

Genetic assessment of the Arabian Oryx founder population in the Emirate of Abu Dhabi, UAE: an example of evaluating unmanaged captive stocks for reintroduction

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INTRODUCTION

The Arabian oryx, *Oryx leucorox*, has become a flagship species in the conservation movement, subject to global efforts to ensure its survival since the 1960s. Despite being declared extinct in the wild in 1972 (Henderson 1974), the success of the World Herd captive breeding programme initiated over 40 years ago in the USA. Private collections across Arabia, have ensured that multiple small captive populations of Arabian oryx are available today to act as potential sources for reintroduction projects. The most well-known project of this type began in Oman at Jeddah Al Harasis, with the first herd of Arabian oryx released in 1982 (Stanley Price, 1989). This was followed by reintroductions in Saudi Arabia, Jordan, Israel and more recently the United Arab Emirates (UAE).

In the UAE, captive populations of Arabian oryx have been established at Al Ain Zoo and a number of different private collections throughout country. In total these now contain over 4,000 Arabian oryx representing more than 50% of the world population (Kiwan et al. 2008). One of these private collections, established by the late Sheikh Zayed bin Sultan Al Nahyan on Sir Bani Yas island, is of particular interest as it is home to *Oryx leucorox*. These animals have been allowed to breed naturally from very small founder populations over as many as ten generations. The high number of Arabian oryx on Sir Bani Yas island offers great potential to support mainland reintroduction projects, but there are clear concerns regarding the level of genetic diversity retained in today's population and potential effects of inbreeding depression.

The UAE captive stocks have recently been used as the basis of two Arabian oryx release programmes. In the Emirate of Dubai, an extended soft release has been undertaken in the Dubai Desert Conservation Reserve, where around 270 animals now live in a 225 km² fenced area receiving partial supplemental feeding and continuous management (Simkins 2008). In the Emirate of Abu Dhabi, a project to establish a free-living population was begun in 2007 on an 8,850 km² site at the Um El Zumool reserve. The research presented here focuses on characterizing the genetic status of the Um El Zumool herd and its source populations. The Um El Zumool reserve is located in the south-east of the Abu Dhabi Emirate, bordering Saudi Arabia to the west and Oman to the south (Figure 1). The reintroduction project was initiated under by H. H. Sheikh Khalifa Bin Zayed Al Nahyan, the President of UAE and is implemented under the patronage of H. H. Sheikh Mohammed Bin Zayed Al Nahyan, Crown Prince of Abu Dhabi emirate. The overall aim of the project is to have a free-living, unsupported population of Arabian oryx. The herd was composed by mixing 98 individuals selected from three sub groups. These were animals from the late Sheikh Zayed's collection kept on Sir Bani Yas Island (YAS; n=38), animals from Al Bahia (BAH), a private collection (n=20) and animals from Al Ain Zoo (AIN; n=32). The animals were translocated to 3 pre-release sites and kept for six weeks to acclimatize prior to release (Al Quarqaz and Kiwan 2007). The release was conducted in Feb 2007 at three sites with supplemental feeding provided for five years.

Consideration of genetic variation in small population management is becoming standard practice for both wild populations and captive breeding programmes. The smaller the population, the more critical it is to ensure that genetic indices such as haplotype diversity, heterozygosity and allelic diversity are maximized in future generations. Nowhere is this more evident than in reintroduction projects, where typically fewer than 100 individuals and sometimes less than 20 individuals, are released to found new populations (e.g. Greth & Schwede 1993; Ogden et al. 2005). Arabian oryx represent an extreme case in which the numbers of founders for both private collections and the world herd stock were remarkably low. Previous genetic studies on Arabian oryx focused on four groups held at San Diego Wild Animal Park (part of the world herd), Oman (the Arabian Oryx Sanctuary) and Saudi Arabia (Thumamah and Tail wildlife research centres). Microsatellite analysis revealed evidence of recent genetic bottlenecks (Marshall et al. 1999) and high levels of inbreeding were correlated with survival to infer inbreeding depression (Marshall and Spalton, 2000), highlighting the need to incorporate genetic data where possible in small founder-number releases.

