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A Molecular Investigation of Inbreeding in Captive Addra Gazelles

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Abstract

Captive breeding of individuals to augment or reestablish a wild population requires the maintenance of maximum possible genetic variation to reflect the genetic variation present in the original wild population and reduce the occurrence of genetic drift or inbreeding in the captive population. Critically threatened addra gazelles (*Nanger dama ruficollis*) have been maintained in a captive breeding program since 1969 (10-15 generations) with no introduction of genetic material beyond the original 22 founders, of which only 8 have recorded descendents in the current population. Results from this study show a strong relationship between infant mortality and inbreeding, and a substantial increase in infant mortality over the first 20 years of the breeding program. In addition, molecular measures of inbreeding were correlated to various historical scenarios and suggest that more founders may have contributed to the population than expected based on pedigree data alone. A genetic sampling of all individuals in the population may be the only way to identify the most genetically distinct individuals in the population, and the best option for maintaining future genetic diversity.

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Introduction

The addra gazelle (*Nanger dama ruficollis*) is an endangered desert antelope (Figure 1) that is likely to go extinct in the wild. Maintaining a viable population in captivity is therefore of critical importance for planned reintroductions of the species if habitat can be restored or reclaimed. The existing captive population faces a number of challenges, including uncertainty in the studbook and potential inbreeding depression due to restrictions on the addition of new founders to the population. This study aimed to investigate the population genetics of this subspecies, to determine the link between inbreeding and infant mortality, and to compare inbreeding and genetic data to the more extensively studied *Nanger dama mhorr* population, which has been maintained in captivity longer with fewer founders.

Captive Population Management

Captive populations of endangered species are often viewed as a safeguard against extinction, and in the case of extinction in the wild, a last chance for revival (Tudge 1992; Rusello 1997). *Ex situ* conservation poses a number of challenges that make it an appropriate option only where other strategies have failed to maintain a viable wild population. These challenges include:

1) Adaptation to captivity may occur due to intentional and unintentional artificial selection, specifically domestication or a loss of predator avoidance and food

acquisition instincts (Frankham 1994) as well as tolerance of humans and loss of resistance to natural diseases or parasites. Measurable adaptation occurs within tens of generations and can be combated by equalizing family size to reduce selection, or mimicking the wild environment as closely as possible (Gilligan & Frankham 2003). This problem grows with the number of generations the population remains in captivity.

2) Founder effects and genetic drift are problems in populations with few founders or those maintained for long periods in captivity. Drift may cause a loss of variation in the population, increasing susceptibility to parasites and environmental stressors. This ultimately decreases evolutionary potential and increases likelihood of extinction (Frankham 2002). Political realities often prevent the introduction of new wild founders into the population.

3) Founders of unknown origin may not represent the whole genetic or regional variation of the species. Founders may be related, but management programs always assume founders to be unrelated and outbred (Toro *et al*. 2003)

4) Inbreeding effects reduce fitness, particularly resistance to parasites and reproductive success (Lacy 1993). Inbreeding depression can have a number of symptoms related to offspring viability, adult reproductive quality, and other factors that reduce the viability of the population as a whole (Cassinello 2001). Ultimately inbreeding also reduces genetic variation in the population (Ballou 1997). Inbreeding is a particularly pressing problem in small populations where mate choice is limited and unrelated individuals may be scarce.

5) The problems that drove the wild population to extinction often still exist, and will almost certainly drive any reintroduced population to extinction as well until the source is addressed (Tudge 1992; Newby *et al.* 2008).

Despite these issues, the maintenance of a captive population provides time to address the problems causing the wild population's decline in the event improvements are made too slowly to keep the wild population at a sustainable level. The number of generations in captivity may impact the viability of reintroduction efforts as small captive populations are prone to genetic drift, inbreeding, and other factors causing a reduction in genetic diversity.

Captive populations of animals, especially those subjected to a bottlenecking event due to low founder numbers, face a high risk for inbreeding and consequent inbreeding depression. Studies done in mhorr gazelles (*Nanger dama mhorr*) and related species found a link between higher levels of inbreeding and both higher parasite susceptibility (Coltman 1999; Cassinello 2001) and reduced reproductive success (Alados 1991; Gomendio 2009). Inbreeding in captive ungulates [including gazelles (Ralls 1979)] is most evident through increased infant mortality over several generations (Lacy 1993; Coltman 1998; Amos *et al.* 2001). Mortality related to inbreeding depression can be counteracted by improved husbandry and veterinary practices (Kalinowski 1999), though probably not indefinitely. Inbreeding in mhorr gazelles has even been shown to decrease both natural reproductive viability and the viability of semen cryopreservation (Roldan 2006). Due to the cost and state of reproductive technology, cryopreservation is not a currently used genetic management strategy, but may become important in the future (Tudge 1992).

Because of the number of negative effects associated with inbreeding and a loss of genetic diversity, captive population managers actively work to minimize inbreeding and loss of genetic variation. Most tools used are based on a studbook or pedigree of animals in the population, but in some critical cases the use of molecular genetics to investigate genetic variation and inbreeding is becoming more common. Actual genetic variation can be measured in either allelic diversity (though for most captive populations, including addra gazelles, the alleles present in the wild population are unknown) or heterozygosity (Ballou 1996). The mechanism of decreased individual fitness due to inbreeding depression is suspected to be related to heterozygosity either through increases in deleterious recessive alleles or the loss of heterozygote superiority (Ballou 1997). Genetic variation can be estimated using pedigree information which is only available in regulated captive environments such as studbook participant zoos, and can be augmented by molecular data. DNA variability for example, from nuclear microsatellites, can be used to clarify pedigrees (Signer 1994; Jones 2002; Russello 2004) and plan for management practices to best retain genetic variability (Signer 1994; Austin 2009). For incomplete pedigrees or those that have questionable paternity assignments (i.e., based on observational inference rather than genetic data), inbreeding can be significantly under- or overestimated based on pedigree data alone (Willis 1993; Pemberton 2008), particularly in small populations. For captive breeding programs with a focus on retaining diversity, it is recommended that individuals of unknown parentage but who have descended from the same group of founders should be excluded as potential mates, due to the added unavoidable inbreeding and uncertainty their mating could contribute to the

population (Willis 1993). In practice this recommendation has not always been followed due to the small population sizes in captivity.

Captive breeding plans are generally based on recommendations made by referencing the species studbook, which includes the following information for all individuals in SSP (Species Survival Plan) member institutions: the sire and dam; dates of birth, transfer to other institutions, and death; and studbook and local identification numbers. This information can be used to calculate relatedness between individuals to avoid inbreeding and identify individuals of particular genetic importance (e.g. for breeding those representing less common lineages or those with a low relatedness to the rest of the population; Ballou 1995).

Although a studbook is the result of significant care and research, sometimes records are spotty, especially for individuals born into the population before the studbook was established. For most species, the studbook is the primary tool for planning breedings based on maintaining genetic variation. In most cases records for paternity are based on behavioral observations of the presumed parents and no notes are made in the studbook of possible alternate paternity or what the likelihood of alternate paternity might be.

Two main strategies in planned breeding include minimization of kinship (MK) and maximization of inbreeding avoidance (MIA). Minimizing kinship reduces average relatedness over the population as a whole, while MIA mates individuals who are the least related to each other. Simulations have found that the MK strategies are most effective at retaining genetic variation by equalizing the contribution of all original founders (Montgomery 1997). This is the strategy most often employed in captive

breeding recommendations, though matings of closely related individuals are still avoided (Read 1986; Tudge 1992).

