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A comparison of pedigree- and DNA-based measures for identifying inbreeding depression in the critically endangered Attwater's Prairie-chicken

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Abstract

The primary goal of captive breeding programmes for endangered species is to prevent extinction, a component of which includes the preservation of genetic diversity and avoidance of inbreeding. This is typically accomplished by minimizing mean kinship in the population, thereby maintaining equal representation of the genetic founders used to initiate the captive population. If errors in the pedigree do exist, such an approach becomes less effective for minimizing inbreeding depression. In this study, both pedigree- and DNA-based methods were used to assess whether inbreeding depression existed in the captive population of the critically endangered Attwater's Prairie-chicken (*Tympanuchus cupido attwateri*), a subspecies of prairie grouse that has experienced a significant decline in abundance and concurrent reduction in neutral genetic diversity. When examining the captive population for signs of inbreeding, variation in pedigree-based inbreeding coefficients ($f_{pedigree}$) was less than that obtained from DNA-based methods (f_{DNA}). Mortality of chicks and adults in captivity were also positively correlated with parental relatedness (r_{DNA}) and f_{DNA}, respectively, while no correlation was observed with pedigree-based measures when controlling for additional variables such as age, breeding facility, gender and captive/release status. Further, individual homozygosity by loci (*HL*) and parental r_{DNA} values were positively correlated with adult mortality in captivity and the occurrence of a lethal congenital defect in chicks, respectively, suggesting that inbreeding may be a contributing factor increasing the frequency of this condition among Attwater's Prairie-chickens. This study highlights the importance of using DNA-based methods to better inform management decisions when pedigrees are incomplete or errors may exist due to uncertainty in pairings.

Keywords: *ex situ* population management, fitness, genetic diversity, heterozygosity, inbreeding, prairie grouse

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Introduction

Although many studies have investigated the effects of population decline on neutral genetic diversity, relatively few have investigated whether such populations have also experienced a subsequent loss in fitness or inbreeding depression (Heschel & Paige 1995; Westemeier *et al.* 1998; Madsen *et al.* 1999; Blomqvist *et al.* 2010). Reduced genetic diversity is common among threatened and endangered species when compared to closely related nonthreatened taxa (Spielman *et al.* 2004). This relationship is not surprising as most endangered species possess a small population size, and neutral theory predicts that there will be a positive correlation between neutral genetic diversity and effective population size

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(Montgomery *et al.* 2000; see also Jamieson & Allendorf 2012). To what extent neutral genetic diversity actually correlates with extinction risk remains an important question (Frankham 2005a), yet contrasting results exist linking loss of neutral genetic diversity with a reduction in fitness (Reed & Frankham 2003; Chapman *et al.* 2009; Harrison *et al.* 2011; Olano-Marin *et al.* 2011).

As populations become smaller, it is more likely that individuals who are more closely related or have alleles that are identical by descent (IBD) will pair and attempt to produce offspring. Consequently, inbreeding depression may result due to the expression of deleterious homozygous alleles (Charlesworth & Charlesworth 1987; Charlesworth & Willis 2009). Evidence of inbreeding depression has been documented across a variety of taxonomic groups in both captive (Ralls *et al.* 1988, 2000; Swinnerton *et al.* 2004; Charpentier *et al.* 2008; Santure *et al.* 2010) and wild populations (Frankham 1995; Westemeier *et al.*1998; Keller & Waller 2002; Richardson *et al.* 2004; Marr *et al.* 2006; Alho *et al.* 2009; Alcaide *et al.* 2010; Grueber *et al.* 2010), and its likelihood of occurring is typically evaluated by examining an individual's inbreeding coefficient (*f*).

An inbreeding coefficient represents the probability that two homologous alleles will be IBD. The impact of inbreeding depression is determined by regressing the values of the trait in question on *f* (Lynch & Walsh 1998). For example, effects of inbreeding depression are more likely during earlier life stages (Ortego *et al.* 2010), as genes with large effects are likely to be expressed on fitness-related traits early in development (Keller & Waller 2002), and traits associated with fitness are subjected to strong natural selection (DeRose & Roff 1999). Relatively few empirical studies exist, however, that document a significant correlation between *f* and inbreeding depression in endangered or threatened species (Frankham *et al.* 2009). This is largely because once populations are of extremely small size (e.g. <100 individuals) and their genetic diversity significantly reduced, it becomes increasingly difficult to identify reduced fitness statistically when limited variability exists within the population overall (i.e. low variance in *f*) or due to a lack of statistical power based on small sample sizes (Kalinowski & Hedrick 1999; Grueber *et al.* 2008; Keller *et al.* 2012).

Despite these difficulties, our ability to investigate inbreeding depression and determine how particular management practices may influence its severity is dependent on accurate measures of inbreeding. The preferred method for estimating inbreeding is to calculate individual *f* based on genealogical relationships using a complete multigeneration pedigree (Slate *et al.* 2004; Pemberton 2008). However, such information is often not available, or errors in the pedigree, such as incorrect or unknown parentage assignment, may exist that limit its utility for accurately measuring inbreeding (e.g. Russello & Amato 2004; Lacy 2009). An alternative approach is to use molecular markers such as microsatellites to estimate genealogical relationships among individuals in the population. In this way, DNA-based estimates of *f* should provide a valuable resource for determining how errors in the pedigree have unknowingly resulted in conditions that allow for inbreeding depression. This may be an especially important approach in captive breeding programmes. However, unless a very large number of loci are analysed (e.g. Oliehoek *et al.* 2006), it is not necessarily correct to conclude that DNA-based measures of inbreeding are more accurate than pedigree-based estimates. Rather DNA-based measures allow a more accurate appraisal of the true variation that may exist within a population in conditions such as those where it has been managed by mean kinship using a pedigree wrongly assumed correct (e.g. Oliehoek & Bijma 2009) and therefore possessing a low variance in pedigree-based measures of inbreeding.

In this study, we investigated whether the critically endangered Attwater's Prairie-chicken (*Tympanuchus cupido attwateri*) shows signs of inbreeding depression using both pedigree- and DNA-based measures of inbreeding. Attwater's Prairie-chickens were once common throughout the coastal tallgrass prairie of Texas and Louisiana with numbers approaching one million (Lehmann 1941). However, by 1967, the population had declined to approximately 1000 individuals (Lehmann 1968), and consequently, the Attwater's Prairie-chicken was added to the endangered species list (32 FR 4001; Morrow *et al.* 2004). A captive population based on 19 founder lineages, or 8.5 founder genome equivalents (see Lacy 1989), was established during 1992–1998, and now, approximately 100–150 breeding individuals exist in six separate breeding facilities (Morrow *et al.* 2004; U.S. Fish & Wildlife Service 2010). Reproductive pairings are determined based on a pedigree while minimizing the average relatedness of an individual compared with all others in the population, often described as mean kinship (e.g. Ballou & Lacy 1995).

Over the past decade, numbers in the wild have fluctuated around 50–110 Attwater's Prairie-chickens among three isolated management areas, all largely dependent on the annual release of captive bred individuals. Although approximately 2100 Attwater's have been released to the wild over the past 17 years, very few of those birds produced chicks that fledged, with only 1 of 31 broods (3.2%) surviving past 2 weeks post-hatch between 2002 and 2008 (Pratt 2010). Brood survival improved during 2009–2012 with 27 of 75 (36%) surviving this critical period. It is not known to what degree reduced chick survival may be the result of

inbreeding or other environmental factors such as habitat or food availability in this species (see Morrow *et al.* 2010; U.S. Fish & Wildlife Service 2010). A lethal congenital defect called wryneck (torticollis), where the neck is twisted and the head is turned under or backward, may also be associated with inbreeding in Attwater's Prairie-chicken. Approximately 4.0 ± 3.6% (mean ± SD; range: 0.3–10.9%) of the 408.7 ± 124.7 Attwater's chicks hatched each year in the captive population between 1998 and 2011 were diagnosed with wryneck (H. Bailey, unpublished; see also Savage & Collins 1971; West *et al.* 2002).

