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# KARYOLOGICAL STUDIES ON SOUTHERN AFRICAN PERISSODACTYLA*

by

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## *Introduction*

### A. *Historical considerations leading to karyotyping*

Although the term "cell" as a descriptive term for the units composing cork was introduced by Hooke in 1665, cytology as a subject was only established after the enunciation of the cell theory by Schleiden and Schwann (1838; 1839). The concept that cells arise by division of pre-existing cells was crystallized by Virchow (1858), but the details could only be worked out during the 'seventies and early eighties, leading to the independent announcements of Hertwig and Strassburger in 1884–1885 that the cell nucleus carried the physical basis of heredity, more particularly in the "chromatin" as Fleming had named the stainable part of the nucleus. He was one of the foremost in describing its behaviour during division, forming threads, splitting lengthwise, shortening and thickening into "chromosomes" (a term introduced by Waldeyer in 1888). Van Beneden in 1883 and Heuser in 1884 established that each of the longitudinal halves of a split chromosome passes into one of the daughter nuclei. During the ensuing years the former demonstrated that the chromosomes of the offspring are derived equally from both parents. Roux and Weissmann interpreted these discoveries in terms of their significance to heredity and evolution (Wilson, 1937; Swanson, 1960). Their full significance only became apparent after the rediscovery of Mendel's classical research on peas by De Vries, Von Tschermak and Correns in 1900. The linking of observations on chromosomal behaviour and hereditary mechanisms which followed may thus literally be said to constitute the science of "cytogenetics". The "rediscovery" of the giant salivary chromosomes of the Diptera greatly aided the understanding of chromosome structure and its relation to genetic effect (White, 1945).

In the interphase somatic cell, no chromosomes are visible as such. They exist in a despiralized, genetically active form of chromatin, namely euchromatin, which at that stage is less stainable by the usual histological

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methods; only scattered granules of heterochromatin, which is spiralized and genetically inactive, are histologically intensely stained. During the prophase of mitosis the chromosomes become visible. Contraction, due to coiling of the chromonemata, reaches a maximum during metaphase and anaphase, consequently metaphase and anaphase chromosomes are the most suitable for counting and for study of their morphology. In animals this applies mostly to chromosomes in the metaphase stage.

The number of chromosomes and their appearance in the different animal species occupied much of the attention of animal cytologists. By the middle 'forties some 1,500 (approximately 0.1 % of the total of known species) had had their chromosome numbers determined, according to an estimate by White (1945).

Makino (1951) listed the chomosome numbers of 2754 invertebrate and 563 vertebrate species. Of the Invertebrata most of the determinations had been done on the Arthropoda (2165) particularly the Insecta (1820). Comparatively less work had been done on the Vertebrata, namely on 563 species. Of these, the Mammalia topped the list, the chromosome number of 176 species, mainly rodents, having been investigated. Of the order Perissodactyla only the horse, donkey and the mule had been studied at that time. The chromosome numbers given for each of these varied greatly, not one of them being correct. This is not surprising, as the relatively high number of chromosomes of mammals and inherent technical problems of handling mammalian chromosomes made accurate determinations extremely difficult. Introduction of improved techniques (Tjio and Levan, 1956; Ford, Jacobs and Lajtha, 1958; Rothfels and Siminovitch, 1958; Nowell, 1960; Sandberg, Crosswhite and Gordy, 1960; Meighan and Stich, 1961) placed this aspect of cytogenetics on a sounder footing and greatly facilitated the work involved in counting chromosomes and studying their morphology.

During the second half of the previous century, concepts concerning evolution had greatly stimulated cytological research. The importance attached to gene mutations in the process of evolution and the realization that these occurred at submicroscopical level, dampened the enthusiasm for knowledge concerning chromosome numbers and morphology. Subsequently the realization of the significance of changes in gene sequence and thus of aberrations of chromosome morphology, once again focused attention on studies of this kind. The finding that certain clinical syndromes in man were linked with chromosomal aberrations intensified the interest of research workers, an interest which was further enhanced by the growing importance of radiation biology in a nuclear era. Karyotyping has again become extremely important, especially when a comparative study is undertaken on the species within a particular order. This is all the more so when there is a danger of some species becoming extinct. With reference to mammals, this importance has been exemplified by the recent publication of "An Atlas of Mammalian Chromosomes" by Hsu and Benirschke (1967). Annual additions to the Atlas are envisaged by

the authors in an attempt to keep it up to date. It is hoped that the present investigations on Southern African Perissodactyla will form an important contribution.

It is also hoped that this work might be of some use in resolving the taxonomic uncertainty prevalent in certain sections of the order Perissodactyla. Furthermore, it may lead to better understanding of karyotypic evolution amongst these animals and to the revelation of possible correlations between karyotypes and other characters used in systematics, be they anatomical, biochemical or behavioural (Levan, Fredga and Sandberg, 1964).

### B. *Sexual dimorphism of the nucleus: the sex chromatin and nuclear appendages*

The discovery of sexual differences visible as chromatic structures in the interphasic nuclei of somatic cells of females aroused considerable interest, both from the academic as well as from the practical aspect. Here would be an eminently useful and simple tool in screening for abnormalities of the sex chromosomes of animals, provided a prior survey had established the regularity of occurrence or absence of such structures in the various cell types of animals of a particular species and sex.