Even if inbreeding is avoided, genetic diversity is lost to genetic drift in small populations. The loss of genetic diversity due to genetic drift in a population for a given generation can be calculated by:

$$GD_t = GD_0(1 - \frac{1}{2Nt});$$

Where GD= genetic diversity, N= population size, t=generation (Lacy 1995).

To delay the effects of genetic drift on the population, it is necessary to increase generation time. This strategy extends the time before genetic variation is lost from the population, but does not ultimately prevent it. The extra time the genetic variation is retained in the population increases the genetic variation still present in the population if or when the population is needed to contribute individuals for introduction. It is assumed that the addition of genetic variation by mutation is negligible in such small captive populations and does not act as a source of additional variation. Genetic diversity is measured as ratio of the current genetic variation to the wild or original variation (assumed to be captured by the variation in the founders). Because it is a ratio and wild populations differ in their genetic variability, most population management analyses rely on the related measure Founder Genome Equivalent (FGE) (Lacy 1995):

$$\frac{GD_t}{GD_0} = 1 - \frac{1}{2(FGE)}$$

Direct measures of genetic variation are better estimates of variation than pedigree data alone. These measures are used to determine heterozygosity for a population, but not to avoid matings of animals with similar alleles at a single locus

(Lande 1987). A combination of molecular data and pedigree data is most effective at reducing homozygosity, as molecular markers are most effective at distinguishing close relatives (Toro 1999), and management of genetic variation is more effective with a combination of partial pedigree and molecular data than either alone (Pemberton 2008). The genetic drift model estimates a worst-case scenario, and can potentially lead to much lower values of genetic diversity than are found by molecular measures of diversity (Earnhardt *et al*. 2004).

Wright (1922) defined a measure of inbreeding that is commonly used in population management. Wright's Coefficient of Inbreeding, F, defines the probability that two alleles at one locus in an individual are the same by descent.

$$F = \sum_{i=1}^{N} \left(\frac{1}{2}\right)^{n_{f,i} + n_{m,i} + 1} (1 + F_i)$$

In this equation, N is the number of common ancestors between the sire and the dam, $n_{f,i}$ is the number of generations between the sire and the common ancestor i, $n_{m,i}$ is the number of generations between the dam and the common ancestor i, and F_i is the coefficient of inbreeding of the ancestor i.

Genetic management is particularly critical for threatened species, as genetic factors are exacerbated in small populations (Spielman 2004). Once genetic variation in a captive population has been lost, augmenting the population with additional founders may be the only way of restoring variation representative of the wild population (Shen *et al*. 2009). Ideally, wild genetic variation should be investigated before a captive population is established, or even before the wild population begins to decline (Blouzin 1998), but this is uncommon in practice. Limited time and resources are dedicated to the

taxon in greatest need of aid, generally after the wild population has declined or disappeared (Tudge 1992).

Maintaining genetic variation in a reproducing population is especially important in cases where breeding success and family size in the wild may be highly unequal. Especially in the first generation, reintroduced populations may experience a loss of genetic variation due to highly variable breeding success (Milinkovitch 2004), which can lead to close inbreeding within a generation (Abaigar 1997).

Nanger dama natural history

The dama gazelle, *Nanger dama* (formerly *Gazella dama*), is a critically endangered and declining migratory desert ungulate species that ranges from Mali to Sudan (Newby *et al*. 2008). The dama gazelle is a desert gazelle, living singly, in pairs, or in harems. Bachelor herds are not observed in the wild in this species (Grettenberger & Newby 1986). Herd sizes have ranged up to several hundred, but now they are rarely seen in groups larger than 10 or 15 (Mallon & Kingswood 2001). Females have a gestation time of 7-7.5 months and give birth to a single offspring per year (Furley 1986). In captivity, birth can occur at any time of year but is most common from April to June (Mallon & Kinswood 2001; Antelope TAG 2008). Females reach reproductive maturity at less than 2 years, while males can reach maturity as soon as 4 months though generally closer to 1 year (Furley 1986; Antelope TAG 2008). This species is considered shy and difficult to study in the wild (Monfort 2001), and most information on the species comes from studies of captive animals.

The species is divided into three subspecies based on geographical location and pelage variation (Perez 1984): the westernmost mhorr gazelle (*Nanger dama mhorr*), the central nominate dama gazelle (*Nanger dama dama*) and the eastern red-necked gazelle or addra gazelle (*Nanger dama ruficollis*). Remaining numbers of *Nanger dama dama* are estimated to be no more than 1000 (Mallon & Kingswood 2001) and *Nanger dama ruficollis* at no more than 1000 but closer to 100 (Mallon & Kingswood 2001; Monfort 2001). *Nanger dama mhorr* became extinct in the wild in 1969, but has been successfully introduced or reintroduced in several locations (Cano 1993; Wiesner and Muller 1998; Abaigar et al. 2007). A single viable captive population of *Nanger dama ruficollis* exists in the United States and is considered a potential safety net for the subspecies, but no captive population exists for *Nanger dama dama*. (ISIS 2008; Newby et al 2008).

Dangers to this species include habitat loss, competition with livestock for food, and massive unregulated hunting with machine guns. While it is regionally extinct in many areas of its former range (Grettenberger and Newby 1986; IEA 1998; Newby et al 2008), there is no coordinated effort to protect this animal in its current range, and the political climate of the region makes any change in this status highly unlikely. The species is currently found only in fragmented habitat and most sightings are of one or two individuals rather than large herds which were formerly observed (Grettenberger and Newby 1986; Monfort 2001). Only four areas across Mali and Chad have a known population of 100 or more (Newby et al 2008). Lack of data, regulation, and secure reserves for this species make continued survival in the wild increasingly unlikely. Managers recognize the potential need for future reintroductions of *Nanger dama mhorr* and *Nanger dama ruficollis* (Antelope TAG 2008).

Captive Management and History of *Nanger dama*

As with most captive populations of endangered species, addra gazelles are managed at a regional level. Managers use information on the populations of the species in captivity and in the wild to make management decisions. Mhorr and addra gazelles in the North American management plans were managed under the 90%/100 I management plan (Sausman 1993), designed to maintain 90% of original population heterozygosity for 100 years from roughly 1995 to 2000. After this point, it was calculated that the maximum variability obtainable for addra gazelles was 79% (Antelope TAG 2008), and this was designated the new goal for this species. In order to achieve this goal, more space was needed to house addra gazelles than was available and mhorr gazelles, being managed viably in elsewhere, were phased out of American zoos to make room for addra gazelles (Antelope TAG 2008). The ideal effective captive population to preserve this amount of genetic variation would be between 200 (Earnhardt 2001) and 500 (Lande 1987), but space constraints keep the actual housable population (K_{target}) at or below 200 (Antelope TAG 2008). The current population exists at closer to 120 (ISIS 2008). For a number of reasons, most zoos do not dispose of non- or post-reproductive individuals, though they occupy space that would otherwise be used to house breeding individuals (Tudge 1992). This leads to a lower ratio of N_e (population effective size, comprised of only reproducing individuals correcting for genetic variation) to N (actual population).