The immediate purpose of the work presented here is to assess the genetic status of the reintroduced Arabian oryx herd at Um El Zumool reserve. Although it was not possible to incorporate genetic data into the selection of candidate animals for reintroduction, it was important to examine the level of genetic diversity within the founder population in relation to previous studies, to act as a reference point both for future supplementation of the herd and to enable comparison with other projects in the UAE (Simkins 2008), Saudi Arabia (Greth and Schwede, 1993), Jordan (Al Zaidaneen & Al Hasaseen 2008) and Israel (Saltz 2008). Research focused on examining data from the mitochondrial control region and a panel of microsatellite loci in order to address the following questions: Is there evidence that the founder population is composed of divergent ancestral lineages and if so how are these related? Is there evidence that the three contributing source populations are genetically distinct and what are the effects of genetic mixing? Is there evidence of recent bottlenecks in the Um El Zumool source populations? How does the level of genetic diversity in the unmanaged source populations compare to Arabian oryx populations managed by stud-books? What are the potential benefits to the ongoing genetic management of Arabian oryx and closely related species in the region? This last question is of wider importance as plans are in place to extend reintroduction programmes across multiple species within the UAE, including the large-scale translocation of individuals from Sir Bani Yas island. This study is the first opportunity to examine the

Methods

Arabian oryx were sampled on their translocation from their breeding sites to release sites. A total of 61 blood samples were collected as AIN n=4, BAH n=20 and YAS n=37. Only 58 were successfully yielding DNA. The mitochondrial control region was targeted for DNA nucleotide sequencing using primers MFRF and MFR-R. Polymerase Chain Reaction (PCR) amplification was carried out in a 20 µl reaction volume. A set of thirteen microsatellite loci, previously described in sheep, cattle or caribou and found to amplify polymorphic alleles in Arabian oryx were used to examine variability in the sample set.

Microsatellite analysis

Results for the thirteen microsatellite loci indicate very low levels of polymorphism (Fig 5). Locus RT3 was monomorphic in all populations and therefore excluded from subsequent analysis. The remaining loci had a mean allelic diversity across all populations of 3.4 and a mean effective allele number of 2.1. Analysis of individual populations, conducted on YAS and BAH only, show even lower levels of diversity within groups, with a further three loci monomorphic in the YAS population. Tests for Hardy-Weinberg Equilibrium (HWE) showed divergence from expectation in only one locus in one population (RBP3, YAS) following Bonferroni correction.

Population structure

The pairwise *F*_{ST} value between YAS and BAH was significant (*F*_{ST} = 0.138; *P* < 0.01), indicating a level of isolation across multiple generations between the two main founder groups. The results of STRUCTURE supported *K*=3 as the most likely number of founder groups in the data. At this level, all individuals were correctly assigned to their true source population in each replicate, with an average proportion of correct cluster membership of 91%. Triangle plots revealed clear evidence of genetic divergence between YAS and BAH and also differentiated the group of four AIN individuals (Figure 3). The results of bottleneck analysis revealed no evidence of recent bottlenecks in YAS or BAH source populations based on the Wilcoxon sign-rank analysis, however there was some support for a contraction in the YAS population based on the mode-shift analysis (Figure 4). The deviation from the L-shaped distribution expected under mutation-drift equilibrium indicates a possible historical bottleneck event.

Effect of founder admixture

By combining individuals from divergent nuclear and mitochondrial gene-pools, the Um El Zumool reintroduced population benefits from a marked increase in the levels of mitochondrial and nuclear genetic diversity when compared to that present in any single source population. In relation to the largest source population (YAS), this represents an increase of 175% in mtDNA diversity, 48% in allelic diversity and 25% in expected heterozygosity. When the Um El Zumool reintroduced population is compared to findings presented in Marshall et al. (1999) that focused on four managed populations (San Diego, Oman, Thumamah and Tail Wildlife), levels of diversity at the five common microsatellite loci are broadly similar (Fig 5). Allelic diversity is the same for Um El Zumool, San Diego and Thumamah and heterozygosity falls between that of the Oman and San Diego populations. Taken together these results suggest that by mixing the three source populations, the Um El Zumool reintroduction population is representative of the level of existing nuclear diversity in Arabian oryx across all populations and includes at least seven different maternal lines.

