Is the endangered Grevy's zebra threatened by hybridization?

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Abstract

Hybridization between an abundant species and an endangered species is cause for concern. When such hybridization is observed, it is both urgent and necessary to assess the level of threat posed to the endangered species. We report the first evidence of natural hybridization between two equids: the endangered Grevy's zebra *Equus grevyi* and the abundant plains zebra *Equus burchelli*. Grevy's zebra now number < 3000 individuals globally, and occur only in northern Kenya and Ethiopia. In recent years, Grevy's zebra have become increasingly concentrated in the south of their range due to habitat loss in the north. Both species are sympatric in the Laikipia ecosystem of northern Kenya, where we have observed purportedly hybrid individuals. Using mitochondrial and Y chromosome DNA, we confirmed the hybrid status of the morphologically identified hybrids and demonstrate conclusively that all first-generation hybrids are the offspring of plains zebra females and Grevy's zebra males. Behaviorally, hybrids integrate themselves into plains zebra society, rather than adopting the social organization of Grevy's zebra. Two hybrids have successfully raised foals to over 3 months in age, including one which has reached adulthood, indicating the fertility of female hybrids and viability of their offspring. We hypothesize that hybridization occurs due to (1) skewed sex ratios, in favor of males, within Grevy's zebra and (2) the numerical dominance of plains zebra in the region where hybridization is occurring. Stakeholders have discussed hybridization as a potential threat to Grevy's zebra survival. We argue, however, based on behavioral observations, that hybridization is unlikely to dilute the Grevy's zebra gene pool in the short term. As a conservation concern, hybridization is secondary to more direct causes of Grevy's zebra declines.

Introduction

Hybridization and introgression between closely related species is a natural phenomenon that has been observed in all major plant and animal taxa (Dowling & Secor, 1997). While hybridization is recognized as an important evolutionary force sometimes leading to the formation of new species (Arnold & Hodges, 1995), increasing rates of hybridization in the last 20 years due to anthropogenically induced habitat decline and/or the introduction of exotic species has become a growing threat (Rhymer & Simberloff, 1996). In animals, hybridization may be more likely in poor habitats or small populations, where low mate availability and skewed sex ratios may lead to exclusion of some individuals from mating (Mace & Waller, 1998; Jansson, Thulin & Pehrson, 2007). Genetic extinction of a rare species may occur by genetic swamping from a more common species or by outbreeding depression leading to loss of locally adapted traits. Even in cases where hybrids are sterile, reproductive effort 'wasted' on hybrid reproduction can still threaten a population (Kanda, Leary & Allendorf, 2002). When hybridization of an endangered species is discovered, management intervention may therefore be useful to limit hybridization, particularly when the species with which it is hybridizing is abundant and when such hybridization appears to be a novel phenomenon (Lande, 1998; Wolf, Takebayashi & Rieseberg, 2001).

Among mammals, hybridization between species of conservation concern and related, more abundant species has frequently been observed (Allendorf *et al*., 2001). Hybridization between parapatric populations of closely related

species is fairly common and often generates narrow hybrid zones that can be relatively stable through time, but hybridization between ecologically distinct species with overlapping ranges is much less frequent (Detwiler, Burrell & Jolly, 2005). In the genus *Equus*, hybridization has been well documented in captivity, with some hybrids bred for commercial use. Hybridization has also occurred where equid species have been introduced outside their natural ranges or where feral equids have interbred with wild equids (Brown & Jenkins, 1987). However, we are unaware of any past reports of hybridization occurring between two historically sympatric equids in the wild.

Here we report the first evidence of natural hybridization between two equids, the plains zebra *Equus burchelli* and Grevy's zebra *Equus grevyi*. Since 2004, we have observed individuals that exhibit physical features intermediate between the two zebra species, raising suspicions that hybridization is occurring The plains zebra is among the most widespread and abundant wild ungulates of east and southern African savannahs. The Grevy's zebra, confined to northern Kenya and Ethiopia is endangered (IUCN, 2008), with the global population numbering <3000 individuals following a decline from 15 000 in 1977 (Williams, 2002; KWS, 2008). Hunting, for meat and trade of skins, may have been a chief cause of earlier declines for Grevy's zebra. Recently, Grevy's zebra are believed to be most strongly limited by intensification of human land use for livestock production. However, local stakeholders including private land managers and the Kenya Wildlife Service (KWS) have become increasingly concerned that this apparent hybridization may threaten the remaining Grevy's zebra (KWS, 2008). To date there has been no genetic or behavioral evidence on which to evaluate these concerns. Here we describe the genetics, morphology, behavior and demography of these putative hybrids and assess whether they pose an additional threat to the endangered Grevy's zebra.

Comparison of plains zebra and Grevy's zebra

Plains and Grevy's zebra have distinct chromosomal structure, morphology, ecology and social systems. Genetically, the Grevy's zebra has 46 chromosomes; while plains zebra has 44 (Ryder, Epel & Benirschke, 1978). There is a close chromosomal correspondence between the two species, with karyotypic differences in only eight chromosomes (Musilova *et al.*, 2007). In captivity, viable male and female hybrid offspring produced from a plains zebra female and Grevy's zebra male had 45 chromosomes (Benirschke, 1977).