The mhorr subspecies has a much longer history of captivity than the addra gazelle. An initial breeding program was maintained by the Spanish military in Spanish

Sahara (Abaigar 1997). In 1969, the plight of the species was recognized and a foundation group of 2 males and 12 females who were likely related (Dolan 1981) were sent to Almeria, Spain at the Arid Zones Experimental Station. By 2000 this station housed 109 mhorr gazelles and was the origin of all gazelles sent to other institutions in Europe and the US (Alados 1988). Almeria's 14 likely related founders falls short of Read and Harvey's (1986) now standard recommendation of 20- 25 unrelated individuals to found a longstanding captive population and Lacy's recommendation of 20 effective founders (Lacy 1989; Willis & Willis 2010). The population has been managed, similarly to the AZA addra population, with genetic considerations such as inbreeding and variability taken into account. However, due to the small founder size, lack of information about the founders, and a relatively small sample size, estimations of inbreeding and related metrics have a substantial margin of error (Alados 1988). The current management recommendation for this captive population in Spain is to delay breeding to keep population levels reasonable given available space while using strategic mating to maintain genetic diversity (Alados 1988). In practice, the population has grown rapidly in order to produce individuals for reintroduction plans (Alados 1988).

Because of uncertainty of origin, not all individuals in captivity are a part of the managed population, even within an AZA institution that participates in the SSP. The AZA population of mhorr gazelles is currently considered unviable and is being phased out to open up space to house addra gazelles, which are considered a viable Species Survival Program. The addra gazelle SSP has a target population of 200 gazelles (Antelope TAG 2008) based on space zoos are willing to contribute to house the species rather than any number derived as sufficient to retain genetic diversity. Not all 200 spaces

are currently available for the housing of this species, and not all 200 spaces will be occupied by breeding members of the managed population.

Nanger dama ruficollis is currently represented in captivity by 121 individuals in managed North American institutions (Current Studbook, 2008). An unknown number exist in private collections outside of AZA (Association of Zoos and Aquariums) institutions, but their number, pedigrees, and history are completely unknown and they are not accessible for purposes of breeding or reintroduction. These unknown individuals are occasionally reintroduced into the managed population, but are descended from the same initial US population founders. For a variety of reasons, it is unlikely that wild-caught individuals will ever be introduced into the captive population, so no additional genetic variation will be added in future generations by outbreeding.

In captivity, the *Nanger dama ruficollis* females are reproductively most active from age 2 years to 9 or later (Antelope TAG 2008). Females are potentially reproductively mature from the age of 2 and males can become reproductively mature before the age of 1 (Addra Gazelle Studbook 2008), leading to some potential questionable paternity in any herd containing females with male offspring. Females produce a single calf per year and appear to be prone to abortions if the herd is subject to significant social stress (Alados 1988). Without intense competition, however, female deaths and abortions appear to be fairly rare, allowing populations to grow in captivity (Alados 1988). The generation time for the species in the wild is 5.25 years, which is longer than most gazelle species but low compared to larger ungulates (Alados 1988). The lifespan of wild gazelles is estimated at 12 years, with gazelles in captivity living as long as 18 (Jones 1993; Antelope TAG 2008; Historical Studbook 2008).

Gazelles are managed in single-male multi-female herds, all-male herds, male-female pairs, or individually (ISIS 2009). To equalize male family size, male-female pairs are preferred, but this is generally not an option given space considerations and minimization of stress by transfer. Only a few institutions have the spatial resources to house a separate single-male breeding herd and an all-male herd. Male herds are costly to maintain in terms of space, as restricted territory increases aggression and injury risk in all-male herds (Cassinello 2000). Such all-male herds are not found in this species in the wild (Grettenberger & Newby 1986).

In the wild, dama gazelles form harems with a single breeding male monopolizing a group of reproductively mature females. This strategy was employed at the beginning of the addra breeding program, with the intent of maintaining the animals in their natural social arrangement (Lande 1987; Lacy 1993). Genetic variability is reduced by this practice, as one male monopolizes breeding females and few other males have the opportunity to breed. This led to one addra male in the AZA population producing over 100 offspring, while many of his contemporary males producing none (Historic Studbook 2008). The most viable and robust individuals were selected for breeding instead of weaker and less attractive individuals. This form of domestication and artificial selection reduces genetic variability within the population. Current management recommendations include obtaining an equal number of offspring from all individuals in the population to reduce loss of genetic variation. This did not occur perfectly in practice due to the spatial requirements of housing hoofstock. It is easier and less space-intensive for zoos, and more appealing to their visitors, if gazelles are housed together in one or two herds rather than singly and doubly. Males can be housed together in bachelor herds, though smaller

enclosures cause increased stress and aggressive interactions (Cassinello 2000), so most zoos keep a single adult male and one or several females, and a few institutions with more available space keep all male herds (ISIS 2008). To introduce new breeding individuals into a herd, it is almost always a male that is sent to other institutions.

Though a population may be divided into discrete herds, as long as one individual travels between groups each generation, the groups may be considered an interbreeding population (Lande 1987). Moving animals between institutions is often minimized for logistical, cost, and safety reasons (Tudge 1992).

Potential for Reintroduction

Captive populations of both *Nanger dama ruficollis* and *Nanger dama mhorr* are considered highly important for the purpose of reintroduction to the wild due to the status of the wild populations of each (extinct and critically threatened). The mhorr program was established first, and as the animal is believed to be extinct in the wild, reintroductions and translocations to similar habitats have already occurred with the hopes of reestablishing a wild population. As the current trend of decline in *Nanger dama ruficollis* populations is unlikely to be reversed, it is highly likely that the captive population will be called upon to augment or reestablish the wild population. Results from the mhorr reintroductions can illuminate potential problems for any future *Nanger dama ruficollis* populations to avoid or overcome.

A small group of *Nanger dama mhorr* founders (2 males and 5 females) was selected to establish a reintroduced population at the Gueumbeul Reserve in Senegal. The

herd was successfully established, though managers noted a high rate of inbreeding, high adult mortality rate biased against females, and a consequent slower than expected population growth rate (Cano 1993). At this point managers recommended introducing additional founders to combat observed inbreeding and improve the sex ratio. Another mhorr translocation, this one an introduction to a natural area, occurred between 1990 and 1994 in the Bou-Hedma National Park in Tunisia, an area similar to but outside of the prior dama range (Abaigar 1997). This introduction also utilized individuals from the Arid Zone Experimental Station captive population, including 4 males and 12 females (Abaigar 1997). Within a few days they showed natural behaviors of eating acacia leaves and hiding in bushes, despite never having encountered these stimuli previously (Wiesner 1998). Within months the animals had completely stopped taking offered water sources and achieved a near wild flight distance, and appeared to have avoided the domestication effects of many generations in captivity (Wiesner 1998). While these observations offer reason for optimism, the population has not been observed long enough to make conclusions regarding long term survivorship or reproductive success.

These cases show that carefully selected and managed animals of the mhorr subspecies, extinct in the wild since 1969 (Abaigar 1997) appear to be able to readapt to a protected wild environment. The population has, however, descended from stock held in captivity for many generations, and have encountered problems due to close inbreeding. The question remains, however, whether the long bottleneck due to captivity will have long-term ramifications, especially in terms of loss of genetic variability. Whether the populations will ultimately stand up to environmental pressures from which they are

currently sheltered [starvation, parasites (Abaigar 1997), predation (Cassinello 2000), poaching, major climactic events and other factors] remains to be seen.

