Karyotype of the South West African Plains Zebra (*Equus burchelli* Gray, 1824 subsp.?) and Mountain Zebra (*Equus zebra hartmannae* Matschie, 1898): their cytogenetic relationship to Chapman's Zebra (*Equus burchelli antiquorum* H. Smith, 1841) and the Cradock Mountain Zebra (*Equus zebra zebra* Linn, 1758)

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ABSTRACT

The plains zebra of South West Africa (*Equus burchelli ?burchelli*) and Chapman's zebra (*E. burchelli antiquorum*) as found in the Kruger National Park have the same chromosome number (2n = 44) and similar karyotypes. A similar relationship holds for the mountain zebra of S.W.A. (*E. zebra hartmannae*) and the Cradock mountain zebra (*E. zebra zebra*) 2n = 32.

INTRODUCTION

This is a further preliminary communication stemming from a cytogenetic survey of the Southern African Perissodactyla (Heinichen, 1967).

The karyotypes of a number of *Equidae* have already been determined, (see Table 3). Regarding Chapman's zebra (*Equus burchelli antiquorum* H. Smith, 1841) Eloff (1966) mentioned in passing that a member of this subspecies from the Kruger National Park had a chromosome number of 44. The present author has been able to confirm this number on seven specimens of Chapman's zebra from the same locality (Heinichen, 1969). Benirschke & Brownhill (1963) and Benirschke & Malouf (in press) have studied the karyotype of Grant's zebra (*E. burchelli böhmi* Matschie, 1892); the chromosome number of this subspecies was also found to be 44. In a personal communication Benirschke (1967) indicated that the Damara zebra, thus to all intents and purposes the plains zebra from South West Africa, has 42 chromosomes.

Because of uncertainty regarding the exact taxonomic status of the plains zebra of South West Africa and in view of the above findings, investigation on the karyotype of *E. burchelli* as occurring in that region has been undertaken.

On the results obtained from a study of the karyotype of an *E. asinus* x *E. zebra* hybrid, Benirschke (1964) inferred that *E. zebra* had 34 chromosomes. King, Short, Mutton & Hamerton (1966) queried this, but later Short (*cit.* Ansell *in lit.*) seems to have accepted it. A study of the chromosomes of five representatives of *Equus zebra zebra* Linn., 1758 (Cradock mountain zebra) showed this subspecies to have 32 chromosomes (Heinichen, 1967). Hartmann's zebra, *E. zebra hartmannae* Matschie, 1898, the mountain zebra of S.W.A., had been studied by both Hamerton and Benirschke and the chromosome number determined as 32 (Benirschke, pers. com.). For comparative purposes a further study on this subspecies has been undertaken as well.

These studies, it is hoped, will enable Hsu & Benirschke (1967) to extend their „Atlas of Mammalian Chromosomes".

MATERIAL AND METHOD

Bone marrow was collected by biopsy needle aspiration from the sternum of four *E. burchelli*, two males and two females, in the Etosha Game Park in northern South West Africa, at the end of July, 1967. As this study was conducted concomitantly with a survey on diseases and parasitisms of wild life in that area, the animals had been shot; the specimens were taken within ten minutes after death.

In the case of *E. zebra hartmannae*, a mare and her colt were tranquillised by succynil choline chloride (100 and 50 mg. respectively, intramuscularly) on a game farm near Windhoek and one stallion and three mares were caught in a corral and tied down on another game farm in the same vicinity. In the latter four cases local anaesthetic (Planocaine) was injected subcutaneously prior to introducing the biopsy needle. All bone marrow specimens were collected between the hours of 8.00 a.m. and 11.30 a.m. All biopsies were submitted to hypotonic treatment immediately after collection and thereafter fixed and preserved in 1:3 acetic alcohol. On arrival in the laboratory at Onderstepoort some weeks later, spreads were made and treated according to the standard procedure in use here (Gerneke, 1967).

RESULTS

The material from the two *E. burchelli* stallions and the one mare was suitable for study. That from the other mare, the only bone marrow specimen collected as early as 8.00 a.m., contained no dividing cells at the metaphase stage.

