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A rapid loss of stripes: the evolutionary history of the extinct quagga

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Twenty years ago, the field of ancient DNA was launched with the publication of two short mitochondrial (mt) DNA sequences from a single quagga (*Equus quagga*) museum skin, an extinct South African equid (Higuchi *et al.* 1984 *Nature* 312, 282–284). This was the first extinct species from which genetic information was retrieved. The DNA sequences of the quagga showed that it was more closely related to zebras than to horses. However, quagga evolutionary history is far from clear. We have isolated DNA from eight quaggas and a plains zebra (subspecies or phenotype *Equus burchelli burchelli*). We show that the quagga displayed little genetic diversity and very recently diverged from the plains zebra, probably during the penultimate glacial maximum. This emphasizes the importance of Pleistocene climate changes for phylogeographic patterns in African as well as Holarctic fauna.

Keywords: ancient DNA; phylogeography; Africa; vicariance; Pleistocene; refugia

1. INTRODUCTION

The extinct quagga was morphologically divergent in coat colour from all extant equids (horses, zebras and asses). The front half of the animal had brown zebra-like stripes, whereas the rear looked more like a horse (figure 1). It was formerly abundant in South Africa, which was also home to the mountain zebra (*Equus zebra zebra*), an extinct population of the plains zebra (*Equus burchelli burchelli*) and a small portion of the range of an extant subspecies of plains zebra in the northeast (*Equus burchelli antiquorum*) and Hartmann's mountain zebra (*Equus zebra hartmannae*) in neighbouring Namibia to the northwest. Of the extant zebra species, the plains zebra is by far the most widely distributed, and is sympatric with Grevy's zebra (*Equus grevyi*) in the north.

Although previous genetic analyses have suggested that the quagga was genetically similar to plains zebras in mitochondrial DNA sequence (Higuchi *et al.* 1984, 1987), not all species of zebra were included in the comparison and genetic diversity in the quagga remained unknown. Morphological analyses of the quagga and all other zebra species have come to conflicting conclusions. In a study based on cranial measurements, the quagga was found to be as different from plains zebra as the plains zebra is from the mountain zebra (Klein & Cruz-Uribe 1999). Another study, based on pelage as well as cranial characters, found the quagga and the plains zebra to be highly similar and argued for subspecific status of the quagga (Groves & Bell 2004).

To determine the amount of genetic diversity present in the quagga before it went extinct, and its relationship with other zebras, we obtained material for genetic analyses from 13 quagga specimens in museums, including 11 pelts, one tooth and one bone fragment (Rau 1974, 1978) plus a pelt from a plains zebra (subspecies or phenotype *E. b. burchelli*).

2. MATERIALS AND METHODS

(a) *Materials*

Small skin, tooth or bone fragments were removed from 13 museum specimens of quagga (*Equus quagga*): Peabody Museum no. 1623; Mainz Museum no. Na1955/14, W1955/11, W1955/13; South African Museum no. 35575; Frankfurt a.M. Museum no.19207; Wiesbaden Museum no. 442; Bamberg Museum no. 236 (mammal catalogue); Berlin Museum no. 38954 (sampled tooth, from different individual from the skin of same number; Rau 1974: 23707; old number An 1407); Basel Museum no. 897; Darmstadt Museum no. HLM, M 719; Munich Museum no. AM541 and Vienna Museum no. NMW-St. 710; and one of a plains zebra probably of subspecies *E. b. burchelli*, from South Africa: Mainz museum no. W1955/12. Except for the Berlin quagga (see above), all catalogue numbers are identical to those in Rau (1974).

Homologous sequences of plains zebra from multiple subspecies were obtained from Oakenfull *et al.* (2000). Traditional subspecies designations were used in Oakenfull *et al.* (2000), which have since been questioned (Groves & Bell 2004). For example, Groves & Bell (2004) suggest that the subspecies *E. b. burchelli* and *E. b. antiquorum* are synonymous. Owing to the variation in results of recent morphological studies involving the taxa studied here (Klein & Cruz-Uribe 1999; Groves & Bell 2004) and the unknown geographical origin of some of the database sequences we have included in our analyses, we defer to the published subspecies designations for the data taken from the literature (Oakenfull *et al.* 2000). It should also be noted that the aim of this study is not an investigation of the genetic relationship of all subspecies of plains zebras but the evolutionary history of the quagga.

