The Quagga and Science.

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Studies on this extinct zebra have twice had major impacts on science. What does its future hold?

ABSTRACT. Quaggas, partially striped zebras from South Africa, have had major impacts on science. In the nineteenth century, the results of mating between a quagga stallion and a horse mare influenced thinking about mechanisms of inheritance for more than seventy years. In the twentieth century, tissue from a quagga yielded the first DNA of an extinct organism to be cloned and sequenced. Selective breeding of plains zebras in South Africa has produced animals whose coat coloration resembles that of some quaggas. This raises the intriguing possibility that quaggas may once again be the focus of scientific investigations.

Quaggas had a striking appearance: the face, neck and the anterior part of their bodies had white stripes like zebras, however, unlike other zebras their legs were not striped. The remainder of the quagga and the background color in the areas with white stripes was a brownish color – sometimes described as light brown, reddish-brown or yellowish-brown

In 1758 Linnaeus created the genus *Equus* to include horses, donkeys, and zebras, and gave the binomial name *E. zebra* to the Cape mountain zebra. This was one of three zebras occurring in South Africa, the others being the plains zebra or Burchell's zebra, *E. burchelli* and the quagga, *E. quagga*. The species name, quagga, was the common name for this zebra and is onomatopoeic: it was the sound of the zebra's barking cry of kwa-ha (the letters "g" are pronounced as "h").

Quaggas ranged through the grasslands and arid interior scrubland (also known as "Karoo") of the contemporary provinces of Free State, Eastern Cape, Northern Cape and Western Cape. Writing in 1840, the hunter Cornwallis Harris, observed: "Moving slowly across the profile of the ocean-like horizon, uttering a shrill barking neigh of which its name forms a perfect imitation . . . long files of Quagga continually remind the early traveler of a rival caravan on its march." Less than forty years later, the "long files of Quagga" were no more: quaggas were extinct in the wild, and the last captive animal died in the Amsterdam Zoo in 1883. Extinction of quaggas was probably caused by several factors: they were hunted by European settlers on a large scale, and excluded by fences from prime watering and grazing sites. This occurred against a background of predation by carnivores, hunting by indigenous people, and droughts.

Not everyone was convinced that quaggas were extinct. There were reports from 1932 and 1941 that zebras resembling quaggas had been seen in Namibia (then South-West Africa, under South African administration). In 1952, Bernard Carp reported his recent unsuccessful attempt to find quaggas in South-West Africa, and concluded that quaggas were, indeed, extinct. Carp had been fully aware of the incongruity of searching for quaggas at a considerable distance from their former range, but he held on to the slim chance that the vast unexplored areas of Namibia might contain isolated populations of quaggas that had escaped human notice – something that would be impossible in the more heavily populated South Africa.

By the late 1950s when even the most optimistic people had accepted that quaggas were extinct, an intriguing possibility began to be discussed, namely, that the genes for the quagga's distinctive coat coloration might exist in populations of plains zebras. An early mention of this possibility was by Lutz Heck who observed in 1955 that some of the plains zebras in Etosha National Park, Namibia, had hindquarters of a reddish-brown color and were less striped than most zebras. It was, of course, such zebras that had given rise to the erroneous reports of quaggas in Namibia. Lutz Heck and his brother Heinz had themselves attempted to breed selectively animals to resemble the extinct auroch (an ancestor of domestic cattle) and the extinct tarpan (an ancestor of the horse), and so were alert to the potential of re-breeding the extinct quagga.

Quagga: a species, or a partially striped plains zebra?

The key issue was whether the quagga was a separate species as proposed by eighteenth century taxonomists, or whether it was a subspecies of the plains zebra.

If the latter were the case then the genes for the distinctive coat coloration of quaggas should be present in populations of plains zebras. Favorable combinations of alleles should be obtainable by mating carefully selected animals whose coat coloration most resembles that of quaggas and then breeding from their most quagga-like offspring. If, however, the quagga were a species distinct from the plains zebra, then quagga genes for coat coloration would have been lost with its extinction.