The *Nanger dama ruficollis* population would seem at the surface to be a better candidate for reintroduction than the mhorr gazelle. The captive population of the addra gazelle in North America had a larger starting founder size, and the founders were wild-caught and less likely to be related than the captive founders of the mhorr population (Historic Studbook 2008; Antelope TAG 2008). The addra gazelle has also been kept in captivity for less time than the mhorr gazelle, which may lead to fewer problems related to loss of genetic variability.

The objective of this study was to quantify the levels of inbreeding in the current population using molecular techniques and compare these to historical records. It is important to assess the reliability of historical records when they are used as the sole source of information for planning population management. With reintroduction programs of related subspecies showing inbreeding-related problems, it is critically important to assess and avoid these problems in *Nanger dama ruficollis* while it is possible to do so.

Materials and Methods

This study used microsatellite data to assess the levels of inbreeding of 33 individual gazelles in the captive population. Hypothetical pedigrees were generated to simulate various historical scenarios. Inbreeding coefficients were calculated from each pedigree scenario and correlated to the individual inbreeding metrics obtained from the microsatellite data.

To evaluate the link between infant mortality and inbreeding in this population, females with two inbred and two non-inbred offspring were compared using a paired t-test. Moderately inbred (0.125) and highly inbred (0.25) offspring were selected to represent inbred births, averaging an inbreeding coefficient of 0.202 . Non-inbred offspring were defined as any offspring with an inbreeding coefficient of 0. A paired test of females was used to control for environmental factors influencing offspring viability.

Institutions housing *Nanger dama ruficollis* were identified using ISIS and the Addra Studbook (2008). Samples were obtained from 5 AZA institutions for a total of 33 individuals. Stool samples were collected in sterile containers for transport and mailed to Trinity University. Samples were to be allowed to dry *in situ* before collection to preserve DNA integrity, but in some cases this was not possible due to risk of contamination by other individuals in the enclosure. In some cases fecal matter that had not been allowed to dry developed minor to substantial fungal growths. Fungus-free samples were used for DNA extraction when possible, and when unaffected samples were unavailable all visible fungus was removed before DNA extraction. All samples were stored at 4° C.

For one individual, DNA was extracted from a tissue sample from the Angelo State University museum collection stored in ethanol using a phenol-chloroform extraction (Sambrook *et al*. 1989). For all other gazelles, DNA was obtained from fecal samples of individuals housed in AZA institutions using the Qiagen Stool Kit (QIAGEN Inc.), with an ungulate pellet extraction protocol modification (Wehausen 2004). DNA was extracted from 1-4 pellets for each sample depending on amount of material available per pellet. As recommended by Wehausen (2004), only the outer mucosal layer of each pellet was used to avoid degradation by undigested plant material present in the interior of each pellet. All feces-extracted DNA was suspended in AE buffer and BSA which increases viability during PCR. When possible, DNA was extracted several times to provide multiple stocks in case of degradation or contamination.

The 8 microsatellite loci used in this study were also used in an investigation of inbreeding in three captive gazelle populations including the *Nanger dama mhorr* population at the Arid Zone Experimental Station (Ruiz-Lopez 2009). Originally these loci were isolated in cattle, sheep, and red deer, and are highly conserved across Cervidae and Bovidae (Slate 1998) and polymorphic in *Nanger dama mhorr* (Ruiz-Lopez 2009). The loci include BM1706 (Bishop 1994), INRA005 (Maudet 2004), CSSM41 (Moore 1994), HU1177 (Viaman 2000), IDVGA29 (Slate 2002), MAF35 (Swarbrick 1991), OARFCB193 (Buchanan 1993), and TGLA94 (Georges 1992). TGLA94 amplified peaks of size 50 and 64 in all samples, and was excluded from study.

Polymerase chain reactions were conducted in 10 µl reactions using 1 µl of suspended DNA of 10-100 mg/ µl in AE or TE, 1 µl of 1µM forward primer, 1 µl of reverse primer, 2 µl of water, and New England Bio Lab's 2x TAQ master mix. DNA

loci were amplified using a PCR protocol of 2 minutes at 95° C, followed by 30 seconds at 95° C, 30 seconds at 45° C, 1 minute at 72° C repeated 35 times and ending with a 10 minute extension at 72° C.

Products from PCR were separated by capillary electrophoresis at the DNA Core Facility at the University of Texas at Austin. Fragment-size analysis was performed on Applied BioSystems 3730 or 3130 DNA Analyzers using a 500-Rox Size Standard (Applied BioSystems). Peaks were viewed in Peak Scanner 1.0 (Applied BioSystems) and scored by hand.

A random selection of sampled loci (n=36) were resubmitted using PCR results from alternate DNA stock to provide a measure of scoring error. Eighty-six percent of alleles showed an exact match when resampled. Of the 5 resubmitted samples that did not display an exact match, 3 displayed alleles that were within 3 bp of the original fragment-size analysis. Microsatellite loci fell within 50 bp of the expected size range based on data from amplification in other species, with the exception of TGLA94 which was excluded from study. Polymorphism at each locus ranged from 6 to 16 alleles.

Pedigree data were taken from the Addra Studbook (2008). These data are the basis for the four pedigree scenarios tested:

1) The original studbook. The completion rate for information regarding the first few generations is poor, leading to uncertainty in the actual founder representation. All founders are assumed to be outbred and unrelated.
2) The hypothetical studbook. This takes the original studbook and augments it following Ballou's (1983; 1996) suggestion for filling uncertainties in studbooks with hypothetical ancestors descended from the same founders

instead of assuming the offspring of unknown origin to be a founder. Hypothetical offspring of founders were created using the overall distribution of founders mated randomly over a number of generations estimated by year of birth of the offspring with the unknown parent. The generated individual was then added as a hypothetical parent in place of "unknown" values. This hypothetical individual and all hypothetical ancestors are added to the studbook. The number of generations was estimated by the average generation time of roughly 5 years.

3) The original studbook with related founders. All founders were acquired over several months in the same location in Chad. Females of the same age group in the same herd are likely to be paternal half-siblings and females of different age groups possible mother-daughters. Founder age groups are not known from the pedigree data. In this scenario, hypothetical founders were created as parents to the female founders (studbook identification numbers 13, 14, 15, 17, and 19) so that all female founders have a relatedness of 0.125%.
4) The hypothetical studbook with related founders. The hypothetical founder parents of the female founders were added to the hypothetical studbook.

Relatedness and inbreeding in the population for each pedigree scenario were calculated using ENDOG 4.6 (Gutiérrez and Goyache 2005).

Following Ruiz-Lopez (2009), the correlations were calculated between Wright's inbreeding coefficient (F) generated from the four pedigree scenarios and three molecular measures of heterozygosity: Standardized Multilocus Individual Heterozygosity (sMLH)

(Coulson et al. 1998), Internal Relatedness (IR) (Coltman et al. 1999) and Homozygosity by Loci (HL) (Aparicio et al. 2006). Standardized Multilocus Individual Heterozygosity measures an individual's proportion of heterozygosity divided by the heterozygosity of the loci. Internal Relatedness is a measure of heterozygosity weighted most heavily for rare alleles in the population. Homozygosity by Loci is a similar measure of homozygosity which is weighted most heavily for common alleles in the population. All three measures were calculated using Excel macro IRmacroN4 developed by William Amos (http://www.zoo.cam.ac.uk/zoostaff/amos). Statistical significance was accepted at $P \leq 0.05$.