Morphologically, Grevy's zebra are larger than plains zebra. The more arid-adapted Grevy's zebra weighs *c*. 400 kg, compared with 240 kg for plains zebra (Kingdon, 1997; Rubenstein, 2001). The Grevy's zebra skull is larger and of greater width, than the plains zebra, with a marked backward extension of the lambdoidal crest. Grevy's zebra has a pattern of thin black and white stripes, a prominent dark dorsal stripe, and a white belly. Plains zebra has broader stripes that extend to the ventral line. The mane of the Grevy's zebra tends to be longer and the hooves broader and its ears are large and rounded, compared with the smaller, more pointed ears of plains zebra.

The two species differ in social organization. The core social group in plains zebra is the harem, comprising a stallion, several females and dependent offspring (Klingel, 1969; Rubenstein, 1986). Multiple harems coalesce into unstable herds, which also contain bachelor males (Rubenstein & Hack, 2004). Male Grevy's zebra, by contrast, seek to control access to unstable groups of females by controlling areas with key resources, primarily waterholes and forage patches. There is debate about whether these social differences are flexible responses to distinct ecological conditions (Rubenstein, 1986) or evolutionarily constrained characteristics, sometimes poorly suited to current conditions (Linklater, 2000)

Both species are grazers that inhabit grasslands and savanna woodlands. Plains zebra favor cooler and wetter localities than do Grevy's zebra (Bauer, McMorrow & Yalden, 1994). Historically, the range of Grevy's zebra range extended into present-day Ethiopia, Somalia, Eritrea and Djibouti (Williams, 2002). The plains zebra range extends from Kenya to South Africa. Northern Kenya and southern Ethiopia are historic zones of overlap for the two species (Hack *et al.*, 2002). Today, both species are also found just north of the Equator in the Laikipia ecosystem, which is one of the last strongholds for Grevy's zebra following a southward expansion of their range in the last 30 years. Today, Laikipia is an area of critical importance for Grevy's zebra conservation (KWS, 2008). In the current overlap zone, plains zebra and Grevy's zebra have been observed associating in mixed herds (Keast, 1965). However, the only evidence of natural hybridization between the two species is from a Kenyan taxidermist who claimed to have the skin of a cross between plains zebra and Grevy's zebra (Keast, 1965) and from recent, unconfirmed reports of apparent solitary hybrids in one other area in Laikipia and around Tsavo National Park in southern Kenya, where Grevy's zebra were introduced outside their historic range.

Methods

Field site and populations

Ol Pejeta Conservancy lies on the Equator (0°00′N, 36°56′E) in Laikipia district, Kenya, at the south-western limit of the range of Grevy's zebra (Fig. 1). The vegetation is a mosaic of savanna grassland, *Acacia* woodland, *Euclea* scrub woodland and riverine woodland (Tatman, Stevens-Wood & Smith, 2000). The area of the conservancy open to wildlife is 305 km². The area supports *c*. 4000 plains zebra while the Grevy's zebra number *c*. 15.

Field methods

Since 2003, we have studied plains zebra and Grevy's zebra behavior and demography on Ol Pejeta. We periodically drive set survey routes, searching for herds. The interval

Figure 1 Geographic range map of plains zebra *Equus burchelli* (shaded gray) and Grevy's zebra *Equus grevyi* (shaded black) in Kenya, with the location of Ol Pejeta Conservancy (white circle) shown (range maps adapted from Hack *et al.*, 2002; IUCN, 2008).

between successive sampling occasions varies from 1 day to 1 month. For each herd sighted, we attempt to identify all individuals present. We identify individual zebra based on unique stripe patterns and a photographic database. We assign foal–mother pairs based on nursing and determine plains zebra harem structure based on social interactions. We first observed apparent hybrids in 2004.

Genetic data collection and analyses

We collected 28 fecal samples representing 25 different individuals immediately after they were observed defecating. Sex, age class and likely familial relationships were recorded at the time of sampling (Table 1). Of the 25 individuals sampled, 10 (four males and six females) were identified in the field as being hybrids, five (two males and three females) as plains zebra and 10 (six males and four females) as Grevy's zebra. To maximize the probability of collecting epithelial cells, 1–2 g of fecal material was removed from the surface of individual dung balls and placed in a 5 mL of RNAlater (Ambion, Huntington, UK). Samples with RNA-later were stored in a cooler in the field, and were moved to 4 °C within 24 h. DNA extraction was performed within 2–5 days of sample collection.

Whole-DNA extraction was performed using a QIAamp DNA Stool Mini Kit (Qiagen, Crawley, UK). Briefly, RNAlater samples were centrifuged for 15 min at 3000 *g* and the supernatant discarded. The pellet was resuspended in 1.6 mL of buffer ASL, then vortexed and incubated for

5 min at room temperature. All intermediate steps followed the manufacturer's instructions. In the final elution step, an extended incubation time of 20 min was used, and samples were eluted into 200 μL of buffer EB.