Errors in the pedigree used to pair individuals for breeding purposes could produce individuals less fit for the release programme as the pedigree may underestimate the true relationship between breeding pairs resulting in a much higher level of inbreeding within the captive population than expected. Therefore, it is crucial that such errors are minimized. Errors can be introduced into the pedigree in many ways including misassigned parentage and poor record keeping. Here, we assess to what extent potential errors in the pedigree may have on levels of inbreeding in the Attwater's Prairie-chicken captive population by determining whether differences exist between pedigree- and DNA-based methods for measuring inbreeding depression. If pedigree errors are frequent enough, using the pedigree to minimize mean kinship may be compromised and require immediate attention to minimize inbreeding depression.

Materials and methods

Sampling and DNA extraction

Attwater's Prairie-chicken tissue samples were collected from three temporal populations: 'historic' (n = 23; collected between 1887 and 1948), 'precaptive' (n = 36; collected between 1990 and 1994) and 'captive' (n = 177; collected in spring 2006). The historic population consisted of toe-pad tissues sampled from museum specimens and have been described elsewhere (Johnson & Dunn 2006). The 'precaptive' population samples were obtained from wild birds just prior to and during establishment of the captive breeding population in the early 1990s (Johnson *et al.* 2007; M. Peterson, personal communication; M. Morrow, unpublished) and may include some founders of the captive population, whereas the 'captive' population consisted of all individuals from the 2006 captive population. For population level comparisons, a subset of 33 individuals were randomly selected from the 2006 captive population after excluding known first- and second-order relatives (i.e. parent/offspring, full- and half-sibs) based on a pedigree maintained by the breeding programme and confirmed with estimates of genetic relatedness using COANCESTRY (Wang 2011; see below). Genomic DNA from blood and toe-pad tissue was obtained following methods described elsewhere (Bellinger *et al.* 2003; Johnson *et al.* 2003, 2004, 2007).

Genotyping and sequencing

Levels of genetic variability for the temporal population analysis were measured using five microsatellite loci (ADL44, ADL146, ADL230, LLSD4 and LLSD9) and 384 base pairs of the mitochondrial control region (domain I) using methods described elsewhere (Bellinger *et al.* 2003; Johnson *et al.* 2003, 2004). Two additional microsatellite loci, SGCA6 and SGCA9 originally developed for Greater Sage-grouse (*Centrocercus urophasianus*; Taylor *et al.* 2003) and a third, LLST1 (see Bellinger *et al.* 2003), were also genotyped for each individual in the 2006 Attwater's captive population. This was done to increase the number of loci (n = 8) used for estimating genetic relatedness values in the captive population. Polymerase chain reaction (PCR) amplifications for SGCA6 and SGCA9 included ~50 ng of genomic DNA, 0.50 μM fluorescently labelled forward and unlabeled reverse primer, 5× Buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs and 1 unit of GoTaq DNA polymerase (Promega Corporation, Madison, WI) for a total reaction volume of 10 μL. Thermal cycling profiles were 35 cycles of 95 °C for 1 min, 62 °C (SGCA6) or 54 °C (SGCA9) for 1 min and 72 °C for 1 min, followed by 5 min at 72 °C (Taylor *et al.* 2003). All PCR products were run on an ABI 3130xl automated sequencer, and allele sizes were determined using GeneMarker (SoftGenetics, State College, PA). No cases of genotyping errors associated with stuttering, allelic dropout and null alleles were observed based on results from MICRO-CHECKER (Van Oosterhout *et al.* 2004). Mitochondrial samples were sequenced using ABI BigDye Terminator chemistry and run on an ABI 3130xl automated sequencer.

All procedures when working with museum samples followed strict guidelines to minimize contamination with contemporary DNA (see Johnson & Dunn 2006; Johnson *et al.* 2007), with all laboratory work conducted in a facility designated for ancient DNA-based analyses. To assess the presence of null alleles and allelic dropout, all museum samples were genotyped a minimum of four times. No cases of genotyping error were observed, which was further confirmed using MICRO-CHECKER.

Statistical analyses

Microsatellite genotypes for each population were tested for linkage equilibrium and departure from

Hardy–Weinberg equilibrium conditions using the program GDA (Lewis & Zaykin 2001) after sequential Bonferroni corrections were applied to account for multiple comparisons (Rice 1989). Mean number of alleles per locus (allelic diversity) and mean expected heterozygosity (H_e) values were calculated using GDA, and measures of allelic richness were calculated using the program FSTAT, v.2.9.3.2 (Goudet 1995), to account for differences in sample sizes across sampling periods and populations (Leberg 2002). Differences in microsatellite genetic diversity estimates between time periods and populations were tested for significance using a Wilcoxon signed rank test. Mitochondrial DNA control region diversity was determined by calculating population estimates of haplotype diversity (h) and Tajima's D using the program Arlequin, v.3.11 (Excoffier *et al.* 2005), and estimates of haplotype diversity were compared between time periods using the t-test described by Nei (1987).

For the 2006 captive Attwater's population, we used multiple pedigree- and DNA-based methods to estimate inbreeding. Pedigree-based inbreeding coefficients ($f_{pedigree}$) were calculated for each individual using PMx (Lacy *et al.* 2012) based on genealogical information from a multigeneration pedigree maintained for captive management. Coefficients of relatedness were calculated using kinship values obtained from PMx and $Kxy = \frac{1}{2}rxy\sqrt{(1+Fx)(1+Fy)}$ (Crow & Kimura 1970), where F_x and F_y represent the inbreeding coefficient of the sire and dam, r_{xy} is the relatedness coefficient of the offspring (or $r_{pedigree}$), and K_{xy} is the kinship coefficient of the pair. Although the captive breeding programme was in its 14th year and Attwater's are capable of reproducing in their first year, the mean number of generations in captivity for the 2006 and 2007 offspring (n = 316) used in this study was 5.386 ± 0.626 (SD).

DNA-based inbreeding coefficients (f_{DNA}) for individuals in the 2006 population and parental relatedness values (r_{DNA}) for offspring produced in 2006 and 2007 were calculated from the microsatellite data (eight loci) using a triadic likelihood method implemented in COANCESTRY, v.1.0 (Wang 2011). TrioML (Wang 2007) was chosen instead of other relatedness estimators (see Wang 2011) because it possessed the lowest variance and highest correlation to the true values associated with identified pairings (i.e. full-sib, half-sib and unknown) based on the results from a simulation study using the genetic data from the 2006 captive population (see Table S1, Supporting information; see also Van de Casteele *et al.* 2001). TrioML has been shown to perform better than other relatedness estimators in cases possessing high inbreeding and closely related individuals, assuming an adequate number of polymorphic markers are available (Wang 2007).

