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Assessment of Non-Metric Skull Characters and Age Determination in the Asiatic Wild Ass *Equus hemionus*: A Methodological Approach

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Assessment of non-metric skull characters and age determination in the Asiatic Wild Ass *Equus hemionus* – a methodological approach[1]

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Abstract

The report presents the first information on the use of non-metric skull characters and age determination by tooth section for Asiatic Wild Asses. It was initiated by a collection of more than 400 skulls of the Asiatic Wild Ass from the Bordzongijn-gobi 2002-2004. The methodological overview gives a first research prospect in Asiatic Wild Ass concerning the use of non-metric skull characters as a morphological tool for assessing genetic variability, determining epigenetic divergences and evaluating fluctuating asymmetry. The time course of tooth eruption and replacement in the Asiatic Wild Ass is described with regard to its use for age determination. To determine the age in years of the older asses tooth sections are made with a low-speed precision saw that reveal annual lines in the tooth cementum.

Key words: Equidae, qualitative traits, epigenetic variability, population genetics, tooth development, incremental cementum lines, population analysis

Introduction

We are now living in times where natural-history museums have established “personal” molecular biological laboratories and electron microscopes, even though museums are often considered to represent the last retreat of classical morphological systematics. For systematic and phylogenetic questions, nowadays many new and modern methods are taken into consideration in addition to the traditionally prepared skin, e.g. recorded bird songs on electronic media and 'frozen collections' of blood or tissues. Against this background, it strikes one as being a true renaissance that, in morphology, non-metric skeletal characters for population genetics as well as fluctuating asymmetry as a measure of developmental stability and age determination through annual layers of tooth cementum are being increasingly used. Non-metric characters and teeth sections have become highly attractive as relatively simple morphological tools, even to non-morphologists, because of the rapid and apparently reliable results for applied research.

These morphological tools, their possibilities and relevance in relation to the extensive collection of Asiatic Wild Ass skulls kept by the Ulan Bator and Halle Universities are presented here in a brief review. This represents the first report on non-metric skull characters and age determination by tooth section for Asiatic Wild Asses.

Wild Ass skulls – the basis of morphological research

More than 400 skulls of the Asiatic Wild Ass *Equus hemionus* (Pallas, 1775) have been collected in the Bordzongijn-gobi since 2002 by zoologists of the Ulan-Bator and Halle Universities (STUBBE et al. 2005). All of them were found as dried carcasses mostly killed by poachers. Only very few seem to have died by natural causes. The skulls were in varying conditions which ranged from the form of mummified heads covered by skin to tidy bones cleaned by necrophagous insects.

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[1] Results of the Mongolian-German Biological Expeditions since 1962, No. 285.

The ass skulls were shipped to Germany for technical processing and scientific research. In the preparation laboratories of the Halle University and the Görlitz Museum of Natural History, the skulls are now being prepared by the odour-free and time-saving method of enzyme maceration. After taking tissue samples for genetic analysis, the skulls are macerated for about one week in warm water of 55°C by addition of enzymatic washing agents. Then the skulls are boiled and cleaned, and subsequently covered with hydrogen peroxide solution to remove any remaining non-bone material.

The prepared skulls will be kept together at one institution, guaranteeing solid and long-term preservation as well as access for further research. This collection of several hundred skulls of the endangered Asiatic Wild Ass represents unique scientific material resulting from the cooperation of Mongolian and German zoologists. It offers major opportunities for biological and ecological research, reported to some extent in this review.

Non-metric skull variability and population genetics

Normally and preferably, mammal skulls are used for taxonomic classification above and below the species level by taking a number of measurements. But further detailed findings can be obtained from through the study of qualitative morphological skull characters. In this context, these non-metric characters represent various kinds of discontinuous variants in different parts of the skull which are obviously independent of growth and not induced by direct external influences. After cautious preliminary investigations, intensive research during the second half of the last century focussed on the genesis and heritability of non-metric traits (e.g. BERRY and BERRY 1967). This presented the basis for the wide use of qualitative morphological variants as epigenetic polymorphisms for evaluating diversity and differences among and within populations and species in manifold lines and numerous mammal species (see ANSORGE 2001).

The use of non-metric traits for population genetics is based on the high heritability of non-metric characters, although this point has been subject to intense discussions BERRY 1978, CHEVERUD & BUIKSTRA 1981, RICHTSMEIER & MCGRATH 1986). Furthermore, the minor variants of non-metric skeletal characters are of lower importance for an organism than selectively more relevant traits concerning feeding or the reproductive system. It has been assumed that such traits are therefore exposed to a minimum of selective pressure (PANKAKOSKI & HANSKI 1989), qualifying them as epigenetic markers.