To determine maternity and paternity, we amplified short fragments of both mitochondrial and Y chromosome DNA. Fragments were chosen so as to facilitate discrimination between the two zebra species. Primers were designed from previously published sequences available on GenBank. A short (350 bp) fragment of the mitochondrial control region was amplified using the primers Eq-CR-1F (5′-CCTCATG TACTATGTCAGTA-3′) or Eq-CR-107F (5′-GCCCCAT GAATAATAAGCATGTAC-3′) and Eq-Cr-534R (5′-CCT GAAGAAAGAACCAGATGCC-3′). A 390 bp fragment of the Y chromosome that spanned four differentiating single nucleotide polymorphisms (SNPs) was amplified using primers Y1F (5′-GAATGCAGCAGTGTAGACTT CTG-3′) and Y390R (5′-GCAGGCCTTGACACTGCTTT GGAC-3′). PCR amplifications were performed in 25 μL total volume using 1.25 U *Taq* DNA polymerase (ABgene, Epsom, UK), 10 × buffer (Invitrogen, Renfrew, UK), BSA (0.2% w/v, Sigma, Dorset, UK), $MgSO_4$ (2 mM), dNTPs (all 25 mM), extract (1 μL) and forward and reverse primers (1 μM each). A 2-min activation step at 94 °C was followed by 45 cycles at 94 °C for 45 s, 55 °C (mitochondrial) and 64 °C (Y chromosome) for 45 s and 72 °C for 1 min 30 s. PCR products were purified using the Qiaquick system (Qiagen Ltd), then directly sequenced using ABI BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Warrington, UK) and imaged on ABI Prism capillary DNA 377 automated sequencers (Applied Biosystems), all according to the manufacturers' instructions. Both forward and reverse strands were sequenced, and fragments were assembled using Geneious v4.5 (Drummond *et al.*, 2006). One hybrid male was sampled twice and one Grevy's female was sampled three times; in both of these cases the results of the replicate genetic analysis (extraction, amplification and sequencing) matched exactly.

To investigate the relationships among the recovered mitochondrial haplotypes, a maximum likelihood (ML) phylogenetic tree was constructed using PAUP v4b10 (Swofford, 2003) using the HKY + G model of nucleotide substitution. A starting tree was generated using neighbor joining (NJ), from which values for the model parameters were estimated. A heuristic search was then performed with TBR branch swapping, followed by re-estimation of model parameters and an additional round of branch swapping. Statistical support for the ML topology was evaluated using 1000 NJ bootstrap replicates with re-sampling, with model parameters fixed to values estimated from the ML tree.

Results

Hybrid morphology

Hybrids demonstrate a mixture of phenotypic traits that reflect their parentage. Quantitative size differences are not available as we used non-invasive techniques. However,

Table 1 The age and sex and field description of each individual for which a maternal genetic haplotype was obtained. All hybrid and Grevy's males had Grevy's Y chromosome haplotypes, and both plains males had plains haplotypes, indicating that all of the hybrid zebra had Grevy's paternal lineages. The mitochondrial DNA sequences were variable within plains zebra, so that there were not two unique haplotypes that could be used to discriminate the maternal lineages. The specimen number is followed by the individual identity number if the individual was referred to in the text.

Specimen number (individual identity)	Morphological field description	Age	Sex	Maternal haplotype	Paternal haplotype	Field relationship
OP0606	Hybrid	F	Fe	Plains		
OP0628 (H04005)	Hybrid	A	Fe	Plains		
OP0629 (H04003)	Hybrid	TYR	Fe	Plains		F2 hybrid
OP0608 (H04002)	Hybrid	A	Fe	Plains		Mother of OP0629
OP0618 (H04007)	Hybrid	A	M	Plains	Grevy's	
OP0622	Hybrid	YR	Fe	Plains		
OP0624	Hybrid	TYR	M	Plains	Grevy's	
OP0626	Hybrid	A	Fe	Plains		
OP0602	Hybrid	A	M	Plains	Grevy's	
OP0603	Hybrid	A	M	Plains	Grevy's	
OP0623	Plains	A	Fe	Plains		Mother of OP0622
OP0625	Plains	A	M	Plains	Plains	
OP0627	Plains	A	Fe	Plains		Mother of OP0626
OP0604	Plains	A	Fe	Plains		
OP0605	Plains	A	M	Plains	Plains	
OP0615	Grevy's	A	Fe	Grevy's		
OP0616	Grevy's	A	M	Grevy's	Grevy's	
OP0617	Grevy's	F	M	Grevy's	Grevy's	
OP0630	Grevy's	A	Fe	Grevy's		
OP0631	Grevy's	A	M	Grevy's	Grevy's	
OP0619	Grevy's	A	M	Grevy's	Grevy's	
OP0607	Grevy's	A	M	Grevy's	Grevy's	
OP0621	Grevy's	A	M	Grevy's	Grevy's	
OP0611	Grevy's	A	Fe	Plains		
OP0612	Grevy's	F	Fe	Plains		

A, adult; TYR, 2-year old; YR, yearling; F, foal; Fe, female; M, male.