To assess heterozygosity–fitness correlations in the 2006 'captive' population, genetic heterozygosity for each individual (n = 177) was calculated using individual heterozygosity (H), or the average heterozygous loci, standardized heterozygosity (SH; Coltman *et al.* 1999), standardized d^2 (Coltman *et al.* 1999; Amos *et al.* 2001), internal relatedness (IR; Amos *et al.* 2001) and homozygosity by loci (HL; Aparicio *et al.* 2006). These values were all calculated using an EXCEL macro (IRmacroN4) available at http://www.zoo.cam.ac.uk/zoostaff/meg/amos.htm#ComputerPrograms (see also Amos *et al.* 2001). Rhh (Alho *et al.* 2010) in R, v.2.15.0 (http://www.r-project.org), was used to test whether HL was correlated among loci based on 1000 random samples. If the microsatellite loci used in this study reflect genome-wide diversity levels with respect to heterozygosity, then this correlation should be positive (Balloux *et al.* 2004; Alho *et al.* 2010).

Cox proportional hazards models (Cox 1972) were used to explore the effects of several potential explanatory variables simultaneously on (i) juvenile and adult Attwater's Prairie-chicken survival in captivity and (ii) their offspring's survival to 14 or 50 days post-hatch (analysed separately; see below). Blood samples from the captive population (n = 177) were obtained March 2006 prior to the breeding season at which time each bird was at least 8 months of age (mean ± SD = 27.0 ± 20.7 months) and then genotyped using eight microsatellite loci. None of their chicks produced during the subsequent 2006 (n = 184) and 2007 (n = 72) breeding seasons were genotyped. Therefore, inbreeding depression was assessed differently in the two data sets.

For the genotyped data set (model 1), hatch facility (n = 6), gender and whether the bird remained in captivity or was released after the 2006 breeding season (herein described as captive/release status) were included as categorical variables, while age (years) and f obtained from both the pedigree ($f_{pedigree}$) and the microsatellite data (f_{DNA}) were included as continuous variables to investigate factors that may influence survival of birds that were at least 8 months of age at time of sampling. Both hatch facility and captive/release status were included as variables to address potential variation in survival due to environmental differences among individual groups.

For offspring of genotyped birds (model 2), hatch facility (Houston Zoo & Fossil Rim Wildlife Center) was included as a categorical variable, while egg storage duration prior to onset of incubation (days), weight at hatch (g), hatch year and both pedigree- and DNA-based chick parental r were also incorporated into the model as continuous covariates to explore chick survival to 14 and 50 days post-hatch. Data were collected at the two facilities that produced the majority of the

chicks (267 of 376 chicks in 2006; 341 of 447 in 2007), and data from the remaining four facilities were not collected or considered for inclusion in this model due to their small sample sizes. Two separate analyses were conducted to assess temporal changes in chick mortality rate (14 vs. 50 days) in captivity. A third analysis was also carried out using a data set where the chicks that had died prior to day 15 were not included in the model. This was done to further investigate whether differences in chick mortality exist between the first 14 days and 15–50 days post-hatch in the study population. Gender was known for only 19 of 132 (14%) and 27 of 146 (18%) chicks that died prior to day 14 or 50 post-hatch, respectively. Therefore, gender was not included as a variable in model 2 due to data limitations.

To identify the best-fit model for each of the sampling scenarios, each variable, including all two-way interactions included separately within each model, was sequentially removed using a backward stepwise elimination process based on the Wald statistic. Both full and minimal models are presented to allow inspection of results prior to backward variable deletion (Whittingham *et al.* 2006). Additionally, both model 1 and model 2 were each run three times containing each predictor separately, that is, once using both pedigree- and DNA-based measures, once with only the pedigree-based measure and once with only the marker-based measure. We conducted all statistical tests using SPSS, v.19.0.0.1 (IBM).

Occurrence of wryneck in Attwater's Prairie-chicken offspring with hatch year, hatch facility, egg storage time prior to incubation, chick weight at hatch, $r_{pedigree}$ and parental r_{DNA} was tested using a generalized estimating equation (GEE) with a binomial probability distribution and probit link function. Repeated parent pairings were identified in the model to control for pseudoreplication. The full model was reduced by sequentially excluding the variables that did not explain a significant part of the deviance. Variables with $p < 0.1$ were included in the final model.

Results

Temporal genetic diversity

The 2006 'captive' Attwater's Prairie-chicken population had low levels of neutral genetic diversity. Both microsatellite (allelic richness) and mtDNA control region (haplotype diversity) genetic variability for the 'captive' Attwater's Prairie-chicken population were significantly reduced compared with 'historic' ($Z = -2.032$, $P = 0.042$; $t = 12.188$, $P < 0.001$, respectively) and 'precaptive' ($Z = -2.041$, $P = 0.041$; $t = 3.145$, $P < 0.01$, respectively) levels, whereas differences in expected heterozygosity levels were not significantly different among the three temporal data sets ('historic' vs. 'precaptive' $Z = -0.674$, $P = 0.500$; 'historic' vs. 'captive' $Z = -0.405$, $P = 0.686$; 'precaptive' vs. 'captive' $Z = -1.214$, $P = 0.225$; Table 1).

Inbreeding and parental relatedness

The pedigree-based individual inbreeding coefficients ranged from 0 to 0.15 ($f_{pedigree}$ mean ± SD = 0.025 ± 0.022, $n = 177$), while the DNA-based coefficients ranged from 0 to 0.65 ($f_{DNA} = 0.087 \pm 0.113$, $n = 177$) showing a wider distribution of values (Fig. 1A). The relatedness coefficients calculated for their offspring ($n = 316$) produced in 2006 and 2007 showed a similar pattern with $r_{pedigree}$ (0.122 ± 0.019, $n = 316$) possessing reduced variability compared with r_{DNA} (0.082 ± 0.109, $n = 316$; Fig. 1B). In both cases, the DNA-based coefficients showed signs of inbreeding in the captive population with a few values ≥0.25 or half-sibling pairs (Fig. 1). Mean $r_{pedigree}$ and r_{DNA} differed significantly between the two consecutive years, but in opposite directions. Mean $r_{pedigree}$ increased from 0.064 ± 0.015 to 0.080 ± 0.017 ($t = -7.493$, d.f. = 279, $P < 0.001$), while mean r_{DNA} decreased from 0.091 ± 0.128 to 0.041 ± 0.110 ($t = 3.686$, d.f. = 279, $P < 0.001$; Fig. 2).

Table 1 Measures of nuclear microsatellite and mtDNA control region domain I genetic diversity (mean ± SE) for the three temporal Attwater's Prairie-chicken populations

	Historic (1887–1948)	Precaptive (1990–94)	Captive (2006)
Microsatellite			
N	23	36	33
Mean no. of alleles	7.8 ± 1.1	7.4 ± 1.0	5.2 ± 0.7
AR*	7.7 ± 1.1	6.8 ± 1.0	5.0 ± 0.7
H_e	0.723 ± 0.078	0.761 ± 0.044	0.697 ± 0.048
mtDNA control region†			
N	19	36	20
No. of haplotypes	12	8	4
h	0.912 ± 0.011	0.751 ± 0.011	0.695 ± 0.014
π	0.009 ± 0.000	0.008 ± 0.001	0.008 ± 0.001
Tajima's D‡	−0.162	−0.042	1.727

N, sample size; AR, allelic richness; H_e, expected heterozygosity; h, haplotype diversity; π, nucleotide diversity.
*Accounts for unequal sample sizes among populations (based on population size $n = 21$).
†Historic mtDNA (Johnson & Dunn 2006); Precaptive mtDNA (Johnson *et al.* 2007); Captive mtDNA (GenBank Accession nos: AY273840, AY273865, DQ027819, DQ027823).
‡None of the values were significantly different from zero.