Implications for the reintroduction programme

In the context of conserving genetic diversity, the decision to combine individuals from multiple sources within the Um El Zumool reintroduction was clearly correct. This is exemplified by the comparison between diversity within the YAS source population individuals and the reintroduced group as a whole. If any one of the source populations had been used in isolation, the observed levels of diversity would be markedly lower. Nevertheless, the history of the species suggests that existing levels of inbreeding at Um El Zumool are likely to be high and that genetic drift is likely to be accelerated through a strongly hierarchical mating system that favours breeding success by only a small proportion of males. The inference of at least two unsampled haplotypes in the mtDNA control region network raises the possibility that further female lineages may exist within Arabian oryx. If so, individuals carrying these haplotypes would be strong candidates for further genetic supplementation of the Um El Zumool herd. The comparison of genetic diversity between the Um El Zumool source populations (YAS & BAH) in this study and the San Diego, Oman and Saudi Arabian populations studied in the late 1990s (Marshall 1998; Marshall et al. 1999) demonstrates the positive effect of stud-book breeding management on maintenance of heterozygosity and allelic diversity, as all four managed populations retain greater diversity than either of the two, larger, unmanaged UAE populations. It is only when the animals reintroduced at Um El Zumool are treated as a single population that the levels of diversity approach that of individual managed populations. Under wider comparison, the mean heterozygosity across polymorphic loci at Um El Zumool (*H* = 0.43) is low relative to other endangered species (Gebremedhin et al. 2009) and this value is likely to decrease due to incomplete founder representation in subsequent generations. Despite this concern, it appears that the reintroduction programme has successfully included a relatively high proportion of the genetic diversity that exists today in the Arabian oryx.

Statistical Analysis

Mitochondrial DNA sequences were edited by eye using CHROMAS v1.6 and aligned using GENEIOUS v4.7.6 (Biomatters Ltd, New Zealand). Gene diversity was examined by estimating pairwise nucleotide distances (uncorrected *p*-distance) using the software MEGA v.2.1 (Kumar et al. 2001). Haplotype relationships were analysed using the median-joining network analysis programme, NETWORK v.4.5.1.0. This approach is designed to investigate intraspecific phylogenies and allows inference of unsampled or extinct sequences within the network (Bandelt et al. 1999). Microsatellite data were examined for departures from Hardy-Weinberg equilibrium using GENALEX 6.0 (Peakall and Smouse 2006), before calculating allelic diversity and effective allele numbers. Population structure was investigated by calculating pairwise *F*_{ST} estimates among original founder populations and through implementation of the Bayesian inference package, STRUCTURE v.2.1 (Pritchard 2000). In the STRUCTURE analysis individuals were assigned to *K*=1-4 populations (burn-in 100k reps, simulation 1 million MCMC reps, no. of reps = 3). The likelihood of the data under increasing *K* values was assessed to determine the most probable number of identifiable genetic units within the data, together with the percentage of times individuals were correctly assigned to their population of origin. Evidence of recent bottleneck events was investigated in the YAS and BAH source populations using the programme BOTTLENECK (Cornuet and Luikart 1996; Piry et al. 1997) that assesses gene diversity (heterozygosity) excess relative to allelic diversity. A two-phase mutation model was implemented assuming 90% stepwise mutation and analysis was based on the Wilcoxon sign-rank test and mode-shift distribution results. To provide a direct comparison to the level of genetic diversity previously observed in *Oryx leucorox*, summary data for five common microsatellite loci from four previously studied oryx populations (Marshall et al. 1999) from San Diego Zoo (n=90), Oman (n=77) and Saudi Arabia (n=34, n=97), were included in the analysis.

RESULTS

Mitochondrial DNA sequencing

A total of 58 control region sequences were produced with fourteen polymorphic sites resulting in seven different haplotypes were determined. Observed haplotypes were labeled alphabetically. The network analysis indicates that all seven haplotypes are closely related, with a maximum of four mutations between observed haplotype pairs and a total of 15 mutations linking all haplotypes. However the four major branches of the network are connected through inferred haplotypes which are now missing from the populations or remain unsampled (Figure 2). The Al Bahia (BAH) population contained the most diversity with five of the seven haplotypes observed in this group. Despite being almost twice the size of the BAH population, the Sir Bani Yas group (YAS) only displayed three haplotypes, only one of which was shared with Al Bahia. The Al Ain zoo animals (AIN) were monomorphic for haplotype D.

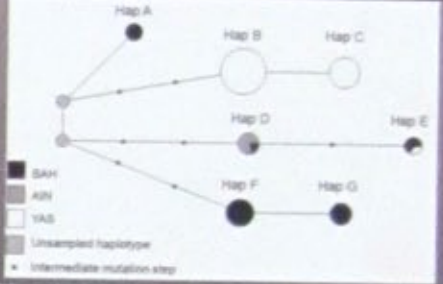


Fig 2) Haplotype network showing the relationships among the seven mitochondrial control region haplotypes observed in the three source populations. Circle size is proportional to number of individuals displaying the haplotype. Distances between haplotypes are relatively small however two haplotypes remain either unsampled or have been lost from all populations.