In the absence of records of matings between plains zebra and quagga, there is no indication of whether their offspring would be fertile, or not. And so morphological evidence for their status as species was sought. The skins of quaggas exhibit various degrees of striping; this is apparent from photographs and painting of livings animals and from the surviving 23 skins. For example, the specimen in Munich (Germany) has striping largely confined to its head and neck, while the specimen in Tring (United Kingdom) has striping over most of its body (Figure 2). In plains zebras coat coloration is variable and forms a cline (a gradual transition) from the northern end of their range (Kenya and Ethiopia) where these animals have stripes over most of their bodies to southern Africa where their stripes are reduced (e.g., the legs are often less striped). The background color of the plains zebra also varies as a cline from black at the northern part of its range to a browner color in southern Africa. Similarities between the most heavily striped quaggas and the least heavily striped plains zebras suggest that the quagga may have been an extreme southern African form of the plains zebra, rather than a separate species.

Another approach compared the morphology of cranial structures between quaggas and other zebras. Measurement of cranial dimensions led Klein and Cruz-Uribe (1999) to conclude that there were similar degrees of difference between the quagga and the plains zebra as between the plains zebra and the mountain zebra. Given that the mountain zebra and plains zebra are generally accepted to be different species, these studies suggested that the quagga might have been a distinct species from the plains zebra.

Eisenmann and Brink (2000) carried out their own morphological comparison between the skulls of mountain zebras, plains zebras and quaggas and came to a different conclusion: that the quagga skulls more closely resembled the skulls of the plains zebra than they resembled the mountain zebra.

Ironically, although careful morphological studies did not yield definitive results about the relationship between plains zebras and quaggas, the museum specimens that were used provided material for analysis by molecular biology. This novel use of molecular biology to study extinct animals was made possible by Reinhold Rau, a German-born taxidermist at the South African Museum. Rau had remounted the skins of three quaggas at the Natural History Museum in Mainz, Germany, and a quagga foal at the South African Museum in Cape Town. The original taxidermy had fallen short of modern standards so that pieces of muscle and connective tissue remained attached to the skins. This tissue, over a hundred years old and having been dried and treated with salt contained, nonetheless, some intact DNA. Rau was not only a careful taxidermist, but also an imaginative and persistent biologist who saved some of the preserved tissue and brought it to the attention of scientists who analyzed some of its proteins and two sequences of mitochondrial DNA. Lowenstein (1985) determined that proteins from quagga skin more closely resembled those of the plains zebra than those of the mountain zebra (*E. zebra*) or of Grevy's zebra (*E. grevyi*), an endangered species occurring in Ethiopia and Kenya.

In 1984 the quagga achieved scientific distinction as the first extinct animal to have its DNA partially sequenced. This analysis by Higuchi et al. (1984,1987) of two short mitochondrial DNA sequences from the muscle and connective tissue of the Mainz quagga aroused widespread interest: Poinar in a 1999 American Scientist article wrote that it "struck like a bombshell" because it indicated that DNA cloning and sequencing could be carried out on extinct organisms. The science of "paleogenomics" or "molecular archeology" was born. Survival of DNA in quagga tissue and in an Egyptian mummy helped inspire Michael Crichton's novel "Jurassic Park".

Higuchi et al. (1984, 1987) showed a close relationship between the quagga and the plains zebra, and demonstrated that the mountain zebra was more distantly related to both the quagga and the plains zebra. The horse was even more distantly related to the quagga. (These comparisons form the basis for a demonstration in molecular biology accessible at http://www.ngbw.org/labs/quagga/quagga.htm.) Leonard et al. (2005) analyzed a much longer sequence of mitochondrial DNA from 8 quaggas (mainly pelts, but also a bone fragment and a tooth) and concluded that "the quagga displayed little genetic diversity and very recently diverged from the plains zebra, probably during the penultimate glacial maximum" which was approximately 120,000 to 290,000 years ago.

Nineteenth-century taxidermy provided material for twentieth century molecular biology and this, in turn, helped to revise eighteenth-century taxonomy. Since mitochondrial DNA analyses indicate that the quagga is an extinct subspecies of the plains zebra, both the plains zebra and the quagga should have the same binomial name. The plains zebra was formerly known as *E. burchelli*, a name given by Gray in 1824. But the name *E. quagga* was proposed for the quagga by Boddaert in 1785 and Gmelin in 1788 and so this name takes precedence over *E. burchelli*. The plains zebra and the quagga are now both *Equus quagga* while the trinomial system distinguishes the quagga (*Equus quagga quagga*) from the plains zebra (*Equus quagga burchelli*). Changes in binomial names often appear unnecessary to non-taxonomists, but the binomial *Equus quagga* for the plains zebra is well deserved and reflects a new understanding of the relationships between zebra. This may not be the last taxonomical change, however, as recent genome studies (Orlando et al., 2009) have suggested that not only are the plains zebra and quagga conspecific, but that they and several other equids belong to a single phenotypically variable species.