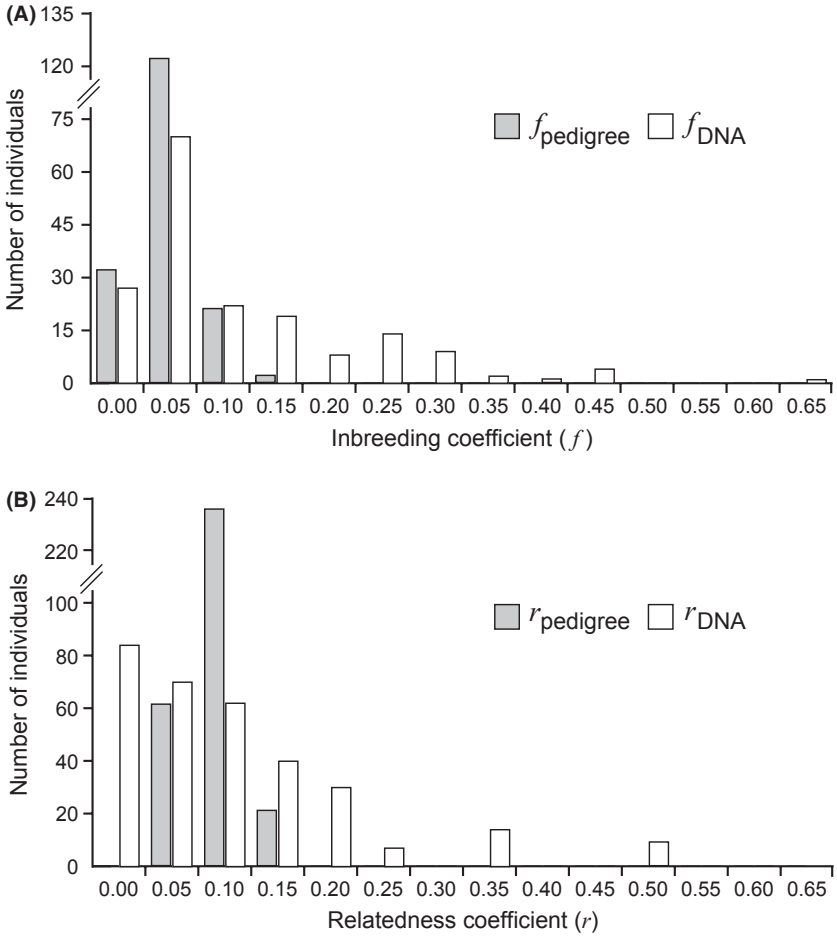

Fig. 1 Distribution of pedigree- and DNA-based (A) individual inbreeding ($f_{pedigree}$ and f_{DNA}, respectively; $n = 177$) and (B) parental relatedness ($r_{pedigree}$ and r_{DNA}, respectively; $n = 316$) coefficients for the 2006 Attwater's captive breeding programme.

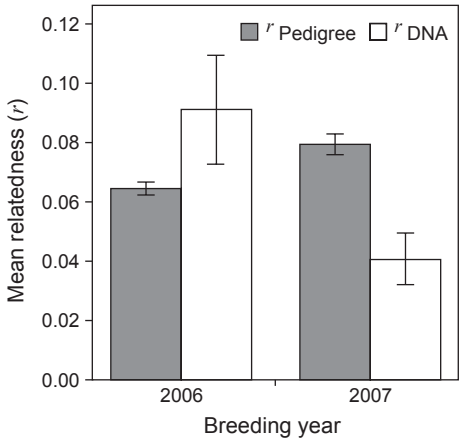

Fig. 2 Mean pedigree- ($r_{pedigree}$) and DNA-based (r_{DNA}) parental relatedness in Attwater's Prairie-chicken offspring in 2006 ($n = 190$) and 2007 ($n = 91$) produced by individuals sampled in 2006. Error bars, ±2 SE.

Inbreeding depression

The assumption of proportional hazards was met for all covariates tested in the Cox proportional hazards models based on scaled Schoenfeld residual analyses (Grambsch & Therneau 1994). Mortality rates for juvenile and adult Attwater's differed for individuals depending on their level of inbreeding as estimated from the microsatellite data (f_{DNA}) while controlling for age and captive/release status (minimal Cox proportional hazards model: $\chi^2 = 96.30$, d.f. = 3, $P < 0.001$; Table 2), while hatch facility, gender, $f_{pedigree}$ and all interaction terms were not shown to be significantly time dependent and were eliminated from the final model. Younger birds had higher mortality rates compared with those that were older at time of sampling as indicated by the odds ratio for the age covariant, where exp(B) < 1 indicates a hazard (or death) decrease per unit increase in the predictor (Table 2; Fig. 3A). The same was shown with captive/release status among individuals following the 2006 breeding season, where those released to the wild ($n = 21$) had higher mortality rate than individuals remaining in captivity ($n = 142$; Fig. 3B). In contrast, when exp(B) > 1, as shown with f_{DNA}, the relative hazard increases per unit increase in the predictor or mortality increased as inbreeding increased after adjustment of the effects of age and

Table 2 Cox proportional hazards models for juvenile (>8 months) and adult Attwater's Prairie-chicken (n = 177) mortality while investigating the effects of hatch facility, gender, pedigree- and DNA-based inbreeding coefficient ($f_{pedigree}$ and f_{DNA}, respectively), age (days) and captive/release status. Critical values for both the full and minimal model are given. Significant P-values are in bold

				Full model		Minimal model			
Variable	d.f.	B	SE	Wald	P	Wald	P	Exp(B)	95% CI
Hatch facility	5	–	–	3.187	0.671				
Gender	1	0.182	0.181	1.016	0.313				
$f_{pedigree}$	1	−8.280	4.682	3.128	0.077	2.714	0.099	0.001	0.000–3.966
f_{DNA}	**1**	**1.628**	**0.706**	**5.322**	**0.021**	**5.239**	**0.022**	**4.935**	**1.258–19.361**
Age	**1**	**−0.541**	**0.079**	**46.690**	**<0.001**	**48.311**	**<0.001**	**0.583**	**0.500–0.678**
Captive/release status	**1**	**−1.363**	**0.284**	**22.943**	**<0.001**	**25.243**	**<0.001**	**0.245**	**0.142–0.424**

captive/release status in the model (Fig. 3C). Similar results were obtained when individuals released to the wild following the 2006 breeding season were eliminated from the data set (data not shown) and were further supported based on the final model selection where f_{DNA} possessed the minimum AIC value (1112.02) compared with a model using $f_{pedigree}$ (AIC: 1113.97).

Mortality rates to both 14 and 50 days post-hatch differed among chicks depending on hatch year (minimal model: $\chi^2 = 38.108$, d.f. = 2, $P < 0.001$; Fig. 4A) and parental r_{DNA} ($\chi^2 = 35.079$, d.f. = 2, $P < 0.001$; Fig. 4B), while egg storage duration prior to incubation, chick weight at hatch, hatch facility, $f_{pedigree}$ and all interaction terms do not significantly affect survival and were eliminated in the final model (Table 3). Probability of mortality increased with increasing parental r_{DNA} (Fig. 4B), suggesting that more inbred individuals had a higher mortality rate, particularly within the first 14 days post-hatch as no variables were significant in a separate analysis when the chicks that had died the first 14 days were removed from the data set (data not shown). These results suggest that the significant pattern observed with Attwater's chick mortality in both the 14 and 50 day analyses was largely driven by the first 14 days post-hatch. Fourteen mortality events (6.5%) were observed in the data set between 15 and 50 days post-hatch, while 101 mortality events (32%) the first 14 days. Similar results were obtained when hatch years were analysed separately (data not shown).