Results

There was a significant difference in infant mortality between inbred and non-inbred offspring of females in this population that bore at least two inbred and two non-inbred offspring (one-tailed $t = -1.92$; $df = 15$, $P = 0.037$; Figure 2). Infant mortality in this population rose from 0% in 1967-1969 (n=28) at the beginning of the breeding program to 39% in 1990 (n=90) (Figure 3). The trend after 1990 becomes much less clear but does not represent a continued increase over time.

Inbreeding coefficients generated for sampled individuals under the four pedigree scenarios ranged from 0.01 to 0.22(Table 1). The Pearson's correlations between individual F values and molecular metrics are summarized in Table 2. The scenario based on the original pedigree with unrelated founders was found to have the highest relationship with IR, sMLH, and HL, but the relationship was not statistically significant ($P \leq 10$; Table 2). The expected relationships between F and molecular metrics were observed, but not statistically significant (Figure 4).

Mean average relatedness by generation showed an increase through generation 6-7 (Figure 5), which is estimated as the point at which management by minimizing mean kinship was initiated based on average generation time (4.7 years). Based on pedigree calculations, mean kinship according to known relationships stabilized in this population.

Inbreeding by generation, as calculated from the original pedigree data in ENDOG (Gutiérrez and Goyache 2005), showed no significant increase through 1983. The original pedigree is believed to represent an underestimation of inbreeding in the

population. Each individual with two unknown parents (N=256) is treated as a founder in this scenario, causing a large overestimation of recorded founders in the population (N=8). In reality all these individuals with unknown parents are descendents of actual founders and are therefore not unrelated to all other individuals in the population. The distribution of genetic contributions of the founders was found to be uneven, ranging from 0.55% to 6.325% with 72.5% unknown (Figure 6).

Discussion

Because inbreeding can have profound impacts on genetic diversity in a small population, it is important to be able to recognize and avoid inbreeding. Indirect measures such as infant mortality can be an indicator of inbreeding. Genetic measures of inbreeding can be correlated to various pedigree scenarios to suggest the most accurate scenario. The best fit scenario can then be used to identify levels of inbreeding in individuals in the population and to plan for future management.

The difference in infant mortality between inbred and non-inbred offspring (Figure 1) supports prior observations of a correlation between inbreeding and juvenile mortality in ungulates (Ralls *et al.* 1979; Ralls *et al.* 1988). Infant mortality in this population rose significantly between 1967 and 1990. Considering the association between inbreeding and infant mortality in gazelles in general and this population in particular, it was expected that infant mortality over time would correlate to average inbreeding in this population as calculated by the original pedigree. However, much of the pedigree is unknown in the first few generations, leading to calculated inbreeding values by year that were not significantly different than 0 through 1983. The relationship between average relatedness by generation as determined from the original pedigree does, however, follow the same trend of increasing through generation 6 or 7 (1985-1995) and remaining roughly level thereafter (Figure 5).

The relationship between molecular metrics of inbreeding and the inbreeding coefficients generated from pedigree scenarios was not significant, possibly indicating that none of the pedigree scenarios adequately illustrate the history of this population.

The original pedigree is known to contain a number of incorrect assumptions; most important the inclusion of 256 individuals considered to be unrelated founders, but which in fact must be descended from the true founders in the population. Despite this known fault, this scenario returned the highest correlation of the four scenarios tested. It is possible that of the four scenarios, the original pedigree generated F values closest to the actual inbreeding levels of the population for sampled individuals due to the fact that there are more founders than those 8 which are recorded as such. Only 22 addra gazelles were ever known to be imported to the United States, making the number of possible founders in this population no greater than 22. Some of these individuals, however, existed outside the managed population, including 8 which resided at Catskill Game Park in New York. These individuals almost certainly reproduced and contributed to the current population, but with no records it is difficult to determine the exact contribution of these founders to the managed population (Table 4 and Figure 6). In 1975 two individuals, numbers 175 and 1579, were moved from Catskill to the managed population. There is no other record of animals moving from the managed population to Catskill or from Catskill to the managed population. Individual 1579 left no known descendents in the current population, but 175 is present in the ancestry of 49% of living individuals, suggesting that it could be acting as an effective founder (Table 5). Other individuals exist outside the managed population and have unknown origin, but 175 seems to be the most likely to have originated from the Catskill group of founders.

This population of *Nanger dama ruficollis* showed similar levels of molecular measures of inbreeding to those found in other captive gazelle populations (*Gazella cuvieri*, *Nanger dama mhorr,* and *Gazella dorcus neglecta*) of similar founder number

sizes (4, 11, and 20) and varying levels of inbreeding (average F = 0.18, 0.10, and 0.05) (Ruiz-Lopez *et al*. 2009). Molecular measures in Nanger dama ruficollis most resembled levels in Gazella dorcus neglecta with 20 founders and an average inbreeding coefficient of 0.05. Working with complete pedigrees, Ruiz-Lopez *et al*. were able to find a significant relationship between F and molecular metrics in *Gazella dorcus* and *Gazella cuvieri* ($P \leq 0.05$) and determine the relatedness of founders in these populations. The study found no significant relationship in *Nanger dama mhorr*, which the investigators postulated to be due to the much longer time this species has been maintained in captivity as well as having captive-born founders, noting the very low levels of heterozygosity in this population. This study found higher levels of heterozygosity in this *Nanger dama ruficollis* population than Ruiz-Lopez *et al.* found in *Nanger dama mhorr* which is consistent with the higher possible number of founders and fewer generations in captivity.

Future Management Recommendations

The remaining founders represented in the population have a large variation in representation, ranging from 16% to 95% (Table 5). Management efforts need to focus on retaining the representation of founder 15 and possible effective founder 175 in order to maintain the genetic variation present in each lineage. Future studies sampling a greater portion of the population may be able to determine the founder contribution in this population and benefit future management decisions.

The demographic management of this species has been successful in reaching the general management goals, including a population growth rate near 0 (-0.08), although still short of the desired population size of 200 (121) (Antelope TAG 2008). The sex ratio is sufficient (51 male:71 female) to roughly equalize family size. To maximize maintenance of genetic variation, generation time (4.7 years) should be lengthened as far as possible without compromising the desired growth rate. With female peak fecundity ranging from 2 to 9 years, delaying breeding to age 4 or 5 should not adversely affect the population growth rate and should delay inbreeding depression in the population.

Overall, the inbreeding in this population appears to be less problematic than it might seem from the pedigree alone. Infant mortality has held steady or decreased in recent years, possibly due to inbreeding avoidance or husbandry practices. It appears that more founders contributed to this population than were recorded in the pedigree. Unfortunately it is impossible to hypothesize about the contribution of these founders without any records. Because records of founder contribution are so unclear, the next step in genetic management of this population would be to sample all individuals in the population to find individuals of genetic importance. Combined with improved recordkeeping and general management recommendations to combat loss of genetic variation, this population should be able to retain enough variation to initiate future reintroduction events if they become necessary.