Two main types of chromatic structures have been found: a relatively small, heteropycnotic mass next to the cell membrane termed the sex chromatin (or Barr body after its discoverer) and nuclear appendages of various shapes attached to the nucleus of polymorphonuclear neutrophil leukocytes. Using the presence or absence of these to determine the genetic sex of an animal led to the terms "nuclear sexing" and "polymorphic sexing" respectively.

#### 1. *Nuclear sexing*

The Barr body, or sex chromatin. was first discovered by Barr and Bertram (1949) in the nucleus of neurons of the cat. Lyon (1962) postulated that this chromatin body represents the heteropycnotic, inactivated X-chromosome. The characteristic sex chromatin for females was found in primates and carnivores. In the Artiodactyla and Perissodactyla sexual dimorphism was seen in neuronal nuclei, but in other cell types the nuclear chromatin was too coarse and the Barr body therefore not clearly distinguishable (Moore, 1964). Sex chromatin was also found in cells of the vaginal mucosa of a female mule (Mukherjee and Sinha, 1964) and in cultured cells from a hybrid between a donkey and a zebra (Benirschke, Low, Sullivan and Carter, 1964). The cell types showing sex chromatin amongst the Perissodactyla were not available for these studies and investigations on the Barr body in these animals, therefore, were not possible.

#### 2. *Polymorphic sexing*

Nuclear appendages in a form resembling drumsticks occurring on the nuclei of some of the polymorphonuclear neutrophil leukocytes of women

were first described by Davidson and Smith (1954). They regarded the occurrence of these "drumsticks" as typical for females. Later research indicated that the "drumstick" represented the late replicating X-chromosome.

The morphology and occurrence of nuclear appendages were studied more closely. Kosenow and Scupin (1956) found that in man they could be classified into four types:

*A-type:* a solid nodule, connected with a thin filament to the nucleus. This is the typical drumstick (1.5 to 2.0 $\mu$ in diameter) and is supposed to occur only in the female.

*B-type:* a sessile nodule, attached to the nucleus without a filament. It has the same size as type A and could be a predecessor thereof (Böhme, 1962). It is also supposed to occur in females only.

*C-type:* intermediate forms, smaller nodules and filaments, occurring in both males and females.

*D-type:* a "racket"- or "ring"-shaped form. It is very rare and can be found in both males and females.

By counting the A, B and C types, disregarding the rare D types, and employing the formula $\frac{A+B}{C}$, Kosenow and Scupin (1956) found that accurate sexing was possible. Values of less than 0.4 indicated a male, and of more than 0.4 a female. On the same basis Böhme (1962) found a sexual difference for sheep, the critical distinguishing value being 0.1.

Clear polymorphic sex differences were found in horses by Porter (1957), Zoaralek (1959), Kraft (1960) and Reutsch, Brüschke and Schulz (1960).

As blood smears were readily obtainable, it was considered worthwhile to investigate the possibilities of polymorphic sexing on the wild perissodactyles occurring in southern Africa.

## C. *Systematics of the order Perissodactyla*

### 1. *General*

The oldest Perissodactyla, *Hyracotherium*, a typical prototype of this group was found in the Eocene in Europe and North America (Thenius, 1966). The classification, adapted from Ellerman and Morrison-Scott (1951), Haltenorth and Trense (1956) and Ansell (1967), of the living and recently extinct forms of "those hoofed mammals with the mid-line of the foot passing through the third toe" (Wood, 1941), is shown in Table 1.

The modern tapirs are considered to be the most primitive living Perissodactyla (Simpson, 1945). From the following quotation of Wood (1941) the relationship of the Rhinocerotidae and Equidae may be deduced: "Hyrachyidae, springing from a common stock close to the ancestry of the horse, represents an Eocene adaptive radiation of the ancestral or, more probably, near ancestral stem rhinoceros."

Table 1

*Classification of the Order Perissodactyla**

| | | | |
|---|---|---|---|
| *Suborder:* | | Ceratomorpha | Hippomorpha |
| *Superfamily:* | Tapirioidea | Rhinocerotoidea | Equoidea |
| *Family:* | Tapiridae | Rhinocerotidae | Equidae |
| | *Tapirus terrestris* Linn., 1766 | *Diceros bicornis* (Linn., 1758) | *Equus caballus* Linn., 1758 |
| | *T. indicus* Desmarest, 1819 | *D. b. bicornis* (Linn., 1758) | *E. przewalskii* Poliakov, 1881 |
| | *T. pinchaque* (Roulin, 1829) | *D. b. holmwoodi* (P. L. Sclater, 1893) | *E. hemionus* Pallas, 1758 |
| | *T. bairdi* (Gill, 1865) | *D. b. somaliensis* (Potocki, 1897) | *E. asinus* Linn., 1758<br>*E. quagga*† Gmelin, 1788 |
| | | *Ceratotherium simum* (Burchell, 1817) | *E. grevyi* Oustalet, 1882 |
| | | *C. s. simum* (Burchell, 1817) | *E. zebra* Linn., 1758 |
| | | *C. s. cottoni* (Lydekker, 1908) | *E. z. zebra* Linn., 1758<br>*E. z. hartmannae* Matschie, 1898 |
| | | *Rhinoceros unicornis* Linn., 1758 | *E. burchelli* (Gray, 1824)<br>*E. b. burchelli* (Gray, 1824) |
| | | *Rhinoceros sondaicus* Desmarest, 1822 | *E. b. antiquorum* H. Smith, 1841<br>*E. b. böhmi* Matschie, 1892 |
| | | *Dicerorhinus sumatrensis* (Fischer, 1814) | *E. b. crawshaii* de Winton, 1896<br>(= *selousi* Pocock, 1897) |

*Adapted from Ellerman and Morrison-Scott (1951), Haltenorth and Trense (1956), and Ansell (1967).
†= recently extinct.