The most commonly used traits among non-metric skull variants constitute the foramina, natural holes in the skull, through which nerves and blood vessels pass. Often, a specific foramen is recorded as only being present or absent but sometimes it is also noted as being single or multiple (fig. 1). Thus, the occurrence of an additional minor foramen is sought. Foramina are so numerous and of such importance that investigations on other ungulates nearly exclusively used this type of non-metric characters (MARKOWSKI & MARKOWSKA 1988).

Nevertheless, further categories of qualitative traits complete this methodological foundation. Besides different kinds of easily recognisable hyperostotic traits, several fusion traits are taken into consideration. However, suture fusion in general or lack of ossification is often connected with the normal growth of the animal, impeding the analysis of epigenetic variation. Therefore, characters of the teeth are especially useful, as they remain very stable during later stages of ontogenesis. The occurrence of small single teeth at the ends of the tooth row (fig. 2), mostly being without any function, shows different frequencies. In addition to to the invaluable information obtained from analysing foramina they give a good impression of epigenetic variation.

Fig. 1: Multiple occurrence of the frontal foramen in the Asiatic Wild Ass skull.

Fig. 2: Occurrence of deciduous and permanent first premolars in the Asiatic Wild Ass skull.

Moreover, fluctuating asymmetry, the non-directional difference in the right and left side of bilateral non-metric characters, offers further opportunities. This results from developmental noise during ontogenesis, meaning environmental or genetic stress in the widest sense, such as chemical or radioactive contamination, food shortage, parasitism or hybridisation. Thus, fluctuating asymmetry is suitable for serving as an integrative measure of developmental stability (PALMER & STROBECK 1986). But the types of stress held responsible for fluctuating asymmetry are often based on subjective assessment. Therefore, the interpretation of fluctuating asymmetry as a biomonitoring tool for detecting loss of genetic diversity should be treated very cautiously (PALMER 1996).

Nevertheless, no study on non-metric skull characters in any species of the Equidae seems to have been carried out to date (see also BACHAU 1988), whereas mostly carnivores or rodents have been considered. As a first effort, a set of 28 non-metric characters were selected using the Wild Ass skulls from the Bordzongijn-gobi prepared as of yet (table 1, fig. 3). Choices focussed on the frequency and variability of skull characters, considering the results of earlier studies on ungulates (MARKOWSKI & MARKOWSKA 1988, MARKOWSKI 1993). Beside 22 foraminal traits, only 2 characters of the teeth and 4 hyperostotic traits were selected, summing up to 28 bilateral traits available to assess fluctuating asymmetry.