(b) *Molecular methods*

DNA was extracted in Leipzig from all samples, except for the Peabody specimen, following the methods of Rohland *et al.* (2004). The Peabody specimen and six of the other specimens were also extracted at the Smithsonian and Yale following the methods of Leonard *et al.* (2000) with extraction volume reduced to 1 ml. All extractions took place in isolated, designated ancient DNA facilities. DNA was amplified by polymerase chain reaction in a series of overlapping fragments with the following primer sets: F1 5′-ATT CAC CCT CAT GTA CTA TGT CAG TA and R2 5′-TTT GAC TTG GAT GGG GTA TGC A; F2 5′-GCA TTA AAT TGT TTG CCC CAT GA and R2 5′-ATG GGC CCG GAG CGA GGA; F3 5′-AAG CCG CGG GAA ATC AGC A and R3 5′-GCA TGA AAC CAC AGT TAT GTG TGA GC; and F4 5′-GGC ATC TGG TTC TTT CTT CAG G and R4 5′-TTA CCA TGG ACT GAA TAA CAC CTT; or F1 and R1a1 5′-ATT ATG TAC ATG CTT ATT ATT CAT GG; and F1a 5′-ATA CCC TGT TAA CAT CCT ATG TAC and R1s 5′-GAC TTG GAT GGG GTA TGC A; F2a 5′-TTA CAT AAG TAC ATT ATA TTA TTG A and R2a 5′-CTG ATT TCC CGC GGC TT; F3a 5′-AAC CCA TAT TCC ACG AGC TTA ATC and R3a 5′-CCT GAA GAA AGA ACC AGA TGC C; F4a 5′-GTG TCC CAA TCC TCG CTC CG and R4a 5′-GTC CAT CGA GAT GTC TTA TTT AAG G; F4 and R3; F6a 5′-CAT CTC GAT GGA CTA ATG ACA G and R6a

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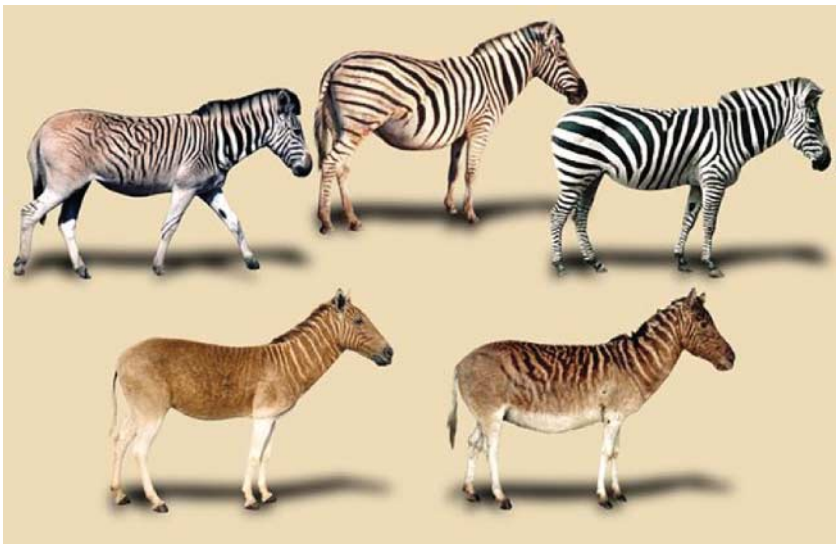

Figure 1. The morphological variability within living plains zebras and the extinct quagga. Upper row, left: mare 'Tracy' from the quagga rebreeding programme, probably the most quagga-like living plains zebra; middle: a plains zebra from the Etosha area; right: *E. b. boehmi*, a subspecies with very pronounced striping and no brown coloration or shadow-stripes in the white parts. Lower row, left: Munich quagga, one of the specimens with the least striping; right: Tring quagga, one of the unquestioned quagga specimens with the most pronounced striping.