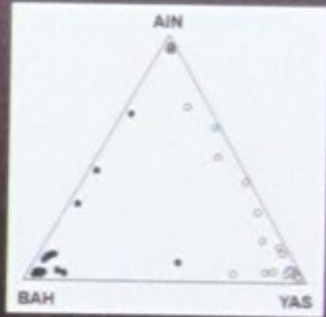


Fig 3) Triangle plot showing the results of structure analysis for 1023 individuals. Circles represent individual animals, shading represents populations corresponding to Sir Bani Yas (YAS; white circles), Al Bahia (BAH; black circles) and Al Ain (AIN; grey circles). Clear differentiation among source populations is observed with the exception of one individual intermediate between YAS and BAH.

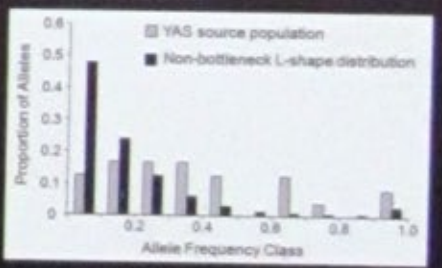


Fig 4) Results of mode-shift analysis for the Sir Bani Yas source population showing evidence of a recent bottleneck based on increased loss of allelic diversity relative to heterozygosity



Map of UAE showing the location of the reintroduction area of Um El Zumool and locations of the three founding populations (squares). Um El Zumool reintroduction occurred at three carefully selected release sites (circles).

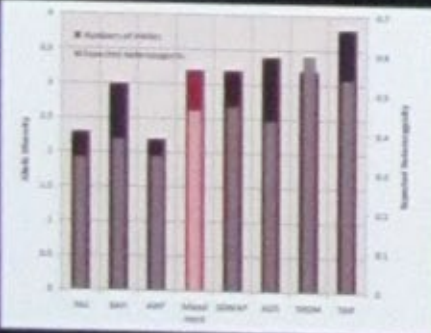


Fig 5) Measures of genetic variability among populations of Arabian oryx describing the number of mitochondrial control region haplotypes (mtDNA), allelic diversity (A) and expected heterozygosity (HE). Mixing the three source populations has increased the level of genetic variation within the Um El Zumool reintroduction population. A comparison of this population with other Arabian oryx populations based on five common microsatellite loci (Marshall et al. 1999) suggests that the genetic variation in the Um El Zumool population is similar to that of previous captive-managed and reintroduced populations.

DISCUSSION
Source population diversity
The genetic distinctiveness of the YAS and BAH populations is supported by data from mitochondrial and nuclear DNA markers. The observation of only one of seven mtDNA control region haplotypes common to both groups, combined with the fact that most females in a herd will typically contribute to subsequent generations, suggests that much of the population differentiation is likely the result of separate sets of founder individuals. The structure observed in the microsatellite data may also be attributed to differences among initial founders, but these will have certainly been amplified through genetic drift, including possible loss of common alleles, during isolated breeding over multiple generations. The differentiation of the AIN animals under STRUCTURE analysis and their observed mtDNA haplotype indicate that these individuals will contribute additional genetic diversity to the reintroduction programme. As both the YAS and BAH populations are assumed to have been essentially unmanaged over a similar number of generations, the lower level of mitochondrial and nuclear variation in the YAS population is also probably explained by the relative levels of diversity present in the founders. This is supported by evidence of a bottleneck in this group, indicating the persistence of a small founder effect signal. The relatively low level of diversity observed in the YAS group (*n*=37, *A*=2.3, *H*_E=0.337, mtDNA haplotypes=3) is important as there have been an estimated 3000 Arabian oryx bred on the island which now represent approximately 50% of the entire species. The way in which the Sir Bani Yas Arabian oryx resource is utilized, requires careful consideration. In the absence of pedigree information for any of these populations, an assessment of the genetic diversity present on the island relative to other potential source populations is recommended as standard practice prior to large scale translocation and reintroduction to maximize the conservation of genetic diversity and minimize the risk of inbreeding. Where possible, the ongoing reintroduced oryx populations should also include a programme of genetic monitoring (Schwartz et al. 2007); such a programme is currently being planned for Arabian oryx at Um El Zumool.