Figure 2 Plains zebra *Equus burchelli*, a hybrid and a Grevy's zebra *Equus grevyi* in a common herd, on one of the few occasions we have observed all three in the same herd. The hybrid (at the forefront of the picture) is about the same size as the Grevy's zebra in the middle. The plains zebra walking just behind the other two are considerably smaller.
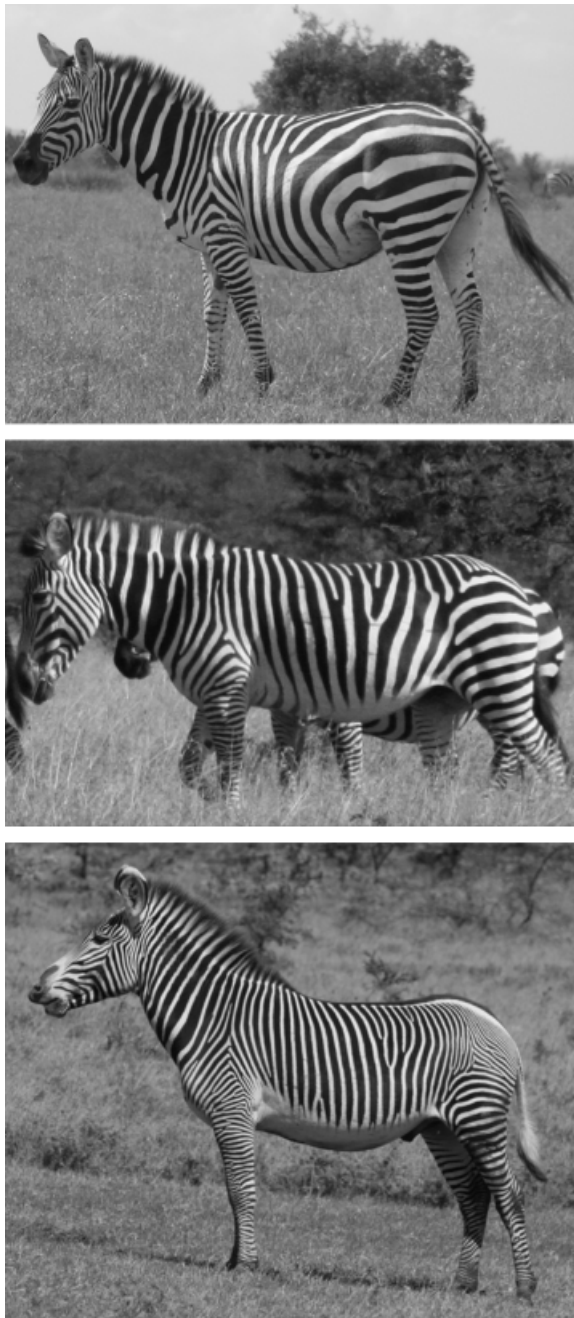
based on visual observations, hybrids are closer in size to Grevy's zebra than plains zebra (Fig. 2). Hybrid yearlings attempted to suckle even when they were larger than their plains zebra mothers. The stripes more closely resemble those of Grevy's zebra, but are broader and they do not touch under the belly (Fig. 3). Although most hybrids have large round ears, typical of Grevy's zebra, the second generation individuals had smaller, pointed ears more resembling plains zebra. This individual also had stripes that extend under the belly (Fig. 4).

Genetics: hybrid parentage

Y chromosome amplification was successful for all 12 males. Grevy's and plains zebra paternal lineages differ at four nucleotide positions (SNPs) within the 290 bp fragments. Based on these four SNPS, we were able to determine the paternal lineage for each male (Table 1).

The relationships between the 25 mtDNA sequences generated here (GenBank Accession Numbers GQ176410–GQ176432) and four previously published mtDNA sequences show that the Grevy's and plains zebra mitochondria fall into two distinct, well-supported clades (Fig. 5). All hybrid zebra have plains zebra mtDNA haplotypes, confirming their maternal lineages. Eight of the 10 individuals identified in the field as Grevy's zebra clustered with the Grevy's mtDNA haplotypes. Two females identified in the field as Grevy's zebra, however, had plains zebra mtDNA