2. *Main morphological distinctions between* Ceratotherium simum *and* Diceros bicornis *and between* Equus zebra *and* Equus burchelli.

To avoid possible misinterpretations, especially amongst the zebras, the main differences between the two rhinoceros and zebra species are given below:

| | *Diceros bicornis* | *Ceratotherium simum* |
|---|---|---|
| 1. Size: | Smaller | Larger |
| 2. Upper lip: | Lip more pointed and prehensile for browsing | Square lip for grazing |
| 3. Behaviour: | Very aggressive | Not aggressive |
| 4. Nuchal hump: | No nuchal hump visible (Ansell, 1967) | Pronounced nuchal hump visible when head is raised (Ansell, 1967) |
| | *Equus zebra* | *Equus burchelli* |
| 1. Size: | Smaller | Larger |
| 2. Dewlap on throat: | Present | Absent |
| 3. "Grid-iron" pattern on croup: | Present | Absent |
| 4. Stripes: | Do not meet on belly | Meet on belly |
| 5. Shadow-stripes: | Absent | Present |
| 6. Ears: | Long, donkey-like | Short, horse-like |
| 7. Tail: | Donkey-like; the tassel starting lower down the tail | Horse-like; the tassel starting nearer to the base of the tail |
| 8. Ground colour of face: | Lower part reddish-brown | All over white to yellow |

*Equus burchelli* resembles the horse to a greater extent than does *Equus zebra*. The latter is more asinine in appearance (Bourdelle, 1941).

### D. *Geographic distribution of Perissodactyla in southern Africa and taxonomic problems concerning species and subspecies of zebra.*

1. *Distribution of the White or Square-lipped Rhinoceros,* Ceratotherium simum simum *(Burchell,* 1817).

Shortly after the end of the 19th century, this species was almost extinct in southern Africa (Ansell, 1967). In 1800 the southern square-lipped rhinoceros was distributed almost throughout southern Africa, but 80 years later only a few were left in the Umfolozi Game Reserve

area in Zululand (Player and Feely, 1960). Since then, with the help of Major Vaughan-Kirby, then Game Conservator for Zululand, they were protected in this area. Numbers increased again, totalling six hundred in 1959 (Player and Feely, 1960). Of these, 86 were transferred to the Kruger National Park in 1965 to ensure a wider distribution (Anon., 1965a). Breeding units were also introduced into the Loskop Dam Nature Reserve (Bigalke, 1963) as well as other smaller reserves in the Transvaal and also to Swaziland (Anon., 1965b) and Rhodesia.

Several hundred of the northern white rhinoceros, *Ceratotherium simum cottoni*, (Lydekker, 1908) may have survived in Uganda, Sudan, Garamba National Park and the Congo. Further information regarding its status is very conflicting and doubtful (Ansell, 1967).

2. *Distribution of the Black or Hook-lipped Rhinoceros*, Diceros bicornis bicornis (*Linn.*, 1758).

Before Europeans entered the African Continent the black rhinoceros was distributed almost throughout Africa. Today their numbers have decreased tremendously and only a few are found in South Africa, South West Africa, the Portuguese Territories, Rhodesia and Zambia. Further north, last strongholds exist in Tanzania and Kenya and only a few specimens remain in Ethiopia, Sudan, Uganda and the Congo (Sidney, 1965).

Several black rhinoceroses are found in three game reserves of South Africa, namely 180 to 200 animals in the Hluhluwe Game Reserve (P. Potter, according to personal communication to Sidney, 1965), 13 in the Umfolozi Game Reserve and about 50 in the Mkuzi Game Reserve (Sidney, 1965). In historic times, rhinoceroses also occurred in the Cape Province, but they are now extinct. Recently a pair have been reintroduced into the Addo Elephant National Park (Bigalke and Bateman, 1962).

According to Sidney (1965) over 200 black rhinoceroses are still living in the Kaokoveld and the Etosha Game Reserve. Gaerdes, however, in an article, circulated privately in 1967, stated that from counts made by the Chief Warden of South West Africa, Mr. B. de la Bat, only approximately 60 black rhinoceroses had been found in the Kaokoveld.

3. *Distribution of* Equus zebra *Linn.*, 1758.

The mountain zebra of today is found in the mountainous regions of the western coastal areas, from about 100 miles north of Mossamedes in Angola, through South West Africa to the southwestern and southern Cape Province (Ansell, 1967). This distribution is not a continuous one. The subspecies *Equus zebra zebra* Linn., 1758 is found mainly in the Mountain Zebra National Park in the Cape and a few animals inhabit adjacent regions. It is threatened by extinction; only some 70 to 80 individuals are still in existence (Bigalke, 1952). At the end of 1970 there were 114 individuals in the Mountain Zebra National Park (Ed.).

*Equus zebra hartmannae* Matschie, 1898, although still found roaming the coastal regions of Angola and South West Africa in some numbers, is at present being protected in a new park, the mountain farm "Naukluft", recently proclaimed in the southern part of South West Africa (Baxter, 1967).

4. *Distribution of* Equus burchelli *(Gray*, 1824).