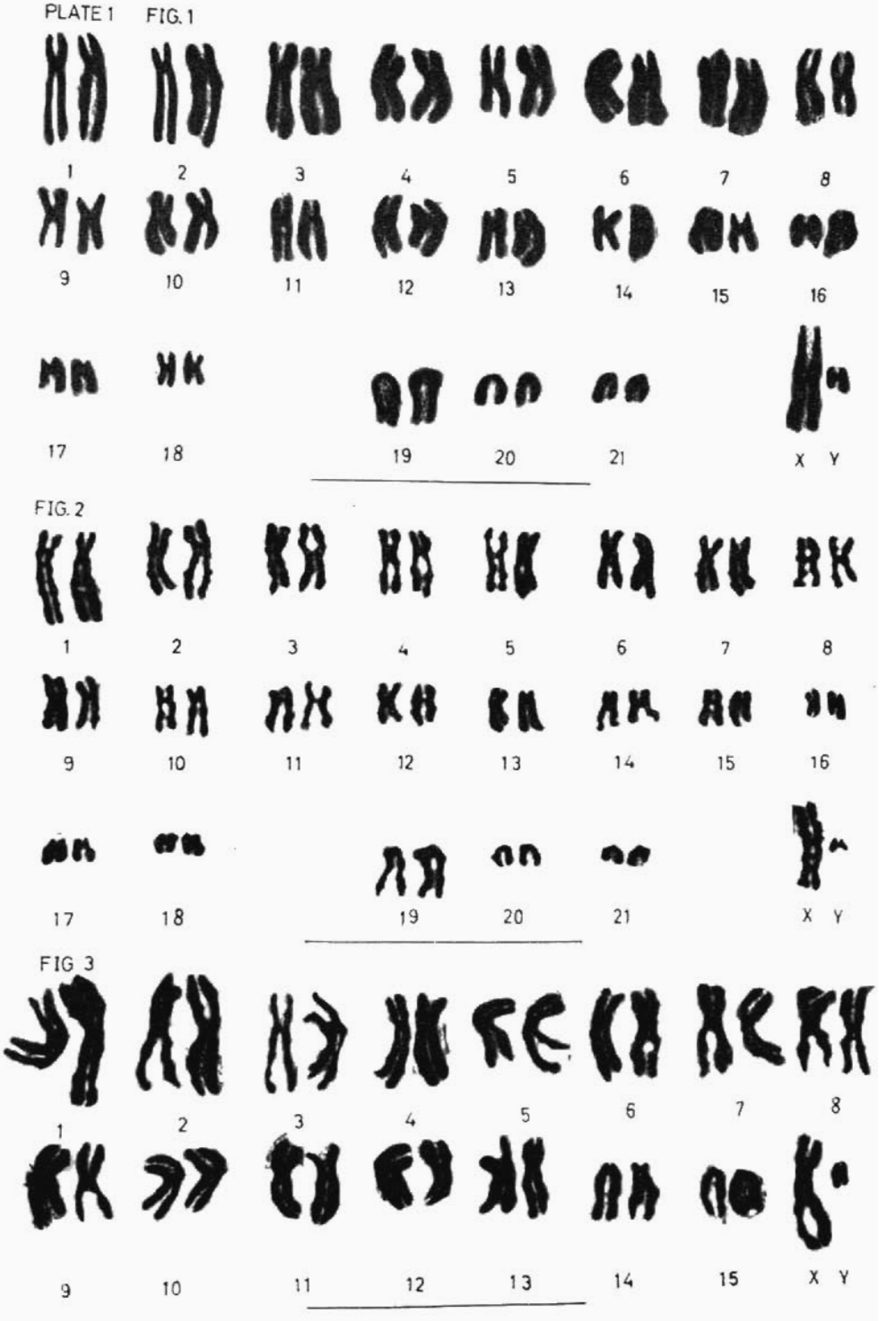

Plate 1. (Touched-up photographs)

Figure 1. Karyogram of *Equus burchelli ?burchelli*.
Figure 2. Karyogram of *Equus burchelli antiquorum*.
Figure 3. Karyogram of *Equus zebra hartmannae*.

The plains zebra from S.W.A. was found to have a diploid chromosome number of 44 (Table 1 & Plate 1, Fig. 1). In fig. 1 autosomes are arranged in descending order of length with centromeres in line. The chromosomes are classified into two groups:
Group 1—18: varies from meta- to submetacentric without definite subdivision being possible.

Group 19–21: all acrocentric.

The large X- and small Y-chromosome are both metacentric.

TABLE 1.

Chromosome counts of *Equus burchelli* (subsp.?)

Sex	42	43	44	45	46	Total
♂	2	8	37	2	1	50
♂	3	5	39	4	0	51
♀	2	5	22	3	1	33
	7	18	98	9	2	134

Of the material from the six *E. zebra hartmannae*, that from one mare (collected at 11.30 a.m.) could not be used, most of the divisions being in the anaphase stage.