Figure 4 Second-generation hybrid female foal, with her hybrid mother. These two individuals were initially observed in a plains zebra *Equus burchelli* harem with a plains zebra stallion male.
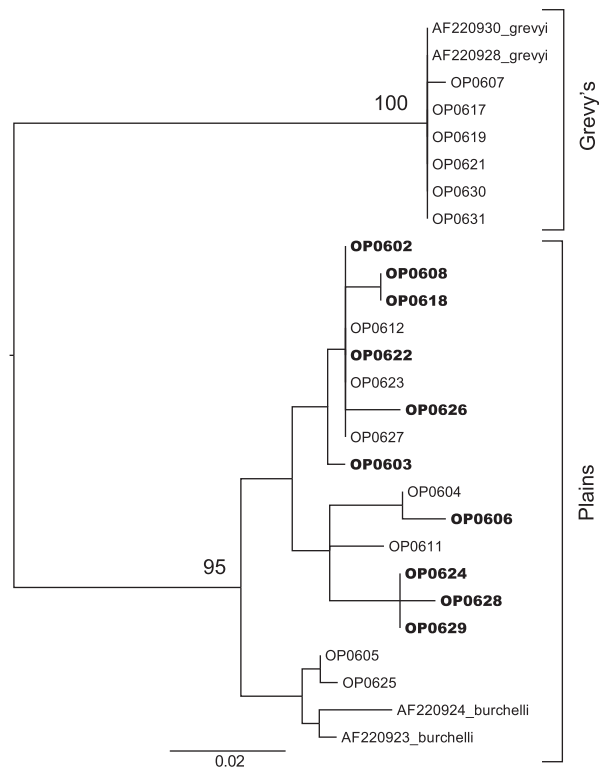

Figure 3 Comparative pictures of the side view of a plains zebra *Equus burchelli* (top), a hybrid (center) and a Grevy's zebra *Equus grevyi* (bottom). Hybrid stripes are less numerous than the Grevy's zebra but do not extend under the belly like those of a plains zebra. The stripes on the rear of the rump have less branching than those of Grevy's zebra, more closely resembling a plains zebra. However, there is a griddleiron pattern on the side of the rump, as is found on Grevy's zebra but not plains zebra. Most hybrids have large round ears, typical of Grevy's zebra.

Figure 5 Maximum likelihood (ML) phylogenetic tree showing the genetic relationships between mitochondrial lineages isolated from plains, *Equus burchelli*, Grevy's *Equus grevyi* and hybrid zebra, with ML bootstrap support values given at the nodes. Grevy's and plains zebra lineages fall into two distinct clusters. All hybrid zebra (bold) have plains zebra mitochondrial lineages.

haplotypes: repeating the mitochondrial amplification using different sets of primers confirmed this finding. Both of these females were sampled from the same herd, which contained one adult female, four males and one female foal. The only male from this group for which it was possible to obtain a sample had a Grevy's zebra mtDNA haplotype.

Molecular analyses confirmed most of our field observations. As of July 2007, 22 hybrids have been individually

identified using their stripe patterns. At that time, their population consisted of nine adult males, five adult females, three juvenile males, one juvenile female and two unsexed infants. Two foals seen in 2005 and 2006 have not been observed since, and are presumed dead. Of the seven hybrids first seen as foals, all had plains zebra mothers, except for two who had hybrid mothers. We have not observed matings between the two species but on three occasions we have seen one of two male Grevy's zebra chasing female plains zebra. On one occasion the female was driven 1 km from the rest of her harem, and isolated for about 20 min. Males of both species typically chase females before mating (Sundaresan, Fischhoff & Rubenstein, 2007). These three sightings by one observer suggest that Grevy's zebra are cuckolding plains zebra stallions by separating plains zebra females from their harem for short periods of time. Of the seven hybrid foals, four were observed as foals in two different plains zebra harems. Both these harems are held by plains zebra stallions. Each harem contains two hybrid foals of different ages (3 months and 6 months in one harem, 6 months and 2 years in the second harem) indicating that a Grevy's zebra male copulated with females in each harem on at least two occasions.

Hybrid reproductive ability

One hybrid female successfully reared a female foal to maturity (Fig. 4). This female is now an adult female in a harem with a plains zebra stallion. A second female hybrid was observed with a suckling foal that closely resembles a plains zebra foal, suggesting the father was a plains zebra. We do not know whether hybrid males are capable of successful reproduction. Observations of hybrid males with mating opportunities, but no apparent foals, suggest they may be infertile.

Social behavior

Hybrids were almost always observed together with plains zebra. In our 220 observations of herds containing hybrids, they were with plains zebra in 118 cases (99.1%): in three of these observations Grevy's zebra were also present. In the remaining two sightings, the hybrids were solitary individuals.

Beyond merely associating with plains zebra, hybrids appear well integrated within plains zebra society, either as harem members or as bachelor males. We infer harem membership based on characteristic social interactions and physical proximity. All female hybrids are part of harems with plains zebra stallions. For example, since 2004 female hybrid H04002 and her foal, H04003, along with three other plains zebra females and their offspring formed a harem held by a plains zebra stallion, OP07483. By May 2007, H04002 was still in the same harem, but 3-year-old H04003 had joined a different harem. At this age, plains zebra females typically leave their natal harem and are fought for by males who are either bachelors or established stallions (Klingel, 1969). Hybrid female H04005 is with plains

zebra stallion OP04064 and three additional plains zebra females.

Three adult hybrid males defend harems of plains zebra females, although we have yet to observe females in these harems give birth. Hybrid male, H04007, was an integral member of a plains zebra bachelor group. Twice, this individual has been observed alone, as is occasionally true of bachelors in both plains zebra and Grevy's zebra. However, in all other sightings he was observed in a group with other bachelors. On one occasion in 2006, H04007, fought for harem ownership with a plains zebra stallion for 2.5 h, both individuals incurring visible wounds. The hybrid took over the harem and exhibited flehmen behavior over urine markings of plains zebra females but was not seen with the harem two days later. We often observe such brief takeovers when a plains zebra bachelor first takes control of females. As of June 2007, this individual was defending a harem of two plains zebra females.