In South Africa, they inhabit the southern savannah zone north of the Orange River. Northwards they formerly occurred in the southern savannah of eastern and northern Botswana. They still occur in northern South West Africa; southern and southeastern Angola; Rhodesia; Mozambique; Zambia; Malawi; southeastern Congo; Tanzania; Ruanda Urundi; southern Uganda; south western Kenya; parts of the Somali arid zone; northern Uganda and the southeastern Sudan (Ansell, 1967). According to Ansell, they are extinct in several of the above mentioned areas, reintroduced into some and present today in considerable numbers in some game reserves.

The distribution of the existing subspecies has not been resolved taxonomically. On the borders of their geographic habitats they are known to intergrade (Ansell, 1967), justifying a separation, if at all, at no higher than the subspecies level. It is generally accepted that the zebra from the Kruger National Park should be called *E. burchelli antiquorum* H. Smith, 1841 and the zebra occurring in central and northern Mozambique, eastern Rhodesia and parts of Zambia and Malawi *E. burchelli crawshaii* de Winton, 1896 (= *selousi* Pocock, 1897) according to Ansell (personal communication 1967, 1968). The former is also known as Chapman's zebra and the latter as the Selous zebra. In his personal communication of 1968, Ansell indicated that the subspecies name *crawshaii* should have priority over *selousi* and this subspecies is referred to as such in this work.

The taxonomic status of their South West African counterpart is uncertain. Ansell (personal communication, 1967) believes that either it is *E. burchelli burchelli* (Gray, 1824) or that *E. burchelli burchelli* is extinct and that, if a subspecies name be given, it should be either *E. burchelli kaokensis* (an end form of *E. burchelli antiquorum*), or *E. burchelli antiquorum*. Purely as a matter of convenience and to avoid confusion between the plains zebra of the Kruger National Park and those of South West Africa, the name *E. burchelli burchelli* will be used in this paper for the plains zebra of South West Africa.

The subspecies, *Equus burchelli antiquorum* H. Smith 1841, is at present distributed throughout the Transvaal, southern Mozambique, western Rhodesia and northern Botswana. Names like *chapmani* Layard, 1865; *wahlbergi* Pocock, 1897; *transvaalensis* Ewart, 1897; *pococki* Brasil and Pennetier, 1909; *kaufmanni* Matschie, 1912; and *kaokensis* Zukowsky, 1924; have been used. Ansell (1967) regards all these as synonyms for *E. burchelli antiquorum*.

## E. *Karyotypes of Perissodactyla*

### 1. *Family: Tapiridae*

Unfortunately no chromosome counts have yet been carried out on the only living genus, *Tapirus*, inhabiting South America and Asia. As they are not indigenous in southern Africa, and the three or four kept in captivity in South African zoos are too valuable to be used for experimental purposes and also because modern methods of immobilization are not absolutely safe, no material for study was available.

### 2. *Family: Rhinocerotidae*

No karyotypes of the Rhinocerotidae are given in "An Atlas of Mammalian Chromosomes" (Hsu and Benirschke, 1967). Recently, Hungerford, Chandra and Snyder (1967) published an article on the chromosomes of a female black rhinoceros, *Diceros bicornis*. Benirschke (personal communication, 1967) investigated the chromosomes of the Indian rhinoceros *Rhinoceros unicornis*, while a preliminary note on the white rhinoceros, *Ceratotherium simum simum* has also appeared (Heinichen, 1967). The northern white rhinoceros, *Ceratotherium simum cottoni*, has not yet been investigated. No studies have been made on the Javanese rhinoceros, *Rhinoceros sondaicus*, nor on the Sumatran rhinoceros, *Dicerorhinus sumatrensis*, of which the only captive specimen is in the Copenhagen zoo.

### 3. *Family: Equidae*

Relatively recent and reliable karyotype studies have been reported on the following species: *Equus caballus*, *E. przewalskii*, *E. hemionus*, *E. asinus* and *E. grevyi* as shown in Table 2.

Benirschke (1964) found the chromosome count of a hybrid between an *Equus asinus* stallion and possibly an *Equus zebra hartmannae* mare to be 48. Neither parent could be examined and only a photograph of the mare was available. Benirschke assumed that it was a specimen of *E. zebra hartmannae*, for which Hamerton (according to Benirschke, 1966) had found a chromosome number of 2n = 32. Since *E. asinus* has a diploid number of 62 chromosomes, it would have meant that *E. zebra hartmannae* had 34 chromosomes, in conflict with Hamerton's finding. Benirschke (1966) concluded that either the stallion or mare had an aberrant karyotype or that the mare was an *E. zebra zebra*, which had not then been investigated. The latter assumption is the one usually accepted in the literature (King, Short, Mutton and Hamerton, 1966; Short, 1967). No further investigation on *E. zebra zebra* has been undertaken until now.

For *E. zebra hartmannae* Benirschke and Malouf (1967) determined the diploid chromosome number as being 32.

Investigation of the chromosome number and karyotype of *E. zebra zebra* was thus an urgent necessity. For this reason it was undertaken, together with a study on *E. zebra hartmannae* for confirmatory and comparative purposes.