Restoration of the quagga

Rheinhold Rau was a tireless advocate for "re-breeding" (also termed "breeding back" or "restoring") the quagga from the plains zebra. The demonstration that the extinct quagga is conspecific with the plains zebra indicated the feasibility of this proposal. The variability in striping and background color that exists within existing populations of plains zebras provided the starting point for a selective breeding program. Beginning in 1987, a total of 18 plains zebras from Etosha National Park (Namibia) and Kwazulu-Natal (South Africa) provided the founder population in a selective breeding program, the Quagga Project, that has led to the birth of zebras with progressively reduced striping in their hindbodies and legs. The degree of striping has been carefully quantified (Harley et al., 2010). Fourth generation progeny have reduced striping of the hindbody and legs when compared to the founder population. Selection for the other distinctive feature of the quagga, the reddish-brown background color of the coat has not occurred so far: the background color has not darkened between the founder population and subsequent generations. In Figure 2 a second generation animal is compared with the Munich quagga (stripes confined to its neck) and the Tring quagga (stripes extend to its hindquarters). Figure 3 features a fourth generation animal from the Quagga Project.

Selective breeding has a long history. Charles Darwin in his book "Variations of Animals and Plants under Domestication" (1868) illustrated the power of choosing a particular characteristic and then breeding from animals and their offspring that best manifested this characteristic. Darwin observed that "it was formerly ordered that the comb of the Spanish cock should be upright and in four or five years all good birds had upright combs." Darwin commented, "Few persons, except breeders, are aware of the systematic care taken in selecting animals, and of the necessity of having a clear and almost prophetic vision into futurity." It is apparent that Reinhold Rau had such a vision when he initiated what would become "The Quagga Project." (Details of this endeavor can be accessed at http://www.quaggaproject.org.) It has been suggested that the restored quaggas should be called "Rau Quaggas" (Harley et al. 2010). This would not only acknowledge Rau's central role in this project, but would distinguish the restored quaggas from the extinct quaggas. This is important because populations of restored quagga will almost certainly have genetic characteristics that differ from populations of extinct quaggas. The extinct quagga evolved in the arid conditions of the Karoo and so possibly had physiological adaptations which were different from those plains zebras living in more favorable conditions. In spite of these possible differences, the extinct quagga was characterized by its coat markings and this is what characterizes the Rau quaggas (Harley et al., 2010.)

In February 2011 The Quagga Project listed 109 animals of which 19 animals are fourth generation. These are maintained at a number of locations, some of which are close to Cape Town. Clearly, these intriguing animals with their interesting history have potential as a tourist attraction.

Lord Morton's quagga and telegony.

While the extraction of DNA from a quagga skin opened up a new area of genomics, observations on the results of mating a quagga with a horse almost 200 years ago seemed to provide scientific evidence for what Charles Darwin termed "the direct action of the male element on the female form." Darwin also referred to the phenomenon as "pangenesis", although it is better known by the term "telegony" coined by August Weismann (Burkhardt, 1979.). Weismann based this new term on two Greek words meaning "offspring at a distance." Telegony holds that progeny can be genetically influenced by the male from an earlier mating. This idea was widely believed from antiquity until the end of the nineteenth century. Examples supporting telegony were anecdotal, for example, descriptions of a widow giving birth by a new husband to a child who resembled her deceased husband. It was observations made on the quagga, however, that appeared to offer scientific evidence for telegony. In a letter read to the Royal Society of London, Lord Morton (1821) described how he had mated a quagga stallion with a virgin mare that was seven-eighths Arabian horse. Unsurprisingly, the hybrid offspring had characteristics of both parents and had stripes. The fate of the hybrid is unknown, but its mother was later bred to a purebloodied Arabian stallion, and produced two offspring: these animals were the subject of Lord Morton's letter. He described as follows a colt and a filly aged one and two years, respectively: "They have the characteristics of the Arabian breed as decidedly as can be expected, where fifteen-sixteenths of the blood are Arabian; and they are fine specimens of that breed; but both in their colour, and in the hair of their manes, they have a striking resemblance to the quagga."