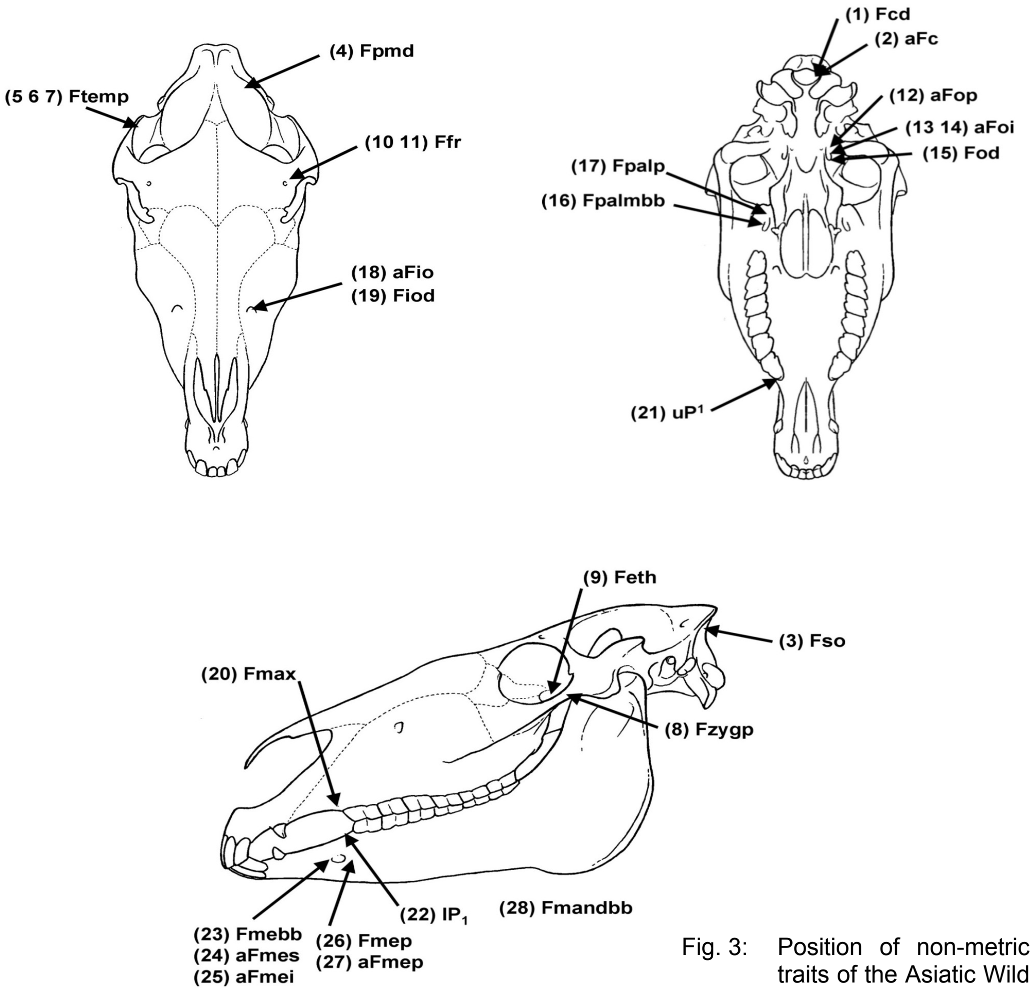

Fig. 3: Position of non-metric traits of the Asiatic Wild Ass skull.

Table 1: Non-metric traits in the Asiatic Wild Ass skull

1	Fcd	Condylar foramen double
2	aFc	Accessory condylar foramen present
3	Fso	Supraoccipital foramen present
4	Fpmd	Major parietal foramen doubled
5	Ftemp2	Two temporal foramina
6	Ftemp3	Three temporal foramina
7	Ftemp4	Four temporal foramina
8	Fzygp	Zygomaticum posterior foramen present
9	Feth	Ethmoidale foramen doubled
10	Ffr2	Two frontal foramina present
11	Ffr4	More than four frontal foramina present
12	aFop	Posterior accessori foramen ovale present
13	aFoi	Interior accessori foramen ovale present
14	aFoi2	Two interior accessori foramina ovale present
15	Fod	Foramen ovale divided
16	Fpalmbb	Major palatal foramen with bone bridge
17	aFpalp	Accessory posterior palatal foramen present
18	aFio	Accessory infraorbital foramen present
19	Fiod	Infraorbital foramen double
20	Fmax	Foramina maxilla present
21	uP^1	Upper first premolar present
22	lP1	Lower first premolar present
23	Fmebb	Mental foramen with bone bridge
24	aFmes	Accessory superior mental foramen present
25	aFmei	Accessory inferior mental foramen present
26	Fmep	Posterior mental foramen present
27	aFmep	posterior accessory mental foramen present
28	Fmandbb	Foramen mandible with bone bridge

Based on these kind of data some cautious prospects for research on the Asiatic Wild Ass can be given, such as determining genetic variability and epigenetic divergences by non-metric traits or assessing developmental stability by fluctuating asymmetry. After preservation of the entire skull collection of the Asiatic Wild Ass is completed, further research can demonstrate the use of non-metric skull characters as a powerful morphological tool (ANSORGE 2001).

Age determination and population analysis

"The ability to determine the absolute age of an animal is one of the most useful techniques available to any wildlife biologists" (HARRIS 1978). Information on the age of individuals is of great importance for studies on diverse aspects of the biology of mammal species. Correct age determination is not only necessary for developing population models, life expectancy or mortality rates (CAUGHLEY 1980). It is also very useful for diverse ecological and evolutionary studies, such as ontogenetic and static allometry in systematic comparisons, physiological parameters, reproduction biology, pathological findings or contamination. This applies to the collection of Wild Ass carcasses found in the Bordzongijn-gobi as well, regardless of their poor condition and shape. Especially population analysis of different parameters up to studies on population ecology should benefit from the precise age classification of the specimens.