As regards *E. burchelli*, Benirschke, Brownhill and McFeely (1963)

Table 2

*Previously Published Chromosome Numbers of the Perissodactyla*

| *Family* | *Species* | *Subspecies* | *2n Chromosomes* | *Metacentric chromosome pairs* | *Acrocentric chromosome pairs* | *References* |
|---|---|---|---|---|---|---|
| Equidae | *E. przewalskii* (Przewalski's horse) | | 66 | 13 | 19 | Benirschke, Malouf and Low (1965) |
| | *E. caballus* (Domestic horse) | | 64 | 13 | 18 | Benirschke, Brownhill and Beath (1962); Trujillo, Stenius, Christian and Ohno (1962) |
| | *E. asinus* (Donkey) | | 62 | 19 | 11 | Trujillo, *et al.* (1962); Benirschke, *et al.* (1962); |
| | *E. hemionus* (Onager) | | 56 | 23 | 4 | Benirschke and Malouf (1967) |
| | *E. grevyi* (Grevy's zebra) | | 46 | 16 | 6 | Mutton, King and Hamerton (1964) |
| | *E. burchelli* | *E. b. antiquorum* | 44 | 18 | 3 | Eloff (1966); Benirschke and Malouf (1967) |
| | (Burchell's zebra) | *E. b. böhmi* | 44 | 18 | 3 | Benirschke, Brownhill and McFeely (1963) |
| | *E. zebra* (Mountain zebra) | *E. z. hartmannae* | 32 | 13 | 2 | Benirschke and Malouf (1967); Hamerton (according to Benirschke 1966) |
| Rhinocerotidae | *D. bicornis* (Black rhinoceros) | *D. b. bicornis* | 84 | 3* | 38 | Hungerford, Chandra and Snyder (1967) |
| | *R. unicornis* (Indian rhinoceros) | | 82 | 0 | 40 | Benirschke (personal communication, 1967) |

* = One metacentric pair has been accepted as representing the X-chromosome.

have studied the chromosomes of *E. burchelli böhmi* and reported the diploid number to be 44.

Eloff (1966) casually mentioned in the legend to a photograph of a chromosome spread that *E. burchelli antiquorum* has a diploid number of 44. The determination had been done on an animal from the Kruger National Park. Benirschke and Malouf (1967), also found the chromosome count to be 44 on one *E. b. antiquorum* specimen that was investigated. Benirschke (1967), in a personal communication, indicated that the Damara zebra—probably the plains zebra from South West Africa, and thus referred to as *E. burchelli burchelli* in this paper—has 2n = 42 chromosomes.

The chromosome numbers accepted until now have been summarized in Table 2.

To obtain greater certainty and clarity, investigations on as many subspecies of *E. burchelli* as possible had to be undertaken. It was also hoped to arrive at findings that would be of assistance to the taxonomist.

## *Methods*

### A. *Immobilization*

Most of the animals from national and private game reserves, farms or zoos, were chemically immobilized, making it unnecessary to kill them (King, Short, Mutton and Hamerton, 1966). In some cases an autopsy was required for other investigations and then the animals were shot. In these instances a more rapid collection of material was possible, since with immobilization some 10 to 15 minutes elapse before the animal becomes incapacitated. Immobilization in many instances was carried out with the morphine analogue, M-99 (Reckitt), which is the code name for the chemical compound 7-α-(1-R-hydroxy-1-methylbutyl)-6, 14-endoethenotetrahydro-oripavine hydrochloride, the central narcotic analgesic activity of which is 5,000 to 10,000 times greater than that of morphine (Pienaar, van Niekerk, Young and Van Wyk, 1966). M-99 has no "nerve-muscle-paralyzing" effect as do the succinyl choline compounds and is therefore safer to use (Harthoorn, 1966). The compound is still being used experimentally and accidents are still to be expected—it was found that some black rhinoceroses, which were to be transported to the Etosha Game Park, died when driven from mountainous regions in the Kaokoveld and immobilized thereafter.

More recently *Fentanyl and *Azaperone were used to immobilize rhinoceroses in the game parks of Natal. Fentanyl, (R4263-citrate) Janssen Pharmaceutica, 1-(2-phenethyl)-4-(N-propionyl-anilino)-piperidine, is a very potent, shortacting, analgesic and anaesthetic with morphium-like action. Azaperone, (R1929) Janssen Pharmaceutica, 1-(3-(4-fluoro-(benzoyl)-propyl)-4-(2-pyridil)-piperazine, is a neuroleptic agent which produces a typical state of catalepsy, characterized by the absence of voluntary movements and by the state of indifference to the environ-

ment, when administered in therapeutic doses. It antagonizes the respiratory depressant effect of morphium-like compounds and is a very potent antitraumatic shock agent. The only practical drawback to both these drugs is the relatively bigger dosage that is required.

Acetylpromazine was used as a tranquilizer, often with hyoscine hydrobromide as a potentiator. The substance or combination of substances was injected by means of dart syringes propelled by a "Cap Chur gun" or the "Van Rooyen Crossbow".