To investigate whether r_{DNA} influenced the results with respect to $r_{pedigree}$, additional analyses were conducted with r_{DNA} excluded from the model because $r_{pedigree}$ and r_{DNA} were negatively correlated ($R^2 = 0.275$, $n = 316$, $P < 0.001$). Both hatch year (B = −7.543 ± 0.313, Wald = 12.240, $P < 0.001$) and hatch facility (B = 0.259 ± 0.104, Wald = 6.177, $P = 0.013$) were significant in explaining chick mortality to 14 days post-hatch, while no significant pattern was observed with $r_{pedigree}$ (B = −7.543 ± 4.315, Wald = 3.055, $P = 0.080$). Although a similar pattern was observed to 50 days post-hatch (hatch year: B = −0.871 ± 0.272, Wald = 10.214, $P = 0.001$; hatch facility: B = 0.196 ± 0.097, Wald = 4.079, $P = 0.043$) when the r_{DNA} data were excluded, $r_{pedigree}$ was also significant (B = −7.975 ± 3.996, Wald = 3.984, $P = 0.046$). In the latter case, however, the model indicated that mortality to 50 days post-hatch decreased with increasing $r_{pedigree}$, and the hazards ratio for $r_{pedigree}$ was 0.000 (95% CI = 0.000–0.866) suggesting very little effect on mortality. In contrast, the hazards ratio for r_{DNA} was 33.412 (95% CI = 7.182–155.439) in the final model investigating mortality to 50 days post-hatch (Table 3). Further, the final model including r_{DNA} possessed the minimum AIC value for both post-hatch survival periods (14 days: 1047.77; 50 days: 1192.70) compared with $r_{pedigree}$ (14 days: 1058.77; 50 days: 1205.03).

While no correlation was observed between *HL* and $f_{pedigree}$ ($R^2 = 0.006$, $t = 1.055$, $P = 293$), a significant positive correlation was documented between *HL* and f_{DNA} ($R^2 = 0.751$, $t = 22.984$, $P < 0.001$), and when included in the proportional hazards model (f_{DNA} excluded, with all other covariates included; see above), mortality increased with increasing *HL* values (B = 1.252, SE = 0.530, Wald = 5.585, $P = 0.018$, Exp (B) = 3.496, 95% CI = 1.238–9.869) when controlling for age and captive/release status. Similar results, although with a negative regression coefficient, were obtained when substituting *HL* with the heterozygosity measures (i.e. H, SH & IR) used in this study, with the exception of d^2, which when included in the model was not a significant variable explaining mortality in the captive population (data not shown). The test for heterozygosity–heterozygosity correlation indicated that the microsatellite loci used to calculate HL were correlated ($r = 0.089$, 95% CI: 0.005–0.200), suggesting that each locus contributed proportionally to the heterozygosity estimate and therefore likely reflected genomic heterozygosity levels for each individual.

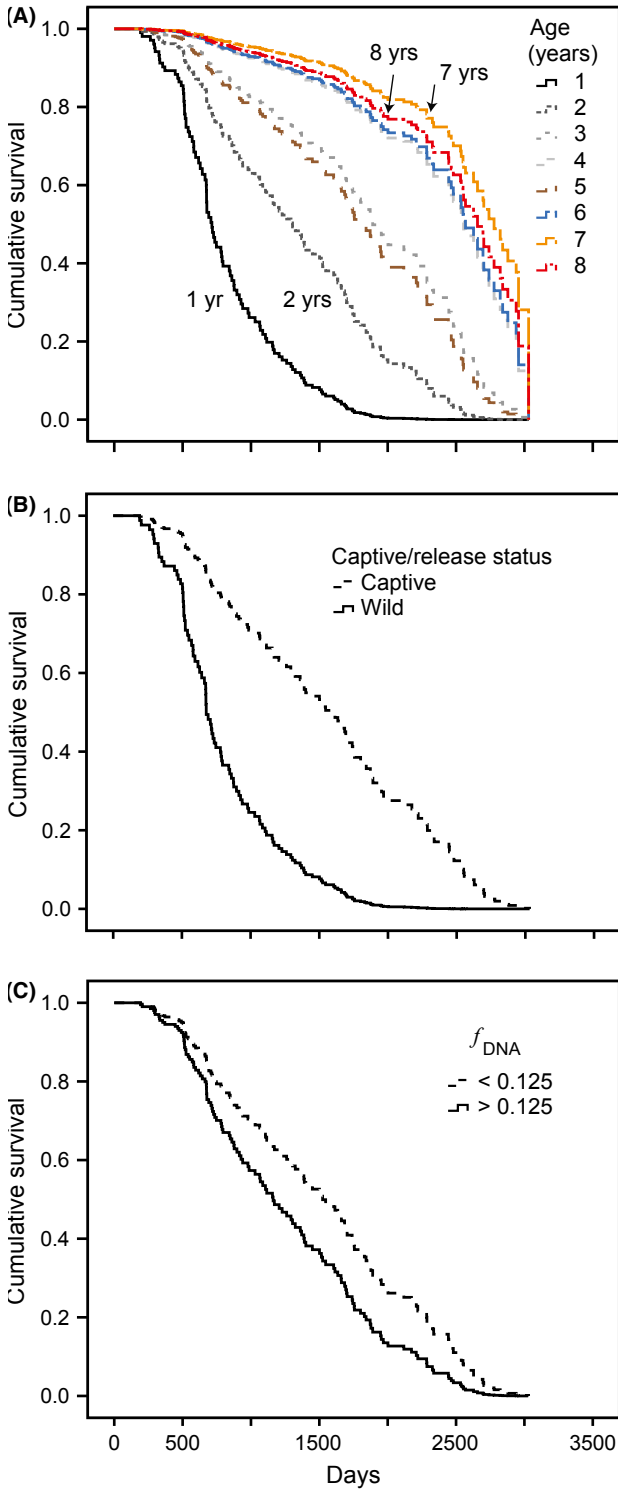

Fig. 3 Cox proportional hazards survival functions of juvenile and adult Attwater's Prairie-chickens ($n = 177$) comparing (A) age (years) at sampling, (B) captive/release status and (C) f_{DNA}. Age and f_{DNA} were modelled as continuous variables, but here they were treated as categories to illustrate the relative effect of age and inbreeding on cumulative survival.

Occurrence of wryneck differed among Attwater's Prairie-chicken offspring, with a greater proportion with wryneck having higher parental r_{DNA} than those without wryneck (Fig. 5; Table 4). In contrast, parental $f_{pedigree}$, egg storage time and chick hatch weight did not differ among chicks possessing wryneck (Table 4). Hatch year and hatch facility were not included in the GEE for wryneck because it was only documented at one of the two captive breeding facilities and was not observed in 2007 among the chicks from the genotyped parental pairs used in this study (Fig. 5).

Discussion

Similar to many species that are threatened with extinction (e.g. Spielman *et al.* 2004), the contemporary Attwater's Prairie-chicken population has reduced levels of neutral genetic diversity. A decline in genetic diversity as shown with the microsatellite and mtDNA sequence data is not unexpected with a founding population comprised of only 19 founding lineages (or 8.5 founder genome equivalents; U.S. Fish & Wildlife Service 2010) after the species' possessed up to a million individuals 100–200 years ago (Lehmann 1968; Morrow *et al.* 2004). Consequently, due to low genetic diversity levels, it is more likely by chance to pair individuals that share a recent common history or identity by descent, thereby allowing the expression of deleterious traits in their offspring as shown with other avian species such as the Greater Prairie-chicken (*T. c. pinnatus*; Westemeier *et al.* 1998), the southern Dunlin (*Calidris alpina schinzii*; Blomqvist *et al.* 2010), the Hihi (*Notiomystis cincta*; Brekke *et al.* 2010), the Black Stilt (*Himantopus novaezelandiae*; Hagen *et al.* 2011) and the Egyptian Vulture (*Neophron percnopterus*; Agudo *et al.* 2012).