5′-AGC TTC AAT TCA ATT GAC TGC GTC; F7a 5′-CTA TGA CTC ACT ATG GAC TGA ATA ACA CCT T and R4.

(c) ***Data analyses***

Sequences were aligned by eye with existing sequences from the neighbouring subspecies of plains zebra and the other species of zebra (Oakenfull *et al.* 2000). The HKY+G model of sequence evolution, with a gamma parameter of 0.6, was selected by MODELTEST v. 3.04 (Posada & Crandall 1998). Neighbour-joining and maximum-likelihood phylogenies were constructed in PAUP* v. 4.0b10 (Swofford 2002) using this model of evolution and the mountain zebra as the outgroup. Maximum-parsimony trees were also constructed through a heuristic search with indels considered as a fifth state in PAUP* v. 4.0b10 (all indels in dataset are 1 bp in length). Confidence was estimated by bootstrap analysis with 1000 pseudoreplicates also in PAUP* v. 4.0b10. Maximum-likelihood phylogenies were also constructed in MRBAYES v. 3.0B4 (Huelsenbeck & Ronquist 2001) with six runs of 1 000 000 steps and one run of 100 000 000 steps with four chains each. Support for monophyly of quagga and subspecies of plains zebra was explored by enforcing monophyly in MACCLADE v. 3.06 (Maddison & Maddison 1992). Nucleotide diversity for each subspecies of plains zebra and the quagga were calculated from the haplotypes in DNASP v. 4.00.5 (Rozas & Rozas 1999).

3. RESULTS

We amplified and sequenced 567 bp of the mitochondrial control region using four to eight primer pairs for the tooth, bone and four pelt samples (Peabody Museum no. 1623; South African Museum no. 35575; Wiesbaden Museum no. 442; Berlin Museum no. 38954; Darmstadt Museum no. HLM, M 719; and Munich Museum no. A.M.541). For two additional quagga pelts and the pelt from the South African plains zebra, we were only able to amplify partial sequences, while the other four samples did not yield amplification products (table 1).

Four different haplotypes were identified in the complete quagga sequences, one in three individuals (**A** in figure 2) and three in each of a single individual (**B–D** in figure 2). Two additional quagga specimens yielded partial sequences, both of which were identical over the obtained sequence length with haplotype **A**. Sequences have been deposited in GenBank (accession numbers AY914318–AY914323). All of the quagga haplotypes were closely related to one another (table 2; average sequence divergence 0.6%, range 0.4–0.9%) and to the plains zebra (range 0.7–2.5%). These data support a close relationship between the quagga and the plains zebra. However, the quagga and the plains zebra did not share any haplotype. The phylogenetic position of the quagga is nested within the much more diverse plains zebra (figure 2). All phylogenies were consistent. No extra steps were required to make the quagga monophyletic. The subspecies *Equus burchelli chapmani* and *E. b. antiquorum* share haplotypes, so it was not possible to constrain them to be monophyletic. To make the subspecies *Equus burchelli boehmi* monophyletic required four extra steps in the parsimony tree. The quagga haplotypes displayed less nucleotide diversity than the plains zebra ($\pi = 0.006$ s.d. ± 0.001 versus $\pi = 0.022$ s.d. ± 0.003). The South African plains zebra differed from the quagga by an average of 1.5% (range 0.7–1.9%) and from other plains zebra by 2.4% (range 1.1–4.4%) in 395 bp.

We estimated the date of the most recent common ancestor for the quagga mtDNA sequences using the substitution rates of 1.0×10^{-8} and 2.4×10^{-8} substitutions/site/year estimated for this region of the mitochondrial DNA for zebra by Oakenfull *et al.* (2000). This indicates that the quagga derived from the plains zebra around 120 000–290 000 years ago.