The method of immobilization used in any particular instance was dependent upon the preferences of the particular game reserve officials, who assisted in this work. Some of these methods were field trials of drugs or drug combinations. The white rhinoceroses, No. *C.s.s.* 1, 2 and 3 (see Material and Results), were immobilized by means of a mixture of 2 mg M-99, 3 mg acetylpromazine and 100 mg hyoscine hydrobromide made up in a solution of 2 ml total volume. During August and September 1968, these drugs were used in the following combinations, during experimental immobilizations carried out by Dr. M. E. Keep.

| *Animal number* | | | *Drugs used* | | | | |
|---|---|---|---|---|---|---|---|
| *White rhinoceroses* | *Sex* | *Age* | *M-99* | *Acetyl-promazine* | *Hyoscine* | *Fentanyl* | *Azapetrone* |
| *C.s.s.* 5 | ♂ | Adult | — | — | 100 mg | 30 mg | — |
| *C.s.s.* 6 | ♂ | Adult | — | — | 100 mg | 52 mg | 150 mg |
| *C.s.s.* 7 | ♂ | Adult | 1 mg | 2 mg | 100 mg | 20 mg | — |
| + injection of | | | ½ mg | 1 mg | — | — | — |
| *C.s.s.* 8 | ♀ | Adult | 1½ mg | 3 mg | 100 mg | — | — |
| First dart unsatisfactory effect, darted again with: | | | | | | | |
| | | | — | — | 75 mg | 20 mg | — |
| *C.s.s.* 9 | ♀ | Juvenile | — | — | 50 mg | 10 mg | — |
| *C.s.s.* 10 | ♀ | Adult | — | — | 100 mg | 30 mg | — |
| + injection of | | | — | — | 75 mg | 22 mg | 150 mg |
| *C.s.s.* 11 | ♀ | Adult | 1 mg | 2 mg | 100 mg | 20 mg | — |
| *C.s.s.* 12 | ♀ | Adult | 1½ mg | 3 mg | 100 mg | — | — |
| *C.s.s.* 13 | ♀ | Juvenile | ½ mg | 1 mg | 50 mg | — | — |
| *Black rhinoceroses* | | | | | | | |
| *D.b.b.* 1 | ♀ | Adult | 1½ mg | — | 100 mg | — | — |
| *D.b.b.* 2 | ♀ | Juvenile | 1 mg | — | 75 mg | — | — |

The *Equus zebra zebra* specimens were immobilized with a mixture of

*Information kindly supplied by Ethnor (Pty.) Ltd.

2 mg M-99 and 15 mg acetylpromazine injected intra-muscularly. Of *E. zebra hartmannae* one mare and her colt (No. *E.z.h.* 3 and 1) were immobilized by intramuscular injection of succynyl choline chloride (100, and 50 mg respectively). A stallion (No. *E.z.h.* 2) and a further three mares (No. *E.z.h.* 4, 5 and 6) were roped in a corral to which they had been confined after capture the previous day.

One adult *E. burchelli antiquorum* stallion (*E.b.a.* 2) in the Kruger National Park was immobilized by means of M-99 and acetylpromazine, at the same dosage level as for *E. zebra zebra*, while the three foals (*E.b a.* 1, 5 and 6) were simply tied down. Three animals (*E.b.a.* 3, 4 and 7) were shot, as were the four zebra in the Etosha Game Park, the three zebra obtained in the controlled hunting area of the Wankie Game Park and the two in the Gorongosa National Park.

### B. *Cytogenetic techniques*

As a preliminary trial, blood cultures of domestic animals were carried out, in which the original culture method of Moorhead, Nowell, Mellman, Battips and Hungerford (1960) was followed with a few adaptations by Eberle (personal communication, 1965). On our first expedition to Zululand, bone marrow as well as blood samples for cultures were collected, but it was found extremely difficult to collect sterile blood under field conditions. As contamination was found to be unavoidable, it was decided that these studies on wild animals were best done using the bone marrow technique, based on that of Sandberg, Crosswhite and Gordy (1960) with some adaptations (Gerneke, 1967).

Bone marrow from either rib or sternum was used. The most common dividing cells in bone marrow are the polychromatophilic erythroblasts and early normoblasts (Maximov and Bloom, 1957). Good metaphase spreads from these cells were used for the counting of chromosomes and for morphological studies.

#### *Collection of material in the field*

For collection of bone marrow specimens in the field, a stout wooden box was constructed, measuring 2 ft. 2 in. by 2 ft. by 9 in. with angle iron reinforcement around the base. To this a $\frac{3}{4}$ in. socket was welded at each corner and a similar piece was inserted into each angle of the unhinged portion of the lid. From $\frac{3}{4}$ in. piping six legs, two long and four shorter ones, were constructed by threading the one end and welding a spike at the other. A side projection was welded onto the junction between pipe and spike, so that the latter could be forced into the ground more easily by foot pressure. A strong metal projection screwed onto the box at one corner provided a firm support for a hand centrifuge. The box and lid were lined with strips of foam plastic, such as is used for insulation in the building trade. The box itself was subdivided by the same material into compartments of convenient size to contain all the bottles and equipment required. Because of this, transport over rough terrain was possible