The primary goal of all endangered species programmes is to prevent extinction (U.S. Fish & Wildlife Service 2010). However, our results suggest that using the current pedigree may not be the most efficient approach for maximizing survivorship in the captive Attwater's population. Both chick survivorship and adult survivorship were significantly correlated with DNA-based inbreeding coefficients and heterozygosity, while pedigree-based measures showed no correlation (Figs 2 and 3). Therefore, this study highlights the importance of using DNA-based methods to better inform management decisions when pedigrees are incomplete or errors may exist due to uncertainty in pairings (e.g. Lacy 2009).

To date, the captive Attwater's Prairie-chicken population has been managed based on its current pedigree initiated in 1992 (Morrow *et al.* 2004; U.S. Fish & Wildlife Service 2010). The 2006 captive population's mean estimate of inbreeding based on the pedigree

Table 3 Cox proportional hazards models of individual offspring (n = 316) time to mortality 14 or 50 days post-hatch (analysed separately) investigating the effects of egg storage time prior to incubation, weight at hatch, hatch facility, $r_{pedigree}$, parental r_{DNA} and consecutive hatch years. Critical values for both the full and minimal model are given. Significant P-values are in bold

				Full model		Minimal model			
Variable	d.f.	B	SE	Wald	P	Wald	P	Exp(B)	95% CI
Egg storage time									
14 days	1	0.021	0.026	0.681	0.409				
50 days	1	0.024	0.026	0.891	0.345				
Weight at hatch									
14 days	1	−0.084	0.072	1.354	0.245				
50 days	1	−0.055	0.067	0.669	0.413				
Hatch facility									
14 days	1	0.172	0.114	2.245	0.134				
50 days	1	0.105	0.107	2.592	0.965				
Parental $r_{pedigree}$									
14 days	1	2.615	4.882	0.287	0.592				
50 days	1	1.255	4.531	0.077	0.782				
Parental r_{DNA}									
14 days	**1**	**3.511**	**0.948**	**13.716**	**<0.001**	**20.130**	**<0.001**	**36.198**	**7.546–173.616**
50 days	**1**	**3.394**	**0.935**	**13.181**	**<0.001**	**20.013**	**<0.001**	**33.412**	**7.182–155.439**
Hatch year									
14 days	**1**	**−1.017**	**0.316**	**10.346**	**0.001**	**9.251**	**0.002**	**0.395**	**0.217–0.719**
50 days	**1**	**−0.802**	**0.275**	**8.473**	**0.004**	**8.126**	**0.004**	**0.470**	**0.280–0.790**

($f_{pedigree}$ = 0.025 ± 0.001, mean ± SE) is comparable or less than other endangered species such as the Mauritius Kestrel (*Falco punctatus*, $f_{pedigree}$ = 0.077 ± 0.007; Ewing *et al.* 2008), the Takahe (*Porphyrio hochstetteri*, $f_{pedigree}$ = 0.068; Jamieson *et al.* 2003), the South Island Robin (*Petroica longipes*, $f_{pedigree}$ = 0.027; Jamieson *et al.* 2007) and the Hihi (f_{DNA} = 0.027; Brekke *et al.* 2010) where inbreeding depression has also been reported. However, the variation of DNA-based inbreeding coefficients in the captive Attwater's population was higher in comparison with the pedigree-based coefficients (Fig. 1), with the mean population f_{DNA} (0.087 ± 0.008) being similar to or greater than other endangered species.

A discrepancy between pedigree- and DNA-based measures is expected, for example, when incorrectly assuming unrelated founders (Keller 1998; Lacy 2009). A total of nine males and 175 eggs from 14 nests were collected from the wild between 1992 and 1998 to initiate the captive breeding programme (U.S. Fish & Wildlife Service 2010). At that time, there were less than 500 individuals remaining in the wild having declined from an estimated 8700 individuals in 1937 and stabilizing around 1000–2000 individuals from the late 1960s through the 1980s (Lehmann 1941; Morrow *et al.* 2004). In establishing the captive population, founder males and eggs collected from different nests were presumed unrelated, while eggs from hens visiting the same booming ground (or lek; and most likely sired by the same male) were presumed half-siblings and those from the same nest were identified as full-siblings (U.S. Fish & Wildlife Service 2010). If the relatedness assumptions of those that survived to reproduce were incorrect and mating among related individuals were unknowingly allowed, pedigree- and DNA-based inbreeding values would likely diverge and thereby provide differing conclusions concerning their correlation with fitness (e.g. Jones *et al.* 2002; Russello & Amato 2004; Oliehoek & Bijma 2009; da Silva *et al.* 2010; but see Rudnick & Lacy 2008; Ivy *et al.* 2009). However, the average generation time for each of the Attwater's chicks at the time of sampling was 5.386 ± 0.626 (SD), which should be sufficient to estimate the levels of inbreeding within the current population based on the pedigree regardless of errors that may have been made in original founder assignments (Balloux *et al.* 2004). Because mating between closely related individuals in recent generations have the largest effect on inbreeding coefficients (Groombridge *et al.* 2012), it is more likely that errors introduced to the pedigree, rather than those based on incorrect founder assumptions, contribute to the high levels of inbreeding observed in captive Attwater's Prairie-chicken population (see also Rudnick & Lacy 2008).

Errors undoubtedly can enter the pedigree from incorrect parentage assignment (e.g. Signer *et al.* 1994; Tzika *et al.* 2009). For example, at least eight individuals in the 2006 Attwater's captive population were misidentified as parents for 12 chicks or 5.7% of those that survived to 8 weeks of age, based on exclusion using

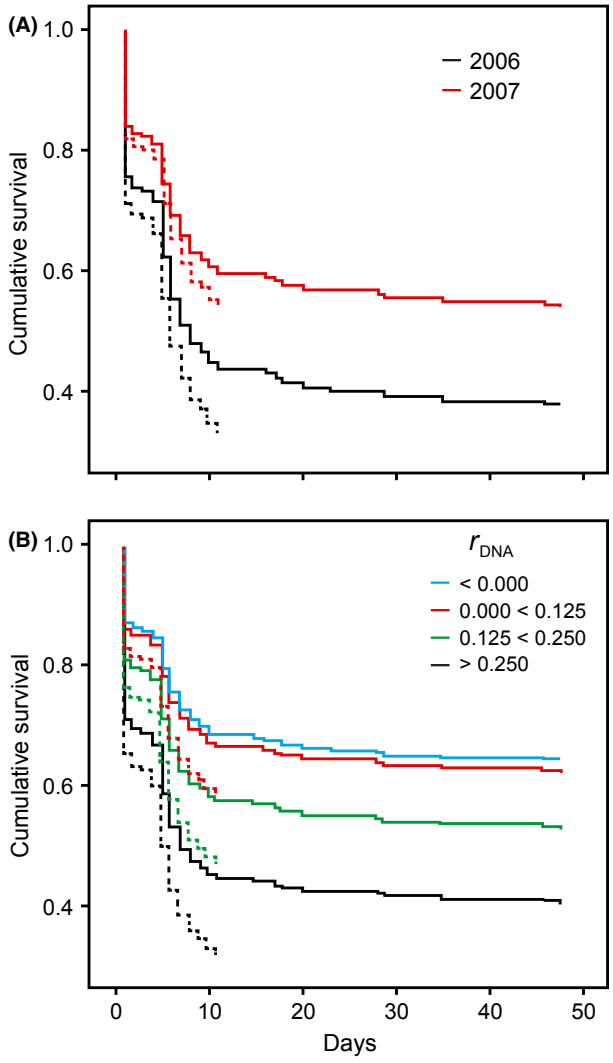

Fig. 4 Cox proportional hazards survival functions to 14 (dotted) or 50 days (solid) post-hatch comparing (A) hatch years and (B) parental DNA-based relatedness (r_{DNA}) for Attwater's chicks (n = 316). Relatedness was modelled as a continuous variable, but here it was treated as categories to illustrate the relative effect of inbreeding on cumulative survival.