The results of this study illustrate how poor or incomplete record-keeping can be detrimental to a management program. Management programs are almost exclusively based on pedigrees alone, making any mistake or omission in the pedigree potentially harmful to the mission of the program by miscalculating inbreeding or genetic diversity

in the population. It is becoming more feasible to use genetic data to augment pedigree data in breeding programs and circumvent problems such as lost records, but most programs still rely on pedigree data only. For critically endangered and declining species kept in small populations in captivity with historically poor recordkeeping, using genetic methods to identify related individuals and genetically distinct individuals may provide a more accurate picture of the population and may prove to be a better basis for population planning.

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Tables

Table 1. Average inbreeding coefficients (F) and standard deviation for *Nanger dama ruficollis* for four pedigree scenarios.

	Original Pedigree	Hypothetical Pedigree	Original Pedigree	Hypothetical Pedigree
	Unrelated Founders	Unrelated Founders	Moderately Related Founders	Moderately Related Founders
Average F	0.057	0.072	0.059	0.078
St Dev	0.059	0.058	0.061	0.061

Table 2. Pearson correlations between inbreeding coefficient (F) generated from four pedigree scenarios and the 3 molecular measures of inbreeding in *Nanger dama ruficollis*. Molecular measures include Standardized Multi Locus Heterozygosity (sMLH), Internal Relatedness (IR), and Homozygosity by Loci (HL).

	Original Pedigree		Hypothetical Pedigree		Original Pedigree		Hypothetical Pedigree	
	Unrelated Founders		Unrelated Founders		Moderately Related Founders		Moderately Related Founders	
	r	P value	r	P value	r	P value	r	P value
sMLH	-0.30	<0.10	-0.14	n.s.	-0.25	n.s.	-0.09	n.s.
IR	0.29	<0.10	0.13	n.s.	0.25	n.s.	0.09	n.s.
HL	0.29	<0.10	0.14	n.s.	0.25	n.s.	0.10	n.s.

Table 3. Distribution of offspring of founders in the *Nanger dama ruficollis* captive population. Recorded offspring based on studbook data, female potential offspring based off of breeding seasons per lifespan in the managed population. Catskill individuals were not retained in the managed population and no data are available on lifespan or offspring. Data from the Addra Gazelle Studbook (2008).

Founder	Sex	Recorded Offspring	Potential Offspring	Population
1	M	24	24+	San Antonio
2	F	0	--	Catskill
3	F	0	--	Catskill
4	F	0	--	Catskill
5	M	15	15+	San Antonio
6	F	0	--	Catskill
7	F	0	--	Catskill
8	M	0	--	Catskill
9	F	0	--	Catskill
10	M	0	--	Catskill
11	?	0	0	San Antonio
12	?	0	0	San Antonio
13	F	2	3	San Antonio
14	F	10	11	San Antonio
15	F	1	8	San Antonio
16	M	3	3+	San Antonio
17	F	2	8	San Antonio
18	F	8	9	San Antonio
19	F	1	9	San Antonio
20	F	0	5	San Antonio
21	F	0	10	San Antonio
22	F	0	5	San Antonio

Table 4. Percentage of current captive *Nanger dama ruficollis* population descended from founders and selected individuals. Data from the Addra Gazelle Studbook (2008).

Founder	Know Descendents Surviving	% of Current Population
1	123	90.44
5	128	94.12
13	109	80.15
14	116	85.29
15	22	16.18
16	129	94.85
17	105	77.21
18	0	0.00
19	87	63.97
175 (Catskill)	67	49.26
1579 (Catskill)	0	0.00

Figures

Figure 1. Adult female San Antonio Zoo addra gazelle, photo by the author.

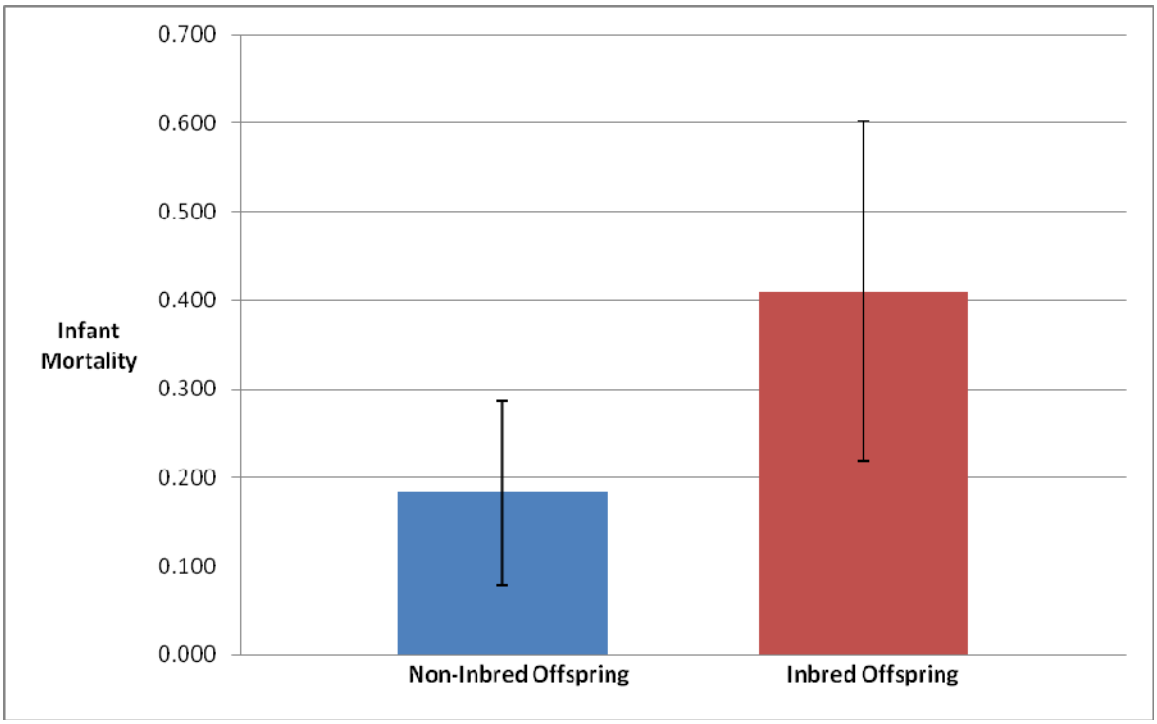


Figure 2. Mean infant mortality in inbred (n=40) and noninbred (n=90) offspring of *Nanger dama ruficollis* females with at least 2 inbred and 2 non-inbred offspring. Error bars represent ± 2 SE.