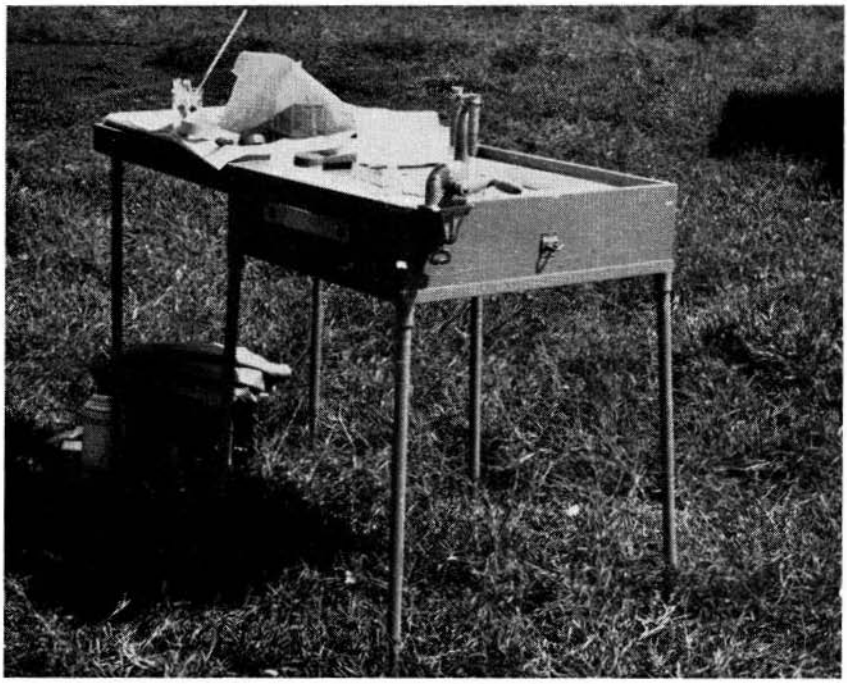


Plate 1. Portable work bench.

without breakage. When opened and with the legs screwed into position, the box provided a combined container, with everything readily available, and a work bench (Plate 1).

*(a) Collection of bone marrow*

In view of previous experience (Gerneke, 1967), all bone marrow specimens were collected between 6.00 a.m. and 12.00 a.m. Subsequent experiences as detailed on following pages suggested the advisability of darting animals without undue excitement and physical exertion on their part as well as of taking biopsy specimens immediately after immobilization. In the case of animals being shot, the specimens were taken within ten minutes after death.

In the case of zebras, bone marrow was aspirated from the caudal part of the sternum by inserting a two to three inch long, gauge 16 Salah sternal puncture needle into the red marrow of the sternebrae, withdrawing the plunger from the needle and applying suction after attaching a syringe. The syringe and needle were previously moistened by rinsing with some of the diluting solution (par. (b) 1) which increased suction and also acted as an anticoagulant. In those cases in which live zebras were merely roped and tied down, a local anaesthetic—1 ml Planocaine (Maybaker)—was injected subcutaneously, prior to performing the biopsy.

In the case of rhinos, it was initially considered too laborious and undesirable to roll the rhinoceroses onto their side. Consequently the bone marrow samples from the first eight white rhinoceroses (Nos. *C.s.s.* 1 to 5 and 8 to 10, as individually identified in the next chapter) were obtained from a rib, any one of the accessible sternal ribs being used. The failure to obtain bone marrow in this way from one female, No. *C.s.s.* 10, despite several attempts, led to the exploration of the sternum as an alternative route. This was found to be less difficult than anticipated and sampling from the last five white rhinoceroses and the two black ones was thus done on the sternum.

Since we only had needles as described above (suitable for relative thin skinned animals such as the domestic animals), it was necessary to inject 5 ml Planocaine subcutaneously and make an incision, about two inches long, through the skin to be able to reach the rib with the needle. The skin had a thickness of almost $1\frac{1}{2}$ inch, with one inch layer of muscle underneath. Considerable effort was needed to force the needle through the compact bone of the rib into the spongy bone underneath. The skin over the caudal part of the sternum was found to be much thinner, than over the rib. A much smaller incision was thus necessary, the only need for it being to overcome the toughness of this structure and thus the amount of force required when inserting the needle. However, there was little difference between rib and sternum in the density and thickness of the compact layer of bone. Biopsies were made as mentioned for the zebra and treated as described below. In the case of the zebra stallion (No. *E.b.c.* 1) collected at Wankie and the one (No. *E.b.c.* 3) in Gorongosa, scrapings were also made from the cut surface of the testis. The material was then subjected to the same treatment as that of aspirated bone marrow.

(*b*) *Treatment of aspirated bone marrow*

1. One to two ml of the bone marrow aspirate was added to 8 ml of a 0.6 per cent dextrose and 0.7 per cent sodium chloride solution and 1 ml of a 10 per cent potassium oxalate solution as anti-coagulant in a conical centrifuge tube (graduated up to 10 ml).

2. The bone marrow suspension was centrifuged at 350 r.p.m. for 5 minutes. Supernatant fluid was discarded and 10 ml of 0.44 per cent aqueous sodium citrate was added. Homogeneous dispersion of marrow cells was obtained by gentle manipulation with a Pasteur pipette.

3. The tubes with the hypotonic suspension were incubated in a water bath at 37° C for 15 minutes. Under field conditions this was improvised by carrying hot water in a Thermoflask and by continually replenishing the warm water in a beaker containing the tubes. The process of centrifugation was repeated to separate the now swollen cells. The supernatant fluid was again discarded.

4. Without disruption, the cell sediment was fixed in ± 5 ml of acetic methanol (1 ml glacial acetic acid + 3 ml methyl alcohol). The cells

were transported to the laboratory in this fixative without any detrimental effect, even after keeping them as long as one month. In fact, it was found that better preparations were obtained after one to two weeks' fixation.

*Procedure continued in the laboratory*

5. In the laboratory, the cells were centrifuged as before, the supernatant fluid was discarded, and 5 ml of a 45 per cent glacial acetic acid solution was added to the cells mixed gently and left in a refrigerator for $1\frac{1}{2}$ hours. Then the suspension was centrifuged once more and the supernatant fluid discarded again.