Lord Morton gave a detailed description of the colt and filly and concluded his letter by emphasizing "…the extraordinary fact of so many striking features, which do not belong to the dam, being in two successive instances, communicated through her to the progeny, not only of another sire who also has them not, but of a sire probably belonging to another species; for such we have very strong reason for supposing the quagga to be." Such was the importance of the foals' appearance that they were painted by the renowned artist Agasse.

Immediately following Lord Morton's paper in the Proceedings of the Royal Society was an account describing a similar phenomenon in pigs: Giles described how a wild boar had been mated with a domestic sow resulting in a litter that resembled both parents. But when the domestic sow was subsequently mated with a boar of the same breed as the sow, the resulting litter had coats of the same chestnut color as the litter produced by the wild boar. Darwin was mindful of these observations and in 1844 wrote in his notebook "…when the dam of one species has borne offspring to the male of another species, her succeeding offspring are sometimes stained (as in Lord Morton's mare by the quagga, wonderful as the fact is) by this first cross; so agriculturists positively affirm is the case when a pig or sheep of one breed has produced offspring by the sire of another breed" (Darwin, 1909).

These observations are mystifying to modern biologists, however, in the case of coat striping in the foals there is a ready explanation. Striping is an ancestral condition in equines (Gould, 1983), and may even occur in purebred horses – though it is more frequent in offspring of crosses between different varieties, and even different species, of equines. The striped foals from the cross between the Arabian stallion and the seven-eighths Arabian mare was a case of atavism, a reversion to an ancestral form; the quagga stallion was not responsible for the striping. Had the mating between the horse mare and stallion not been preceded by mating with a quagga, the striped foals would have been a curiosity of little note. As it was, Lord Morton's observations seemed to offer scientific evidence supporting popular and long-standing ideas about heredity. Middle class or upper class Victorian parents might fear contamination of a family's bloodline if a daughter were to have a dalliance with a working class male, even if there were no resulting pregnancy. By the same token, Victorian fathers might worry about the legitimacy of their offspring since a child from a wife's extramarital liaison would resemble her husband, rather than the child's biological father. This sense of pollution was evident in Darwin's use of the word "stained" (quoted above) and in Francis Galton's phrase "the taint of the quagga". The influence of Lord Morton's quagga on the mare's subsequent offspring was well known even outside of the sciences. In August Strindberg's play "The Father" written in 1887 the Captain summarizes Lord Morton's observations: "Is it true that if you cross a mare with a zebra you get striped foals?...And that if breeding is then continued with a stallion, the foals may still be striped?...So the offspring's resemblance to the father proves nothing." Lord Morton's observations had found a receptive audience

In "Variations of Animals and Plants under Domestication" Charles Darwin (1868) summarized Lord Morton's observations and concluded "Hence there can be no doubt that the quagga affected the character of the offspring subsequently begot by the black Arabian horse." Darwin believed that the mechanism of this inheritance were "gemmules" (or "pangenes"), minute particles that were capable of dividing and of transmitting information from the parents to the offspring and subsequent generations. In Darwin's ideas about pangenesis, gemmules produced by the different cells of the body divide and circulate through the body. Some of these gemmules collect in the gonads and are transmitted to offspring during reproduction when, in Darwin's words, "…their development in the next generation forms the new being; but they are likewise capable of transmission in a dormant state to future generations and may then be developed." Based on the hypothesis of pangenesis, gemmules from the quagga would have been transmitted to the horse mare by quagga semen or from the embryonic hybrid foal. Some of these gemmules would remain dormant within the mare and would later be transmitted to her subsequent offspring sired by the black Arabian stallion where they would become active and confer characteristics of the quagga to the foals.

As Burkhardt (1979) has noted, the breeding experiments with Lord Morton's quagga also influenced the thinking of such prominent scientists as Louis Agassiz, Claud Bernard, George John Romanes, Herbert Spencer and August Weismann. In 1883 Weismann had argued that the germ line was separate from the somatic cells. This would, of course, preclude the transmission of gemmules from the body into the germ cells, however, Spencer's belief in telegony and gemmules was so strong that he argued against Weismann's ideas, stating that germ cells and somatic cells "are in communion" (cited by Burkhardt, 1979).