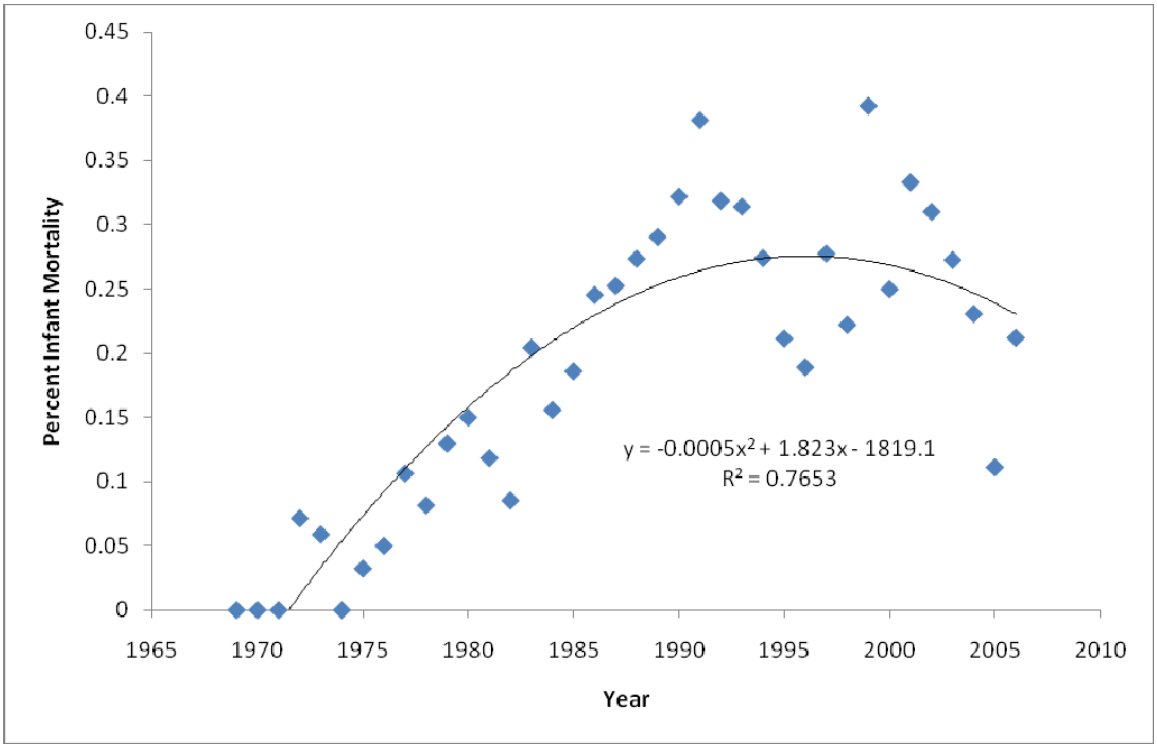
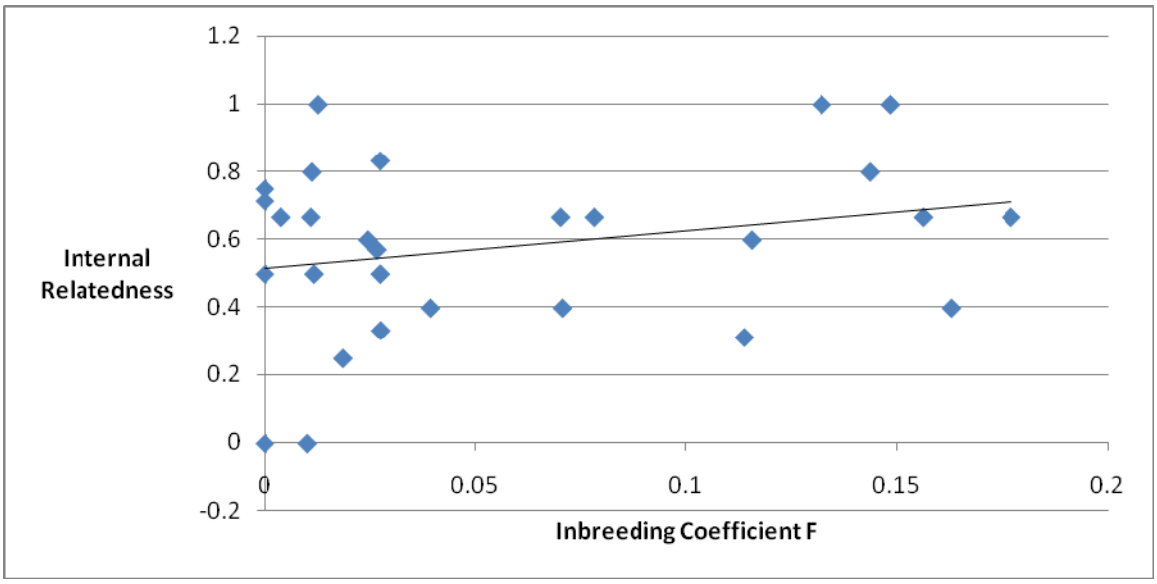
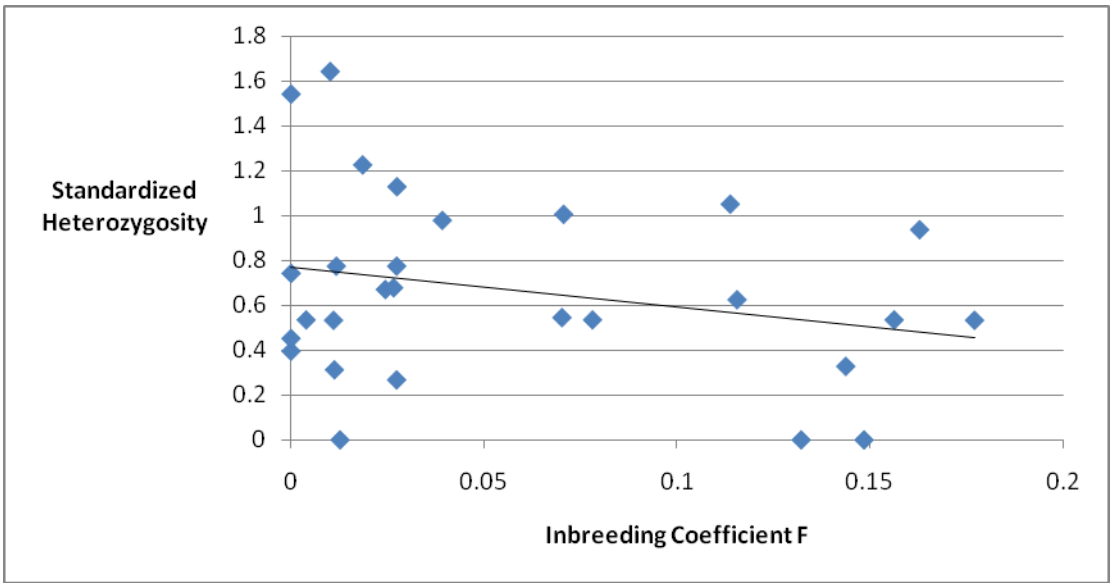


Figure 3. Infant mortality (death at age <30 days) by year of birth as a proportion of births in the AZA addra gazelle (*Nanger dama ruficollis*) population.

a)


Internal Relatedness
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
0.05
0.1
0.15
0.2
Inbreeding Coefficient F

b)


Standardized Heterozygosity
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.05
0.1
0.15
0.2
Inbreeding Coefficient F

c)