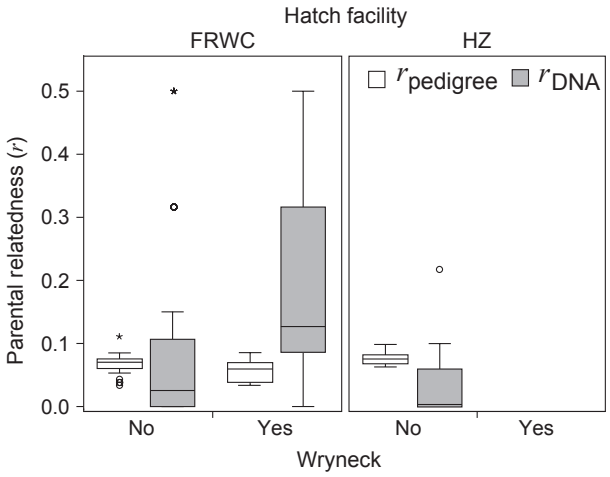

Fig. 5 Boxplot of Attwater's Prairie-chicken offspring (n = 225) parental r [pedigree (white) and DNA (grey)] and occurrence of wryneck at two hatch facilities in 2006. No occurrence of wryneck was observed in 2007 from the individuals sampled in 2006. FRWC, Fossil Rim Wildlife Center; HZ, Houston Zoo.

Table 4 Generalized estimating equations (GEE) for the occurrence of wryneck in captive Attwater's offspring (n = 316). Variables in the final minimal model after sequentially eliminating variables with $P < 0.1$ are shown in bold

	Occurrence of wryneck				
Variable*	d.f.	B	SE	χ^2	P
Full model					
Egg storage time	1	0.063	0.042	2.340	0.126
Weight at hatch	1	0.039	0.091	0.183	0.669
Parental $r_{pedigree}$	1	11.121	15.342	0.525	0.469
Parental r_{DNA}	1	4.841	1.449	11.163	0.001
Minimal model					
Parental r_{DNA}	**1**	**4.358**	**0.907**	**23.112**	**<0.001**

*Inclusion of hatch year and hatch facility produced either quasi-complete or complete separation of the data and therefore not included in the model (see results).

microsatellite genotypes (J. Johnson, unpublished). According to the pedigree, four of those individuals including their subsequent relatives comprised approximately 40% of the total captive population in 2010 (APC studbook, unpublished). Therefore, allowing errors to accumulate in the pedigree has important implications for minimizing inbreeding depression, with consequences magnifying in intensity across multiple generations. If left unchecked, such errors may become frequent enough to eliminate the utility of a pedigree for minimizing mean kinship in the population entirely (Lacy 2009; Oliehoek & Bijma 2009).

Inbreeding depression in birds is often recorded as a decline in hatching success or chick survival (Keller & Waller 2002; Briskie & Mackintosh 2004; Heber & Briskie 2010; Hemmings *et al.* 2012). Twenty-seven percent of captive Attwater's chicks fail to survive their first 10 days post-hatch, with 'failure to thrive' being the most common cause of death (APC studbook, unpublished). Similar to other populations exhibiting signs of inbreeding depression in early life stages (e.g. Swinnerton *et al.* 2004; Szulkin *et al.* 2007), Attwater's chick

mortality was positively correlated with parental DNA-based relatedness values (r_{DNA}), with the majority of the effect shown during the first 14 days post-hatch. This was further supported by comparing the cumulative mortality difference per day to 14 or 50 days post-hatch as shown by the steeper slope observed with the former data set (Fig. 4B) and also the fact that no significant correlation was observed between mortality, and any of the study variables when the chicks that died during the first 14 days post-hatch were removed from the analysis. The later effect, however, could be due to low power to detect a pattern because only 14 chicks died between 15 and 50 days post-hatch.

The occurrence of wryneck in chicks was also more likely to occur with higher parental r_{DNA} values (Fig. 5), suggesting that inbred individuals were more likely to possess wryneck than noninbred individuals. Because not all individuals with high parental r_{DNA} possessed wryneck, additional variables are important for the expression of this phenotype, and parental relatedness may be a possible contributing factor increasing the likelihood of wryneck occurrence in Attwater's Prairie-chickens (see also Savage & Collins 1971).

While inbreeding depression is often more noticeable in early life stages, it can also be detected throughout the lifespan of an organism (Daniels & Walters 2000; Kruuk *et al.* 2002; Szulkin *et al.* 2007; Bilski *et al.* 2013). A significant correlation in survivorship with age and f_{DNA} was observed in Attwater's Prairie-chickens that had reached reproductive maturity (≥1 year; Table 2, Fig. 3). These results suggest that a proportion of presumed less fit individuals (i.e. inbred) did not breed or died prior to the onset of the 2007 breeding season, corresponding with a decreased mean parental r_{DNA} (Fig. 2) and an overall increased chick survivorship (Fig. 4A) among the subset of captive breeding birds monitored in this study. In fact, no cases of wryneck were observed in chicks produced by the subset of individuals that also bred in the second year of the study. Although it is not known whether purging of deleterious alleles associated with the above fitness traits occurred before the second year's breeding season (e.g. Larsen *et al.* 2011), their persistence in the captive population is likely due to equal representation of each of the founder lineages by minimizing mean kinship in the captive breeding programme each generation, assuming that wryneck has a strong inheritance pattern.

A similar example was documented with the California Condor (*Gymnogyps californianus*) captive breeding programme. Ralls *et al.* (2000) identified carriers of chondrodystrophy, a lethal autosomal recessive skeletal disorder, tracing it back to four of the 16 genetic founders of the captive population. Because complete elimination of carriers would severely impact the species' population size, the captive breeding programme reduced its occurrence by preventing pairings that have a high probability of producing chicks with this condition (Ralls *et al.* 2000; Ralls & Ballou 2004). A similar strategy has been undertaken for the Attwater's with respect to wryneck. Any bird having a history of chicks with >10% wryneck has been excluded from the breeding pool. Having an accurate pedigree would allow a more robust approach for exploring inheritance patterns associated with the occurrence of wryneck in Attwater's Prairie-chicken and thereby determine whether environmental conditions, such as nutrition or artificial incubation, are playing any significant role influencing this condition in the captive population. More work is required to explore this relationship in more detail.

In addition to finding inbreeding depression associated with f_{DNA} in the captive Attwater's Prairie-chicken population, mortality was positively correlated with individual homozygosity (*HL*). These results can also be interpreted as evidence of inbreeding (Hansson & Westerberg 2002; Mainguy *et al.* 2009; see also Keller *et al.* 2012), which is further supported by a positive heterozygosity–heterozygosity correlation (HHC; Balloux *et al.* 2004). Although the HHC seen in Attwater's was weak ($r = 0.089$), a correlation between *HL* and inbreeding was expected (see Balloux *et al.* 2004) particularly as the correlation between the two values is strongest when there is a high variance in f (Slate *et al.* 2004; Szulkin *et al.* 2010) as was shown with Attwater's. Additionally, a significant positive correlation was observed between f_{DNA} and *HL* among the sampled juveniles and adults. Individuals with high inbreeding coefficients are more likely to be more homozygous (Charlesworth & Charlesworth 1987). Given that f_{DNA} and *HL* were correlated, it was not unexpected that they were both significant variables in the proportional hazards model explaining mortality.