The diploid chromosome number of the Hartmann zebra was found to be 32 (Table 2 & Plate 1, Fig. 3). The chromosomes are also classified into two groups:

Group 1–13: meta- to submetacentric.
Group 14–15: all acrocentric.

The sex chromosomes consist of a large metacentric X- and a small submetacentric Y-chromosome.

TABLE 2.

Chromosome counts of *Equus zebra hartmannae*.

Sex	30	31	32	33	34	Total
♂	0	2	12	1	1	16
♂	1	4	44	2	0	51
♀	0	3	21	2	0	26
♀	0	0	7	0	1	8
♀	0	0	12	1	2	15
	1	9	96	6	4	116

A few spreads clearly revealed the late replicating X-chromosome. Its identity and morphology could therefore be clearly determined and resembled that of the horse very closely.

DISCUSSION

In the light of previously published results by various authors and of my own findings, it is clear that *E. burchelli* has 44 diploid chromosomes and *E. zebra* 32. The Cradock mountain zebra and the mountain zebra of South West Afrfica seem to be conspecific: they have the same chromosome number and their karyotypes have an identical appearance (Measurements have not yet been undertaken to determine the relative arm lengths of the respective chromosomes. Even if this were the case, it would still only afford supportive evidence and not conclusive proof). The practice of regarding the one as *E. zebra zebra* and the other as *E. zebra hartmannae* is purely a subspecific taxonomic differentiation and one on which most taxonomists agree (Ansell, pers. com.).

Similarly, the plains zebra of South and South West Africa, as well as Grant's zebra (occurring north of the middle Zambezi, Luangwa and Rovuma rivers (Ansell, 1967) are conspecific judging from their karyotypes (see also Plate 1, Figs. 1 & 2). The status of the existing subspecies has not been resolved taxonomically. It is generally accepted that the zebra from the Kruger National Park be called *E. burchelli antiquorum* H. Smith, 1841, i.e. the so-called Chapman's zebra. The taxonomic status of its South West African counterpart is unsure, one idea being that it is *E. burchelli burchelli* (Gray, 1825), the other, that *E. burchelli burchelli* is extinct and that if a subspecies name be given, it should either be *E. burchelli kaokensis* (an end form of *E. burchelli antiquorum)*, or *E. burchelli antiquorum* (Ansell, 1967). The karyotypes do not assist in this degree of distinction. To avoid possible misinterpretation, an illustration is appended of each of the two extremes of markings found in plains zebras from which material was obtained for this study. (Plate 2, Figs. 4–7). For general confirmation it is intended to study the karyotype of *E. burchelli selousi* Pocock, 1897 (= *crawshaii)*.

Of special interest is the fact that the diploid chromosome numbers in the family *Equidae* range from 32–66. Decrease of chromosome number often appears to be accompanied by a decrease in number of acrocentrics and an increase in number of metacentrics, as illustrated in Table 3.

It has been postulated that a decrease in acrocentrics could be due to Robertsonian fusion. As study of the karyotypes of *E. przewalskii* and *E. zebra* (both with 26 metacentric chromosomes) proves, the decrease of acrocentrics is not necessarily accompanied by an equivalent increase of metacentrics. Some of the smaller chromosomes possibly could have been lost during the evolution of the *Equidae* since their origin in the Eocene of Europe and North America (Thenius, 1966). Higher organisms, however, are very sensitive to chromosome loss, hence a more acceptable explanation could be that translocations had occurred with subsequent loss of heterochromatic centromeres. Such translocations need not necessarily lead to the formation of metacentric chromosomes: such chromosomes may revert to acrocentric ones especially as result of pericentric inversion.

It is interesting to note that the White Rhino, *Ceratotherium simum simum* Burchell, 1817 and the Indian Rhino, *Rhinoceros unicornis* Linn., 1758 have a chromosome number of 82 (Heinichen, 1967 and Benirschke, pers. com.). Only 8 autosomal chromosome pairs in the White Rhino and 10 in the

TABLE 3.