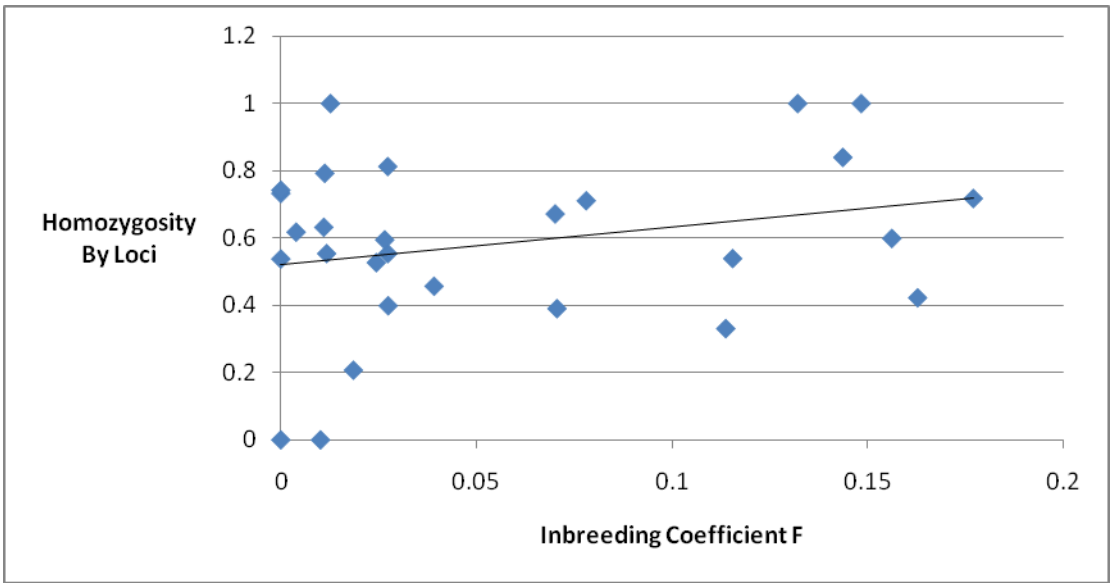


Figure 4. The relationship between individual Wright's inbreeding coefficient (F) and molecular metrics of inbreeding a) Internal Relatedness (IR), b) Standardized Multilocus Heterozygosity (sMLH), and c) Homozygosity by Loci (HL) for the scenario of original pedigree with unrelated founders in *Nanger dama ruficollis*.

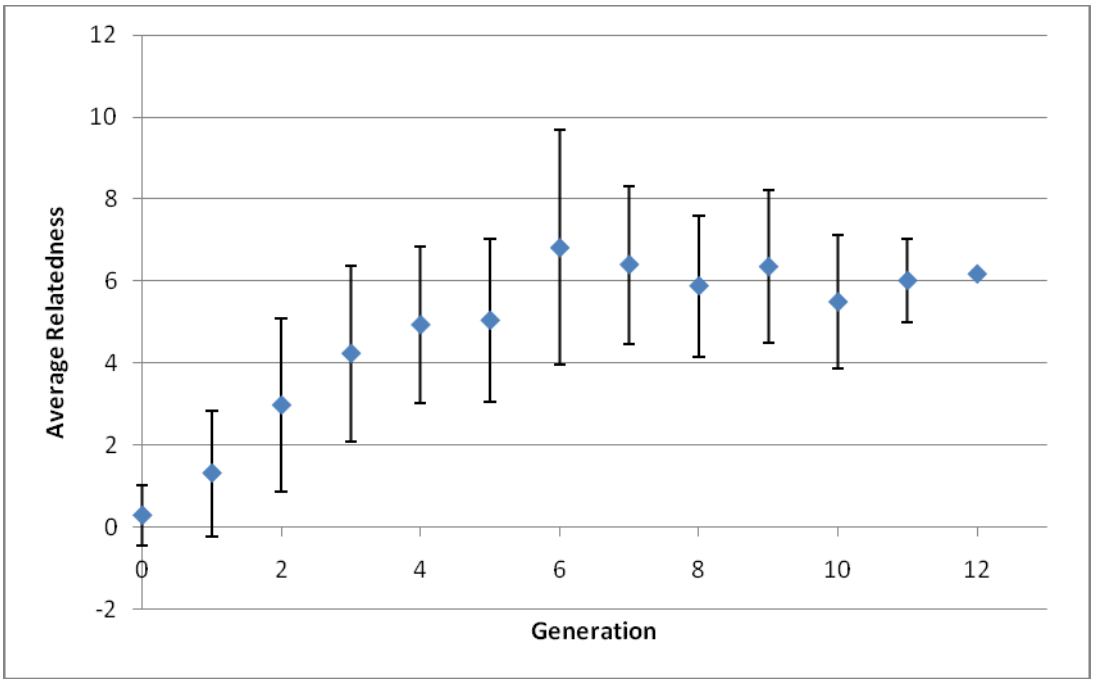


Figure 5. Mean Average Relatedness by maximum generation, calculated from pedigree data of captive AZA *Nanger dama ruficollis*. A "minimization of kinship" strategy was applied beginning at generation 6-7. Error bars represent one standard deviation.

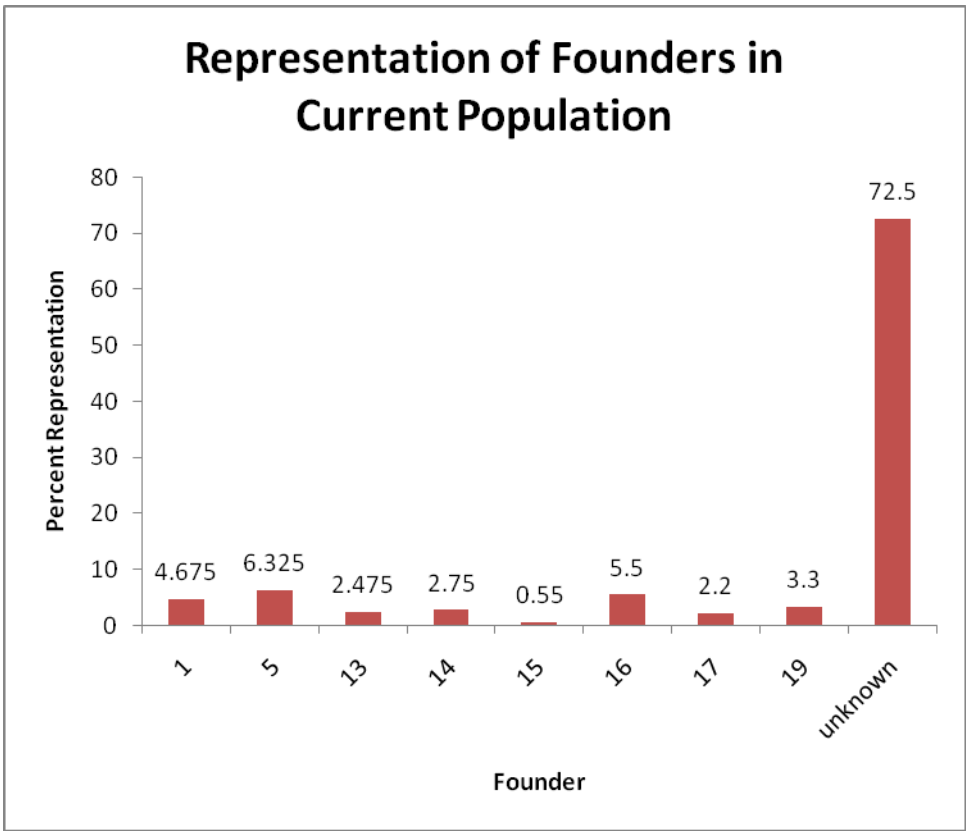


Figure 6. Representation of the 8 known founders in addra gazelles (*Nanger dama ruficollis*) contributing to the current population including 3 males (1, 5, 16) and 5 females (13, 14, 15, 17, 19). Only 25.5% of total founder contributions are known due to gaps in record keeping in the first few generations of breeding. Data from the Addra Gazelle Studbook (2008).