While multiple studies have observed heterozygosity–fitness correlations (HFC; Amos *et al.* 2001; Acevedo-Whitehouse *et al.* 2003; Forstmeier *et al.* 2012), the use of pedigree-based analyses should provide a more robust approach for investigating fitness correlations with inbreeding (Slate *et al.* 2004; Pemberton 2008; Taylor *et al.* 2010). As described above, the discrepancy between pedigree- and DNA-based approaches as observed in this study is likely due to errors that exist in the pedigree and their compounding effect across generations due to managing the population to minimize mean kinship while assuming the pedigree was correct. Consequently, a high variance in inbreeding exists in the captive population as shown with DNA-based methods (Fig. 1), thereby allowing more statistical power to identify a significant HFC in the Attwater's Prairie-chicken population (Kalinowski & Hedrick 1999;

Grueber *et al.* 2008; Szulkin *et al.* 2010; Keller *et al.* 2012). More work is needed to determine whether inbreeding or loss of heterozygosity is playing a more significant role in reducing survivorship in this population; however, this task may prove difficult given that the two variables are significantly correlated using the available data set, including low genetic diversity existing within the population. Recent advances in generating large data sets using SNP markers should provide a more robust approach for addressing the above questions after minimizing errors that currently exist within the pedigree (e.g. Santure *et al.* 2010).

All breeding pairs in the Attwater's captive population are chosen to minimize overall mean kinship in the population. Therefore, our ability to detect inbreeding depression is much reduced when using the pedigree because of the low variance in *presumed* inbreeding for the population (Fig. 1A; see also Keller *et al.* 2012). While Bilski *et al.* (2013) documented inbreeding depression based on measures obtained from a pedigree for a captive population of bush dog (*Speothos venaticus*), their study population's mean inbreeding coefficient ($f_{\text{pedigree}} = 0.155$) and range (0–0.5) were much higher than that observed in the Attwater's population ($f_{\text{pedigree}} = 0.025$, range: 0–0.15), suggesting that low statistical power may explain the lack of support for inbreeding depression observed with Attwater's. However, our results also suggest that numerous errors exist within the pedigree as a significant negative correlation between mortality to 50 days post-hatch and f_{pedigree} was shown when f_{DNA} was excluded from the Cox proportional hazards model. Certainly, given the results from the DNA-based measures and the identified errors in parentage assignment, it is not reasonable to conclude that we should promote inbreeding as a valid approach for minimizing mortality in Attwater's Prairie-chicken (e.g. Leberg & Firmin 2008). These results highlight the uncertainty involved for choosing breeding pairs when sufficient errors exist in the pedigree.

Conservation and management implications

DNA-based measures of inbreeding and relatedness were used to show evidence of inbreeding in the Attwater's Prairie-chicken and that individuals with high inbreeding coefficients exhibited inbreeding depression or reduced longevity and lower offspring survival. These results, however, do not indicate recovery efforts for Attwater's Prairie-chickens are inconsequential. Other species have experienced similar bottlenecks and loss of genetic diversity and subsequently recovered with the assistance of captive management (Groombridge *et al.* 2001; Ralls & Ballou 2004; Swinnerton *et al.* 2004). The percentage of birds in the contemporary Attwater's population exhibiting high DNA-based inbreeding and relatedness coefficients, >0.25 or half-siblings, was relatively low (9.6% and 7.2%, respectively). In fact, observing inbreeding depression in the captive population is also positive confirmation that variability in fitness exists depending on the level of inbreeding despite an overall temporal decrease in neutral genetic diversity in the population. The range of inbreeding values and their significant correlation with mortality indicate that deleterious alleles associated with these traits have not gone to fixation or have been purged from the population (i.e. genetic load; Kruuk *et al.* 2002), otherwise, no pattern or correlation would have been observed (Keller *et al.* 2012).

To provide Attwater's Prairie-chickens with the highest likelihood of persisting and increase their probability of survival, careful selection of individuals for breeding purposes is critical not only to maintain current levels of genetic diversity (Willi *et al.* 2006), but to reduce inbreeding in the captive population (Hedrick & Kalinowski 2000; Witzenberger & Hochkirch 2011). With careful genetic management of breeding pairs, managers of the critically endangered Attwater's Prairie-chicken can minimize inbreeding in the captive population, thereby increasing the number of individuals available for release. Kaplan–Meier estimates of survival to 1 year post-release have averaged 17%, ranging from 8% to 43% since the release programme was initiated in 1995 (M. Morrow, unpublished). Increasing the number of birds released each year would increase the number that survive and breed the following season provided that sufficient resources are available to sustain a larger population (see Morrow *et al.* 2004, 2010; Pratt 2010; U.S. Fish & Wildlife Service 2010).

Increasing the number of individuals in the wild should also reduce the probability of inbreeding depression post-release, particularly in offspring produced in the wild. The Attwater's Prairie-chicken is a lek breeder characterized by a high variance in male mating success (Johnson *et al.* 2011), where a small percentage of males sire the majority of offspring in a given breeding season. Because pairings cannot be managed in the wild, inbreeding is more probable when the population is small (e.g. Ewing *et al.* 2008) and would most likely contribute to increasing local extirpation (O'Grady *et al.* 2006). In addition, inbreeding depression is often more pronounced in the wild compared with captivity (Crnokrak & Roff 1999) and in response to increased stress (Frankham 2005b; Fox & Reed 2010; Reed *et al.* 2012), particularly in populations with reduced genetic diversity (Bijlsma & Loeschcke 2011).

The Attwater's Prairie-chicken recovery plan identified poor chick survival in the wild as '…the single-most factor limiting significant progress toward

recovery' (U.S. Fish & Wildlife Service 2010: 40; see also Peterson & Silvy 1996). Therefore, minimizing inbreeding in the captive population is essential to improve the likelihood of producing a self-sustaining population, assuming other limiting factors such as habitat and food availability have been adequately addressed. Molecular methods are currently being used to reconstruct the pedigree and more accurately estimate kinship to better inform management decisions concerning future pairings and reduce inbreeding depression within the Attwater's Prairie-chicken captive population. With an increasing number of *ex situ* conservation programmes, it is highly recommended that a periodic assessment of pedigrees are conducted based on molecular methods (Lacy 2009; Oliehoek & Bijma 2009; Witzenberger & Hochkirch 2011), thereby increasing their ability to maintain healthy populations suitable for release once the cause of the species' decline is resolved.

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J.A.J. conceived the basis for this study. J.A.J. generated the molecular data. M.E.M. collected the survival data and provided historical perspective for Attwater's prairie chicken recovery. S.C.H. and J.A.J. performed data analyses. S.C.H. and J.A.J. wrote the initial drafts, while all coauthors provided editorial comments.

Data accessibility

Individual genotypes, mtDNA control region sequence alignments and mixed model SPSS input files for each of the data sets (i.e. chick and adult) are archived at Dryad: doi:10.5061/dryad.22 g66.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 A simulation of 17 dyads representing three relationship categories (full-siblings, half-siblings, and unknown) resulted in the following mean and variance values for each of the 7 estimators used in COANCESTRY.