Discussion

Our molecular analyses confirmed that individuals with intermediate phenotypes are, indeed, the results of crosses between plains zebra females and Grevy's zebra males. This evidence of *in situ* hybridization between plains and Grevy's zebra is unprecedented for zebra. Yet because these species are evolutionarily closely related, such interspecific matings can occur. These zebra species diverged <1 million years ago (Yang *et al.*, 2003; Carbone *et al.*, 2006) and only differ in chromosome number by one – Grevy's zebra have 23 haploid chromosomes and plains zebra possess 22 haploid chromosomes. [Correction added after online publication <13th October 2009>: 'Grevy's zebra have 23 pairs and plains zebra have 22.5.' corrected to 'Grevy's zebra have 23 haploid chromosomes and plains zebra possess 22 haploid chromosomes.'] Moreover, based on chromosomal banding comparisons, the genetic material appears to be conserved across the two species, but rearranged by a series of centric fissions and fusions (Musilova *et al.*, 2007). Because translocated segments of different chromosomes can occasionally pair up successfully even when more than one centromere is involved, viable hybrid offspring can be produced as is the case from matings between domestic and Prezewalskii's horses whose karyotypes also differ by only one chromosome (Ahrens & Stranzinger, 2005). Two Grevy's–plains zebra hybrid females have given birth to foals, one of which has grown to be an adult. The occurrence of a female hybrid with a foal that closely resembles a plains zebra foal indicates that it may not be possible to identify all second generation hybrids based on phenotype alone.

The anomalous result of plains zebra mtDNA haplotypes in two Grevy's zebra females probably reflects an error made collecting the sample in the field. It was not a result of a laboratory error, as the mitochondrial amplification was repeated a number of times. These individuals were in the only group that was sampled in relatively thick and grassy *Acacia drapanalobium* woodland, and a large plains zebra herd was nearby. It is likely that a fresh plains zebra

fecal sample was wrongly attributed to a Grevy's zebra individual. An alternative explanation could be that at some time in the past, environmental and population conditions favored the introgression of plains zebra genes into the Grevy's zebra gene pool. Further genetic sampling may allow us to test for this possibility. For now, we believe the most parsimonious explanation is that we mistook the species source of the dung samples.

Hybridization is a conservation threat for some species, such as red wolf *Canis rufus* hybridizing with coyote *Canis latrans* (Adams, Kelly & Waits, 2003) or bison *Bison bison* with domestic cattle (*Bos* spp.) (Freese *et al.*, 2007). If one species is abundant and the other is rare, introgression may leave an ever-smaller fraction of 'pure' individuals in the rare species. For example, the gray duck *Anas superciliosus* of New Zealand has hybridized with (alien) mallards *Anas platyrhynchos* to such an extent that it almost certainly no longer exists in pure form (Rhymer & Simberloff, 1996). In the case of the Laikipia zebra, hybridization does not appear to be a threat to Grevy's zebra. Hybrids seemingly integrate into and seek mating opportunities within plains zebra society, rather than adopting the social organization of Grevy's zebra: this suggests that backcrossing is unlikely to occur. Four hybrid males have mating opportunities as defenders of harems, although there is no evidence to date that they have bred. This effectively unidirectional gene flow means that hybridization poses no immediate threat to Grevy's zebra.

We hypothesize that the hybridization is a consequence of limited intraspecific mating opportunities for Grevy's zebra males living sympatrically with a high density of plains zebra. The Ol Pejeta Grevy's zebra population is at the southern end of the species' historical range and consists of four adult females and six sexually mature males. The four females are consistently either pregnant or lactating. However, the sex ratio is strongly male-biased relative to other Grevy's zebra populations. For example, on nearby Mpala Ranch, there are six Grevy's zebra adult females for every territorial adult male. The combination of limited mating opportunities and a large plains zebra population may prompt Grevy's zebra males to seek out plains zebra females. Their larger body size may allow them to briefly fend off plains zebra stallions and thereby gain access to females. If demographic and density conditions promoting hybridization develop in other areas, due to decreases in numbers of Grevy's zebra, hybridization will likely become more widespread. For now, this hybridization is more a symptom than a cause of rarity and endangerment. The chief threats to the species are habitat degradation, competition with people and livestock for water and grass, and poaching (Sundaresan *et al.*, 2008; Low *et al.*, 2009). In the immediate future, ensuring the survival of Grevy's zebra depends on addressing these more direct pressures that could drive the species to extinction long before hybridization would threaten the genetic integrity of Grevy's zebra. Elimination of hybrids, as proposed by some stakeholders, may be an unnecessary waste of conservation resources. Removal or sterilization of hybrids can be problematic

either when hybrids are not morphologically distinct (Lancaster, Goldsworthy & Sunnucks, 2007) or when hybridization has occurred naturally and thus is likely to continue (Hedrick & Fredrickson, 2008).