Species	Subspecies	Chromosome Number 2n.	Metacentric Chromosome pairs	Acrocentric Chromosome pairs	References
E. przewalskii (Przewalskii horse)		66	13	19	Benirschke, Malouf & Low (1965)
E. caballus (Domestic horse)		64	13	18	Benirschke, Brownhill & Beath (1962) Trujillo, Stenius, Christian & Ohno (1962)
E. asinus (Donkey)		62	19	11	Trujillo, Stenius, Christian & Ohno (1962)
E. hemionus (Onager)		56	23	4	Benirschke (1967)
E. grevyi (Grevy's zebra)		46	16	6	Mutton, King & Hamerton (1964)
E. burchelli (Burchell's Zebra)	*(E.b. ?burchelli)* (S.W.A.)				Present investigation
	(E.b. antiquorum)	44	18	3	Eloff (1966); Heinichen (1969)
	(E.b. böhmi)				Benirschke and Brownhill (1963)
	(E.b. selousi)				Not yet investigated
E. zebra (Mountain zebra)	*E.z. zebra* *E.z. hartmannae*	32	13	2	Heinichen (1967) Benirschke & Malouf (1967) present investigation

Indian Rhino are submeta- to subtelocentric, while the others are all acrocentric. It is generally believed that the *Equidae* are the most specialised of the three families of the order Perissodactyla: *Tapiridae, Rhinocerotidae* and *Equidae* (Thenius, 1966). From these results the suggestion could possibly be made that the more specialised the animal, the smaller the chromosome count and the rarer the number of acrocentric chromosomes. Such a suggestion has also been made for a number of other animal groups (Bender & Chu, 1963).

SUMMARY

(1) Supportive evidence on cytogenetic grounds is brought forward that Chapman's zebra *(Equus burchelli antiquorum)* as found in the Kruger National Park is conspecific to the plains zebra of South West Africa, both having a chromosome number of 2n = 44, with 18 groups of meta- to submetacentric chromosomes, three groups of acrocentrics and a large metacentric X- and a small submetacentric Y-chromosome.

(2) The South West African mountain zebra or Hartmann's zebra *(Equus zebra hartmannae)* has the same number of chromosomes as previously found for the Cradock mountain zebra *(Equus zebra zebra)*, namely 2n = 32, and their karyotypes, consisting of 13 meta- to submetacentric pairs of chromosomes, two acrocentric pairs, a large metacentric X- and a small submetacentric Y- chromosome, appear to be identical. This similarly lends support to their being classified as belonging to the same species.

(3) The confusion regarding the subspecific naming of the plains zebra of South West Africa is mentioned.

(4) The present information concerning the cytogenics of Perissodactyla is reviewed briefly. It would appear that in this order, too, evolutionary progress has been accompanied by reduction in chromosome number, although this reduction cannot be ascribed solely to Robertsonian fusion. Rather, translocations with subsequent loss of heterochromatic centromeres may have taken place.

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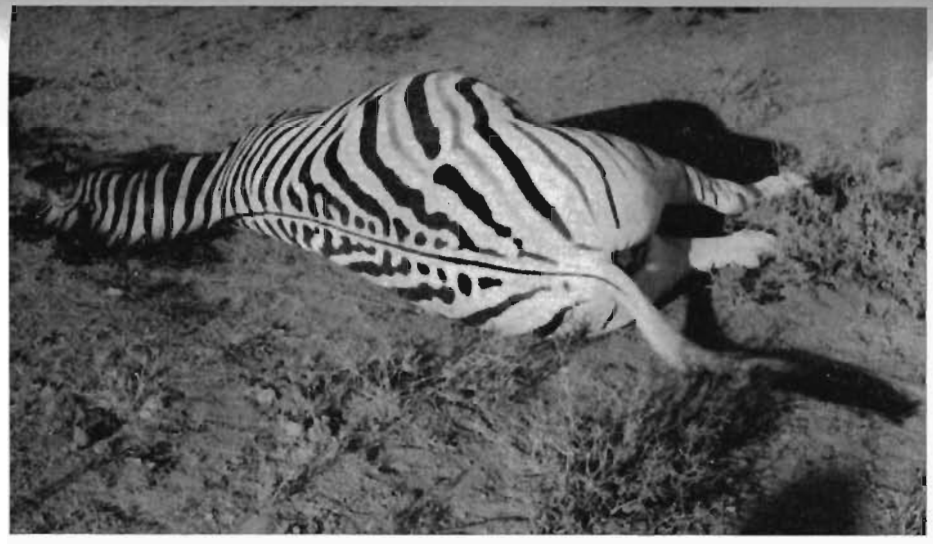