Therefore, diverse methods have been developed to estimate the real age of dead individuals as precisely as possible (see MORRIS 1972, HARRIS 1978, HABERMEHL 1985, LÜPS et al. 1987). In many cases, however, only a few age classes have been separated, often merely distinguishing between juveniles and adults by diverse skull characteristics. In the Asiatic Wild Ass, several features enable such a rough age classification (fig. 4):

- the proportions of the skull in general (ORLOV 1961),
- formation of the bone crests,
- obliteration of nasal and frontal sutures,
- rugosity of the cranium surface.

Fig. 4: Skull characteristics of Asiatic Wild Asses of different age classes.

However, in the case of the Equids, an extensive period of tooth eruption and replacement fortunately enables the correct determination up to an age of five years. But no serious description of tooth development in Wild Asses exists in the literature, except for some short remarks by ORLOV (1961) and the vague note of HEPTNER et al. (1966) "die Milchzähne werden mit dem 4. Jahr durch bleibende ersetzt" ("milk dentition is replaced by the permanent teeth as of the forth year"), which describes tooth replacement in the Khulan very incorrectly. According to the well known situation in domestic horses (HABERMEHL 1985), donkeys (SVENDSEN 1997) and zebras (JOUBERT 1972) and by examining the extensive material of Asiatic Wild Asses from the Bordzongijn-gobi, a simple outline of tooth development can be drawn for this species.

It can be assumed that at birth all premolars have erupted, including the small first deciduous premolar which only sometimes appears and probably does not cut through the gum. This stands in contrast to the general opinion of eruption of only the permanent P1 without any deciduous predecessor in the Equids. This is just as incorrect as JOUBERT's (1972) interpretation of the permanent P1 in the Hartmann zebra, which appears within two months as the first milk premolar with no successor, as being a persisting milk tooth. Our study supplies evidence for a normal diphyodont dentition in *Equus hemionus*, including both tooth generations of the first premolar.

This is indicated in fig. 2, which shows the replacement. These findings correspond with the situation in the dentition of other mammal orders such as carnivores (ANSORGE 1991).

The deciduous incisors, the deciduous canine and the permanent first premolar appear within the first year, whereby the latter two do not regularly cut through the gum. Eruption and replacement of the rest of the permanent teeth is given in table 2. Being the last permanent tooth, the corner incisor replaces the respective milk tooth at an age of about 4.5 years. This knowledge allows correct age determination up to an age of at least five years. The age of elderly asses can usually be estimated by the degree of tooth abrasion. Although tooth wear indicates the age of long-living animals quite well, this depends on certain influences such as nutrition, soil composition or even disease. Age estimation by observing tooth abrasion produces only roughly data, being insufficient for population analysis.

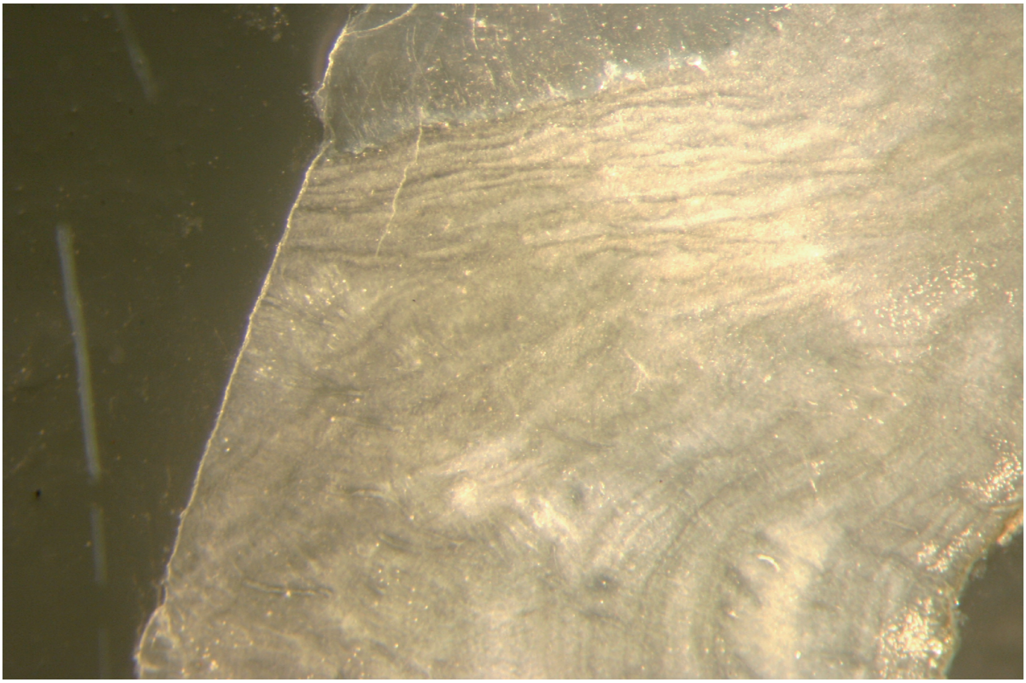

Fig. 5: Longitudinal section of M^1 of *Equus hemionus* from the Bordzongijn-gobi.