Our discovery of hybridization between zebra species emphasizes the importance of long-term monitoring for determining changing threats to species of conservation concern. Eliminating a potential threat as needing immediate management action can allow limited resources to be allocated to more direct conservation threats. Additionally, understanding the factors that lead to hybridization will allow us to minimize the likelihood of it happening in other populations, especially when translocations are conducted (KWS, 2008). For example, we may be promoting conditions for hybridization if a small number of Grevy's zebra were to be translocated to a location where plains zebra are abundant. To reduce this risk, we may bias the sex ratio of the translocated population toward females. Our study supports the growing literature emphasizing the importance of combining non-invasive molecular genetic analyses with behavioral studies to accurately detect the occurrence of hybrids in animals (Allendorf *et al.*, 2001). By determining the apparent direction of gene flow within the two species we have been able to assess the level of threat these hybrids pose to an endangered species. In this case, the occurrence of morphologically indistinct second generation hybrids necessitates further investigation of the incidence of both maternal and paternal Grevy's zebra haplotypes across plains zebra populations in overlap zones to see whether hybridization has occurred elsewhere. This may provide additional information about the conditions that lead to hybridization. We will continue to monitor the hybrid population on Ol Pejeta Conservancy, in particular the second generation hybrids, to assess the fitness of these individuals and therefore the potential threat they pose to Grevy's zebra.

Acknowledgments

References

Adams, J.R., Kelly, B.T. & Waits, L.P. (2003). Using faecal DNA sampling and GIS to monitor hybridization between red wolves (*Canis rufus*) and coyotes (*Canis latrans*). *Mol. Ecol.* **12**, 2175–2186.

Ahrens, E. & Stranzinger, G. (2005). Comparative chromosomal studies of *E. caballus* (ECA) and *E. przewalskii* (EPR) in a female F1 hybrid. *J. Anim. Breed. Genet.* **122**, 97–102.

Allendorf, F.W., Leary, R.F., Spruell, P. & Wenburg, J.K. (2001). The problems with hybrids: setting conservation guidelines. *Trends Ecol. Evol.* **16**, 613–622.

Arnold, M.L. & Hodges, S.A. (1995). Are natural hybrids fit or unfit relative to their parents? *Trends Ecol. Evol.* **10**, 67–71.

Bauer, I.E., McMorrow, J. & Yalden, D.W. (1994). The historic ranges of 3 equid species in northeast Africa – A quantitative comparison of environmental tolerances. *J. Biogeogr.* **21**, 169–182.

Benirschke, K. (1977). An unusual zebra hybrid: the zeebraa. *Zoonooz* **50**, 15.

Brown, C.J. & Jenkins, A.R. (1987). Hybridization between a Hartmann's mountain zebra and a donkey. *Madoqua* **15**, 193–194.

Carbone, L., Nergadze, S.G., Magnani, E., Misceo, D., Cardone, M.F., Roberto, R., Bertoni, L., Attolini, C., Prias, M.F., de Jong, P., Raudsepp, T., Chowdhary, B.P., Guerin, G., Archidiacono, N., Rocchi, M. & Giulotto, .E. (2006). Evolutionary movement of centromeres in horse, donkey and zebra. *Genomics* **87**, 777–782.

Detwiler, K.M., Burrell, A.S. & Jolly, C.J. (2005). Conservation implications of hybridization in African cercopithecine monkeys. *Int. J. Primatol.* **26**, 661–684.

Dowling, T.E. & Secor, C.L. (1997). The role of hybridization and introgression in the diversification of animals. *Annu. Rev. Ecol. Syst.* **28**, 593–619.

Drummond, A.J., Kearse, M., Heled, J., Moir, R., Thierer, T. & Ashton, B. (2006). Geneious v4.5. Available at http://www.geneious.com/ (accessed 26 October, 2006).

Freese, C.H., Aune, K.E., Boyd, D.P., Derr, J.N., Forrest, S.C., Gates, C.C., Goyan, P.J.P., Grassel, S.M., Halbert, N.D., Kunkel, K. & Redford, K.H. (2007). Second chance for the plains bison. *Biol. Conserv.* **136**, 175–184.

Hack, M.A., East, R., Rubenstein, D.I. & Moehlman, P.D. (2002). Status and action plan for the plains zebra (*Equus burchellii*). In *Equids: zebras, asses and horses: status survey and conservation action plan*: 43–60. Moehlman, P.D. (Ed.). Gland, Switzerland: IUCN/SSC Equid Specialist Group.

Hedrick, P.W. & Fredrickson, R.J. (2008). Captive breeding and the reintroduction of Mexican and red wolves. *Mol. Ecol.* **17**, 344–350.

IUCN. (2008). IUCN red list of threatened species. Available at http://www.iucnredlist.org/ (accessed 29 February, 2009).

