2002) |
| AF532426 | <i>Dendragopus obscurus</i> | Utah (UT) | Outgroup | Drovetski (2002) |
| AF532427 | <i>Dendragopus obscurus</i> | Washington (WA) | Outgroup | Drovetski (2002) |