Eventually, the compelling evidence against telegony was again provided by a zebra. To examine the inheritance of striping in equids, Professor J.C.Ewart of Edinburgh University repeated Lord Morton's breeding experiment using another variety of the plains zebra, *Equus burchelli chapmani* as the striped progenitor, in place of the extinct quagga. This zebra was mated with mares from several breeds of horses. These mares were later mated with purebred stallions of their own breed. Ewart (1899) showed both that mating with a zebra need not result in striped offspring in subsequent matings, and that striped offspring could be produced by matings between horse stallions and mares which had never been mated with zebras. Based on his findings, Ewart viewed stripes as the ancestral markings of horses and consequently concluded that the occasional striping of horse offspring is due to a reversion to the ancestral condition.

Lord Morton had also noted a resemblance between the mane of the quagga and the manes of the horses born from the subsequent mating of the Arabian mare and stallion. Ewart also critically examined these observations and presented a series of arguments rejecting any influence of the quagga on the horses' manes

Ewart's findings published in 1899 were curiously prescient of the rediscovery of Mendel's experiments in the following year and the publication in that year of papers by Hugo de Vries, Carl Correns and Erich von Tschermak. Telegony which had influenced nineteenth century biology was replaced by modern genetics.

The influence of telegony continues, however, in some quarters."Mole", a columnist in the Journal of Cell Science, described a conversation with an official of a kennel club who, though knowledgeable about genetics, nonetheless declared that a bitch would lose her pedigree status if she were mated with a non-pedigree dog (Mole, 2006). And an internet search of the word "telegony" reveals many contemporary instances where telegony is applied to humans – sometimes in advocacy of premarital female celibacy.

Rau's quaggas and science.

To return to the Quagga Project and the Rau quaggas. What are the possibilities that these animals will contribute to science? The distinctive coat coloration of Rau's quaggas suggests several possibilities for research. Black and white striping of zebras has been suggested as a selective advantage in thermoregulation, protection against both predators and flies, and to aid in social interactions (Ruxton, 2005). These possibilities could be tested by making use of the striped coat of the forebody and the unstriped reddish-brown coat of the hindbody. For example, Ruxtion (2002) cites experiments using a series of models to determine that tsetse flies were more attracted to solid colors than to stripes. A Rau quagga provides this coloration in a living animal: are tsetse more attracted to the uniform hindbody or the striped forebody?

The different degree of cooling caused by black versus white stripes might give rise to air movements over the surface of zebras and so contribute to their thermoregulation (Ruxton, 2005). Rau quaggas would provide a model for studying possible differences in coat temperatures between the striped forequarters and the unstriped hindquarters.

Continuing improvements in genome analysis suggest that eventually it may be possible to sequence part or all of the nuclear genome of the extinct quagga. It has been speculated that because quagga lived in semi-arid areas they might have had physiological adaptations to drought. Genomic comparisons between the extinct quagga and populations of plains zebras from non-arid regions might uncover genetic adaptations to aridity; understanding such mechanisms might be important in protecting zebras and other animals against the effects of climate change.

Rau quaggas will be introduced into historical habitats of the quagga and will have a beneficial effect on the biota of these localities. The range of the extinct quagga included the Karoo with its diverse flora of succulents, and areas of south-west South Africa that are home to the distinctive fynbos flora with its approximately 6,000 endemic species (Raimondo, 2005.) Many of these native plants require grazing in order to preserve their habitats. Consequently, the native flora has been affected by the extinction of the quagga, the reduction of the populations of other native grazing animals, and the introduction of non-native woody plants which often compete with the low-growing natural vegetation (Milewski, 2000.) Introduction of Rau's quaggas could be part of a comprehensive restoration program including such ongoing efforts as eradication of non-native trees. Quaggas, wildebeest and ostriches which occurred together during historical times in a mutually beneficial association could be kept together in areas where the indigenous vegetation has to be maintained by grazing. The presence of Rau quaggas in the Karoo National Park not only returns animals to their historic range, but also helps to restore the original vegetation. For many people in South Africa there is a strong desire to recognize and redress old wrongs. This was a powerful aspect of the Truth and Reconciliation Committee set up after the return to democracy in 1994. The re-breeding of quaggas, their return to their former ranges, and the restoration of these damaged habitats all reflect the same intent to put things right.

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