Jansson, G., Thulin, C.G. & Pehrson, A. (2007). Factors related to the occurrence of hybrids between brown hares *Lepus europaeus* and mountain hares *L-timidus* in Sweden. *Ecography* **30**, 709–715.

Kanda, N., Leary, R.F. & Allendorf, F.W. (2002). Evidence of introgressive hybridization between bull trout and brook trout. *Trans. Am. Fish. Soc.* **131**, 772–782.

Keast, A.C. (1965). Interrelationships of two zebra species in an overlap zone. *J. Mamm.* **46**, 53–66.

Kenya Wildlife Service (KWS). (2008). *Conservation and management strategy for Grevy's zebra (Equus grevyi) in Kenya 2007-2011*. Kenya: Kenya Wildlife Service.

Kingdon, J. (1997). *The Kingdon guide to African mammals*. London: Academic Press.

Klingel, H. (1969). The social organisation and population ecology of the Plains Zebra (*Equus quagga*). *Zool. Afr.* **4**, 249–263.

Lancaster, M.L., Goldsworthy, S.D. & Sunnucks, P. (2007). Multiple mating strategies explain unexpected genetic mixing of New Zealand fur seals with two congenerics in a recently recolonized population. *Mol. Ecol.* **16**, 5267–5276.

Lande, R. (1998). Anthropogenic, ecological and genetic factors in extinction and conservation. *Res. Popul. Ecol.* **40**, 259–269.

Linklater, W.L. (2000). Adaptive explanation in socio-ecology: lessons from the Equidae. *Biol. Rev. (Camb.)* **75**, 1–20.

Low, B., Sundaresan, S.R., Fischhoff, I.R. & Rubenstein, D.I. (2009). Partnering with local communities to identify conservation priorities for endangered Grevy's zebra. *Biol. Conserv.* **142**, 1548–1555.

Mace, R.D. & Waller, J.S. (1998). Demography and population trend of grizzly bears in the Swan Mountains, Montana. *Conserv. Biol.* **12**, 1005–1016.

Musilova, P., Kubickova, S., Zrnova, E., Horin, P., Vahala, J. & Rubes, J. (2007). Karyotypic relationships among *Equus grevyi*, *Equus burchelli* and domestic horse defined using horse chromosome arm-specific probes. *Chromosome Res.* **15**, 807–813.

Rhymer, J.M. & Simberloff, D. (1996). Extinction by hybridization and introgression. *Annu. Rev. Ecol. Syst.* **27**, 83–109.

Rubenstein, D.I. (1986). Ecology and sociality in horses and zebras. In *Ecological aspects of social evolution: birds and mammals*: 282–302. Rubenstein, D.I. & Wrangham, R.W. (Eds). Princeton: Princeton University Press.

Rubenstein, D.I. (2001). Horses, asses, and zebras. In *Encyclopedia of mammals*: 482–487. McDonald, D.W. (Ed.). Oxford: Oxford University Press.

Rubenstein, D.I. & Hack, M. (2004). Natural and sexual selection and the evolution of multi-level societies: insights from zebras with comparisons to primates. In *Sexual selection in primates: new and comparative perspectives*: 266–279. Kappeler, P.M. & van Schaik, C.P. (Eds). Cambridge: Cambridge University Press.

Ryder, O.A., Epel, N.C. & Benirschke, K. (1978). Chromosome banding studies of the Equidae. *Cytogenet. Cell Genet.* **20**, 332–350.

Sundaresan, S.R., Fischhoff, I.R., Hartung, H., Akilong, P. & Rubenstein, D.I. (2008). Habitat choice of Grevy's zebras (*Equus grevyi*) in Laikipia, Kenya. *Afr. J. Ecol.* **46**, 359–364.

Sundaresan, S.R., Fischhoff, I.R. & Rubenstein, D.I. (2007). Male harassment influences female movements and associations in Grevy's zebra (*Equus grevyi*). *Behav. Ecol.* **18**, 860–865.

Swofford, D.L. (2003). *PAUP**. *Phylogenetic analysis using parsimony (*and other methods)*. *Version 4*. Sunderland, MA: Sinauer Associates.

Tatman, S.C., Stevens-Wood, B. & Smith, V.B.T. (2000). Ranging behaviour and habitat usage in black rhinoceros, *Diceros bicornis*, in a Kenyan sanctuary. *Afr. J. Ecol.* **38**, 163–172.

Williams, S.D. (2002). Status and action plan for the Grevy's zebra (*Equus grevyi*). In *Equids: zebras, asses and horses: status survey and conservation action plan*: 11–27. Moehlman, P.D. (Ed.). Gland, Switzerland: IUCN/SSC Equid Specialist Group.

Wolf, D.E., Takebayashi, N. & Rieseberg, L.H. (2001). Predicting the risk of extinction through hybridization. *Conserv. Biol.* **15**, 1039–1053.

Yang, R., Ru, B., O'Brien, P.C.M., Robinson, T.J., Ryder, O.A. & Ferguson-Smith, M.A. (2003). Karyotypic relationships of horses and zebras: results of cross-species chromosome painting. *Cytogenet. Genome Res.* **102**, 235–243.