Table 2: Mean age of eruption and replacement in the permanent dentition of *Equus hemionus*

P 1	4 weeks	M 3	3 years
M 1	1 year	I 2, P 4	3.5 years
M 2	2 years	C 0	4 years
I 1	2.5 years	I 3	4.5 years
P 2, P 3	2.5 years		

The approximately accurate age of an older ass can be determined only by the incremental cementum lines of tooth sections. This well-established method (LAWS 1962, KLEVEZAL & KLEINENBERG 1967, KLEVEZAL 1988) is based on the periodic growth of the tooth root cementum. For the demands of population analysis and large sample sizes, an efficient application of tooth cutting has been developed (JOHNSTON & WATT 1980, DRISCOLL et al. 1985, ANSORGE 1995). Longitudinal sections (50-100 µm) of the teeth roots are produced by the diamond wheel of a low-speed precision saw without any pre-treatment. In unstained sections, annual lines can be counted using reflected light (lateral illumination) under a dissecting microscope (fig. 5). The age of the animal at the time of tooth eruption has to be added to the number of annual lines. In case of the Asiatic Wild Ass, only cheek teeth are suitable for checking cementum annuli, since the incisors close their pulp chambers only later. This method is used here for the first time to determine the age of Asiatic Wild Asses and there even is little experience in dissecting teeth of equids in general. Only SANTIAPILLAI et al. (1999) report on age determination by vertical sections of the molar cementum of African Wild Asses as well as GRUE & JENSEN (1979) on a small number of domestic horses which were aged by tooth sections too.

Since only the season of death – winter respectively summer – is known for the given animal, the middle of winter or summer is accepted for estimating the years of age by general skull characteristics. In this way, a preliminary picture of the age structure of the Wild Ass sample could be drawn (fig. 6), but the precise age of the older animals will be completed after preparation and tooth section of the entire collection material. This impressively demonstrates the usefulness of this morphological tool for population biology and wildlife research.

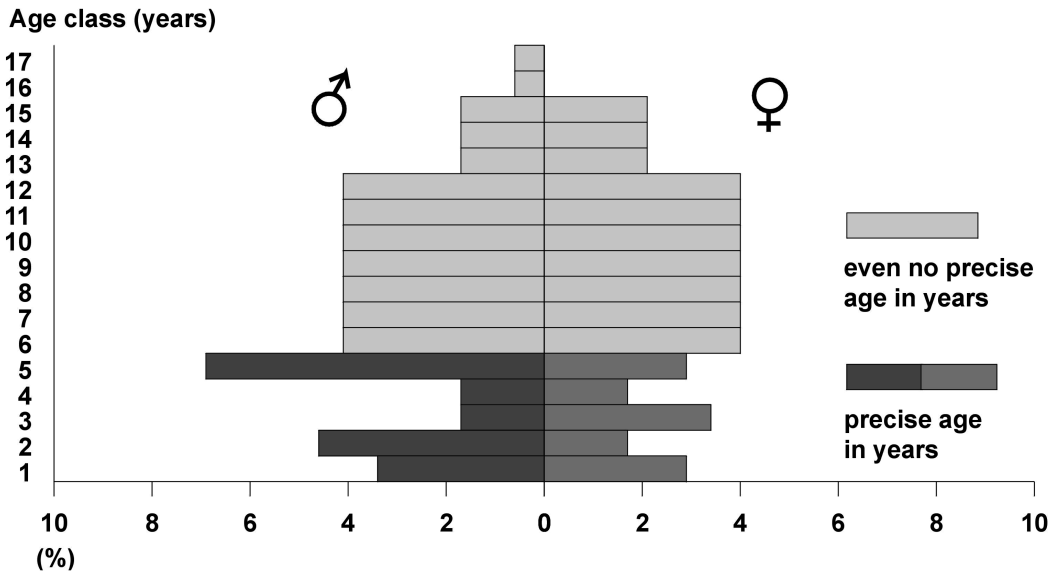

Fig. 6: Preliminary age and sex structure of the collection of Asiatic Wild Ass from the Bordzongijn-gobi 2002-2004.

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