4. DISCUSSION

The quagga has alternatively been described as a species and a subspecies of the plains zebra (Rau 1978; Klein & Cruz-Uribe 1999; Groves & Bell 2004). Our analyses did not identify any shared haplotype between the quagga and the plains zebra. Since the plains zebra was living adjacent to the quagga (Rau 1978), they probably would have interbred if they had been subspecies and would thus have shared haplotypes, as some of the other

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Table 1. All samples of quagga (*Equus quagga*) and plains zebra (*Equus burchelli*) included in analyses.
(Subspecies designations for extant zebra as in Oakenfull *et al.* (2000). Number is museum number for all museum specimens, and sample code as reported in Oakenfull *et al.* (2000) for extant plains zebra specimens. Museums are abbreviated; P for Peabody Museum, Ma for Mainz Museum, SA for South African Museum, F for Frankfurt a.M. Museum, W for Wiesbaden Museum, Bm for Bamberg Museum, Br for Berlin Museum, Bs for Basel Museum, D for Darmstadt Museum, Mu for Munich Museum and V for Vienna Museum. Locations for museum specimens are from Rau (1974). In the column 'sequence', either the haplotype as it is represented in figure 1 is listed in bold, or 'partial' or 'none' for samples from which partial or no sequence was obtained. Laboratory where each sequence was obtained or replicated indicated in parentheses: M for Max Planck, S for Smithsonian and Y for Yale.)

species	subspecies	number	location	sequence
E. quagga		P no. 1623	given as 'Syria'	**A** (S, Y)
E. quagga		Ma no. Na1955/14	unknown	partial (M, S, Y)
E. quagga		Ma no. W1955/11	unknown	none (M, S, Y)
E. quagga		Ma no. W1955/13	unknown	none (M, Y)
E. quagga		SA no. 35575	Nelspoort, Beaufort West District, Cape Province	**D** (M, Y)
E. quagga		F no. 19207	unknown	partial (M)
E. quagga		W no. 442	unknown	none (M)
E. quagga		Bm no. 236	unknown	none (M)
E. quagga		Br no. 38954	unknown	**C** (M)
E. quagga		Bs no. 897	Shiloh/Whittlesea, Eastern Cape Province	none (M)
E. quagga		D no. HLM, M 719	unknown	**B** (M)
E. quagga		Mu no. A.M.541	unknown	**A** (M, S, Y)
E. quagga		V no. NMW-St. 710	unknown	**A** (M)
E. burchelli	*burchelli*	Ma no. W1955/12	unknown	partial (M, S, Y)
E. burchelli	*boehmi*	1	unknown	AF220917
E. burchelli	*boehmi*	2	unknown	AF220917
E. burchelli	*boehmi*	3	Masai Mara, Kenya	AF220920
E. burchelli	*boehmi*	4	Maralel, Kenya	AF220917
E. burchelli	*boehmi*	5	Tsavo West, Kenya	AF220916
E. burchelli	*boehmi*	6	Tsavo West, Kenya	AF220917
E. burchelli	*boehmi*	7	Tsavo West, Kenya	AF220918
E. burchelli	*chapmani*	1	unknown	AF220919
E. burchelli	*chapmani*	2	Gwaii Forest, Zinbabwe	AF220923
E. burchelli	*antiquorum*	1	West Okavango, S. Africa	AF220923
E. burchelli	*antiquorum*	2	Umfolozi, S. Africa	AF220919
E. burchelli	*antiquorum*	3	Umfolozi, S. Africa	AF220921
E. burchelli	*antiquorum*	4	Vernon Crookes, S. Africa	AF220922
E. burchelli	*antiquorum*	5	unknown	AF220924

subspecies of the plains zebra do. However, one of the living subspecies of the plains zebra, *E. b. boehmi*, also carries exclusively private haplotypes in the current dataset (figure 2). This observed lack of shared haplotypes could either indicate a long enough population separation to result in unique haplotypes in the quagga and Boehm's plains zebra or insufficient sampling. Thus, a measure of genetic divergence alone is not conclusive about the taxonomic status of the quagga.