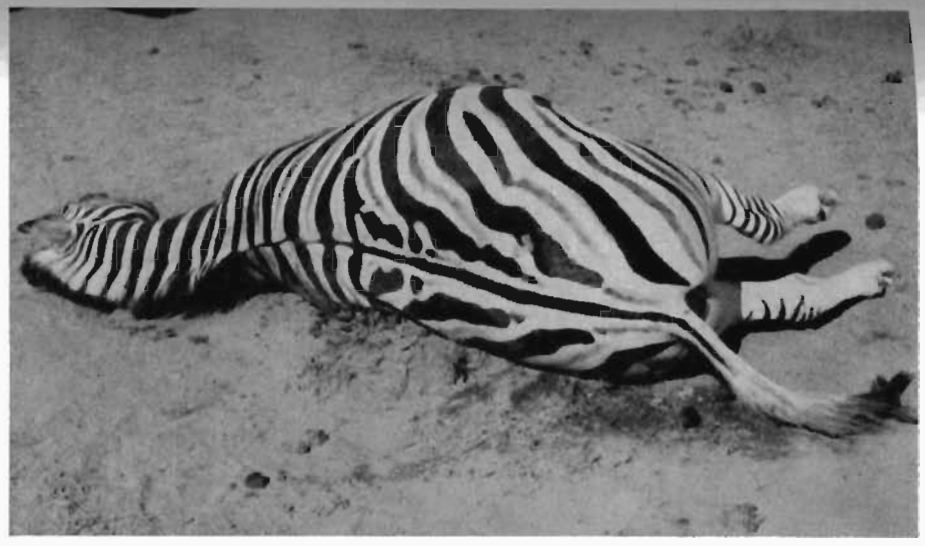

Plate 2.

Figures 4 & 5. Dorsal and ventral views of lightly striped specimen of *E. Burchelli ?burchelli* female.

Figures 6 & 7. Dorsal and ventral views of darkly striped specimen of *E. burchelli ?burchelli* male.

REFERENCES

ANSELL, W.F.H.
1967 Personal communication.

BENDER, M.A., & E. H. Y. CHU
1963 "The Chromosomes of Primates" in "Evolutionary and Genetic Biology of Primates". J. Buettner — Janusch. New York & London, Academic Press. Vol. 1 p. 303.

BENIRSCHKE, K.
1964 Corrigendum. Chromosoma 15: 1—13.

BENIRSCHKE, K., McFEELY, R. A. & L. E. BROWNHILL
1963 The chromosomes of the Grant zebra, *Equus quagga*. Mammalian Chromosomes Newsletter 10: 82.

BENIRSCHKE, K., BROWNHILL, L. E. & M. M. BEATH
1962 Somatic chromosomes of the horse, the donkey and their hybrids the mule and the hinny. J. Reprod. Fert. 4: 391—326.

BENIRSCHKE, K., MALOUF, N., & R. J. LOW
1965 Chromosome Complement. Differences between *Equus caballus* and *Equus przewalskii*. Poliakoff Science 148: 382—383.

BENIRSCHKE, K & N. MALOUF
Chromosome studies of Equidae. Zool. Garten. In press.

BENIRSCHKE, K.
1967 Personal communications.

ELOFF, G.
1966 The passing of the true Quagga and the little Klibbolikhonnifontein Burchell's zebra. Tydskr. Natuurwet. 6: 193—207.

GERNEKE, W. H.
1967 Cytogenetic investigations on normal and malformed animals, with special reference to intersexes. J. S. Afr. vet. med. Ass. 34 (1): 219—300.

HEINICHEN, IRMGARD, G.
1967 Karyotype of *Ceratotherium simum simum* and *Equus zebra zebra:* A preliminary note. J. S. Afr. vet. med. Ass. 38 (3): 247—248.

HEINICHEN, IRMGARD, G.
1969 Karyological Studies on Southern African Perissodactyla. J. S. Afr. vet. med. Ass. 40 (1): 99—100.

HSU, T. C. & K. BENIRSCHKE
1967 An Atlas of Mammalian Chromosomes. Vol. I. New York, Springer-Verlag.

KING, J. M., SHORT, R. V., MUTTON, D.E., & J. L. HAMERTON
1966 "The reproductive physiology of male zebra-horse and zebra-donkey hybrids" in "Comparative Biology of Reproduction in Mammals" Ed. I. W. Rowlands New York & London, Academic Press.

MUTTON, D. E., KING, J. M., & J. L. HAMERTON
1964 Chromosome studies in the genus *Equus*. Mammalian Chromosomes Newsletter 13: 7.

SHORT, R. V.
1967 cit. Ansell.

THENIUS, E.
1966 Die Vorgeschichte der Einhufer. Z. Säugetierkunde 31 (2): 15—171.

TRUJILLO, J. M., STENIUS, C., CHRISTIAN, L. C. & S. OHNO
1962 Chromosomes of the horse, the donkey and the mule. Chromosoma. 13: 243—248.