Separate analyses of quagga remains based on cranial morphology (Klein & Cruz-Uribe 1999) and cranial morphology and pelage (Groves & Bell 2004) have come to very different conclusions with regard to the specific status of the quagga. Cranial morphology of only the most securely documented quagga specimens lead Klein & Cruz-Uribe (1999) to find the quagga to be as different from the plains zebra as the plains zebra was from the mountain zebra. However, owing to their stringent conditions for including specimens, they were left with only four quagga. In addition to cranial morphology, Groves and Bell (2004) included pelage characters in their analyses. With a slightly larger sample size ($n=5$), which was entirely non-overlapping with the sample used by Klein & Cruz-Uribe (1999), they found no difference between quagga and plains zebra. Morphological as well as genetic analyses of the quagga have been confounded by a dearth of well-documented remains that are clearly attributable to *E. quagga* (Klein & Cruz-Uribe 1999; Groves & Bell 2004). Because the quagga is extinct, it is most probable that this situation will continue, and controversy over the status of specific samples will continue. However, our results could be consistent with the quagga and the plains zebra being synonymized, as suggested earlier (e.g. Rau 1978; Groves & Bell 2004). Owing to priority, the correct name for plains zebras would thus be *E. quagga*, with, according to Groves & Bell (2004), five living and one extinct subspecies, the quagga (*E. quagga quagga*). A genetic investigation of these proposed subspecies, including the status of the supposed *E. b. burchelli* specimen from Mainz, must await further studies.

The phylogenetic position of the quagga haplotypes within the diversity of the plains zebra haplotypes

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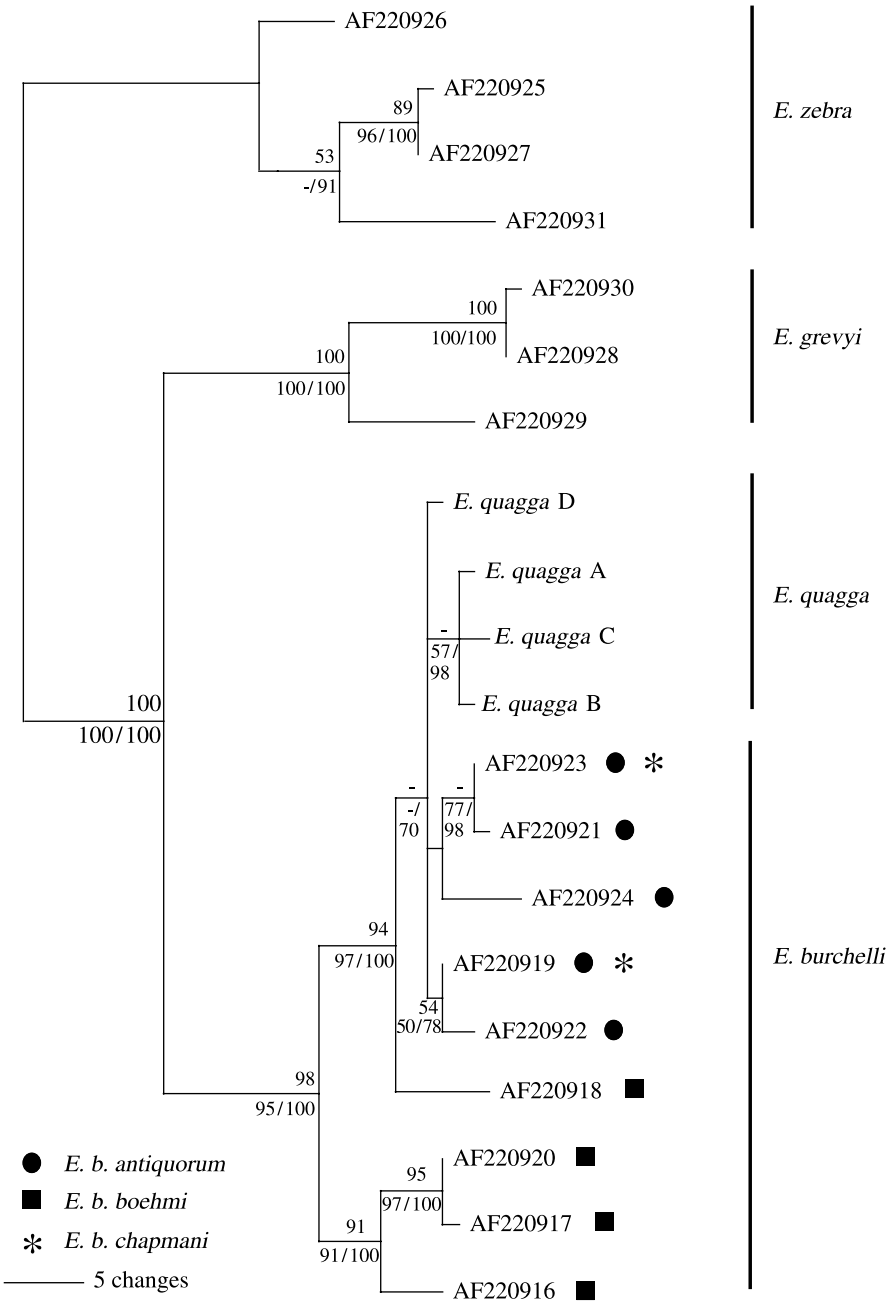

Figure 2. Phylogeny of all zebra species and quagga. One of 64 most parsimonious trees (136 steps). Node support is indicated when above 50% for parsimony (above branch) and neighbour-joining bootstrap and maximum likelihood from a long run of MrBayes (below branch). GenBank numbers for sequences are from Oakenfull *et al.* (2000).

Table 2. Average sequence divergence between haplotypes (from figure 1) in the zebra species.

species	average distance (%)
E. zebra	2.2
E. grevyi	2.5
E. burchelli	2.3
E. quagga	0.6

together with the observation of only private haplotypes in the quagga indicate that it descended from a population of plains zebras that was isolated some time ago. We estimate that this divergence took place in the Pleistocene, about 120 000 to 290 000 years ago, possibly during the penultimate glacial maximum (Dawson 1992). Therefore, the distinct coat colour of the quagga (Bennett 1980; figure 1) must have evolved quite rapidly. Existing plains zebras show a geographical gradient in coloration with progressive reduction in striping from north to south, which has been explained as an adaptation to open country and for which the quagga represented the extreme limit of the trend (Rau 1974, 1978). In this context, it is also noteworthy that quaggas vary in the extent to which they show 'quagga-typical' features such as the lack of stripes and the darkness of the brown coloration at the rear (Rau 1974, 1978; figure 1). Thus, the rapid evolution of coat colour in the quagga may be explained by either of two factors, or a combination of them: the disruption of gene flow owing to

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geographical isolation and/or an adaptive response to a drier habitat.

Some other large African ungulates also seem to have differentiated at about the same time in Africa, including the kob (*Kobus kob*) and puku (*Kobus vardoni*), the red lechwe (*Kobus leche leche*) and kafue lechwe (*Kobus leche kafuensis*) and the common waterbuck (*Kobus elliprymnus elliprymnus*) and the defassa waterbuck (*Kobus elliprymnus defassa*; Birungi & Arctander 2001). In all cases, these African bovids, both species and subspecies, show a pattern of morphological differentiation. Although the ranges of these bovids did not overlap with the range of the quagga, the same evolutionary force may have been at work in all of these cases. These results are further evidence that Pleistocene climate shifts had a strong influence not only on Holarctic species (Hewitt 2000) but also on African species (e.g. Matthee & Robinson 1997; Flagstad *et al.* 2001; Matthee & Flemming 2002).

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