6. Sufficient acetic methanol fixative (see par. 4) was added to the sediment to give a moderately turbid cell suspension. Drop preparations were prepared with a Pasteur pipette, using clean glass slides, steeped in iced water. The cell suspension was allowed to drop onto the slide still covered by a thin film of cold water. Spreading of the cells is caused by the methanol-water reaction and is further aided by blowing on the slides. Rapid evaporation was obtained by intermittent gentle heating over a small flame and blowing.

7. When the slides were dry, they were placed in methyl alcohol for three minutes, transferred to 100 per cent May-Grünwald solution for three minutes, then to a 50 per cent May-Grünwald solution for three minutes and finally stained for two to three hours in a 10 per cent Giemsa water solution, alkalinized by addition of a few drops of ammonia. Slides were rinsed in running distilled water, cleaned underneath and rapidly dried in a warm air draught.

8. Any stain deposits were removed by dipping individual slides briefly into a jar of oil of cloves. The slides were then blotted dry and rinsed four times in xylol.

Spreads were selected under low power of the microscope and counted under oil immersion. Approximately 50 spreads were counted, or all, if less than 50 good spreads were available.

Mounting of preparations was found unnecessary. In case of fading, the staining procedure was repeated.

Suitable spreads were photographed using Agfa Isopan IFF (15 Din) film. Du Pont 9D, high contrast developer was used at 20° C for 5 minutes.

Prints were made on hard, mat paper. Karyograms were compiled by cutting out and pairing the homologous chromosomes. They were arranged in descending order of size, according to the Denver-system (Boök and 14 co-workers, 1960). In most cases the chromosomes were touched-up to reveal more clearly finer detail seen on focusing and not brought out in the photographs. The karyogram thus compiled was photographed again.

In classifying the chromosomes, the standardized nomenclature suggested by Levan, Fredga and Sandberg (1964), was followed according to which metacentric, submetacentric, subtelocentric and acrocentric chromosomes are recognized. As, however, the original binomial system

Plate 2 (1 and 2). Frontal and lateral facial views of *Ceratotherium simum simum*. (3 and 4). Frontal and lateral facial views of *Diceros bicornis bicornis*.

of metacentric—acrocentric, as described earlier by White (1945), is very convenient and popular but liable to confusion when used along with the Levan-Fredga-Sandberg system, the terms "meta-submetacentric" and "acro-subtelocentric" will be used whenever it is convenient to refer to two classes only.

(*c*) *Polymorphic sexing*

Blood smears were made from each animal, stained in 10 per cent Giemsa for ± one hour and thereafter examined under oil immersion for nuclear appendages of the polymorphonuclear leukocytes. Five hundred such cells were examined for each animal.

*Material and Results*

The details regarding material are more conveniently described under this heading.

A. *Ceratotherium simum simum* (Burchell, 1817)

1. *Material*

During February 1967 bone marrow and blood were collected and blood smears made from one male and one female white rhinoceros

(Nos. *C.s.s.* 1 and 2) in the Umfolozi Game Reserve, Zululand, Natal. On a subsequent expedition in April, bone marrow was collected from a male (No. *C.s.s.* 3). Bone marrow was also collected and blood smears made some two to there hours after death from a female (No. *C.s.s.* 4), killed by a train in the Sabie Sand Game Reserve.

Plate 2 (1 and 2).

Similar collections were made during August and September 1968 in an area adjoining the Umfolozi Game Reserve from two adult males and one juvenile male (Nos. *C.s.s.* 5 to 7) as well as from four adult females and two juvenile females (Nos. *C.s.s.* 8 to 13).

2. *Results*

(*a*) *Chromosome number and karyotype*

Due to technical difficulties initially experienced under field conditions, unsatisfactory spreads were obtained from rhinoceros Nos. *C.s.s.* 1 and 2. In the case of No. *C.s.s.* 4, too long a time had elapsed after death to obtain any cells at the metaphase stage. Despite several attempts, no bone marrow could be aspirated from the rib of animal No. *C.s.s.* 10. The poor results obtained from the bone marrow of cases Nos. *C.s.s.* 5, 6, 8 and 9 led to re-appraisal of the whole technique. In all these cases the animals had been chased a considerable distance before being darted. To test the effect of excitement and physical stress, a pilot test was done on a horse. The results of this test are given under section H at the end of this chapter.

Because other observations had to be made as well, anything from one-half to one hour had elapsed between the time the above-mentioned animals had come to a standstill and drawing of the sample. This also applied to animals Nos. *C.s.s.* 7 and 11, which had been chased only a couple of yards before being darted. In view of these experiences, every effort was made in the case of Nos. *C.s.s.* 12 and 13 to reduce excitement and physical exertion to a minimum before darting and to perform the biopsy immediately after immobilization. This was achieved in the case of No. *C.s.s.* 12, but in the case of No. *C.s.s.* 13 about three-quarters of an hour had elapsed between achievement of complete immobilization and performance of the biopsy.

The results of the chromosome counts and times of bone marrow collection are given in Table 3.

The karyotype is illustrated in Plate 3 by means of typical spreads (1 and 2) and karyograms (3 and 4). From Table 3 it is clear that the white rhinoceros has a chromosome count of $2n = 82$. As seen in the karyograms, Plate 3 (3 and 4), the autosomal chromosomes are all acro-subtelocentric, although numbers 6 and 8 could be submetacentric. The acro-subtelocentric chromosomes can be divided into two groups: Group A chromosomes 1–8: Subtelocentric; numbers 6 and 8 could also be placed under a submetacentric group. Group B chromosomes 9–40:

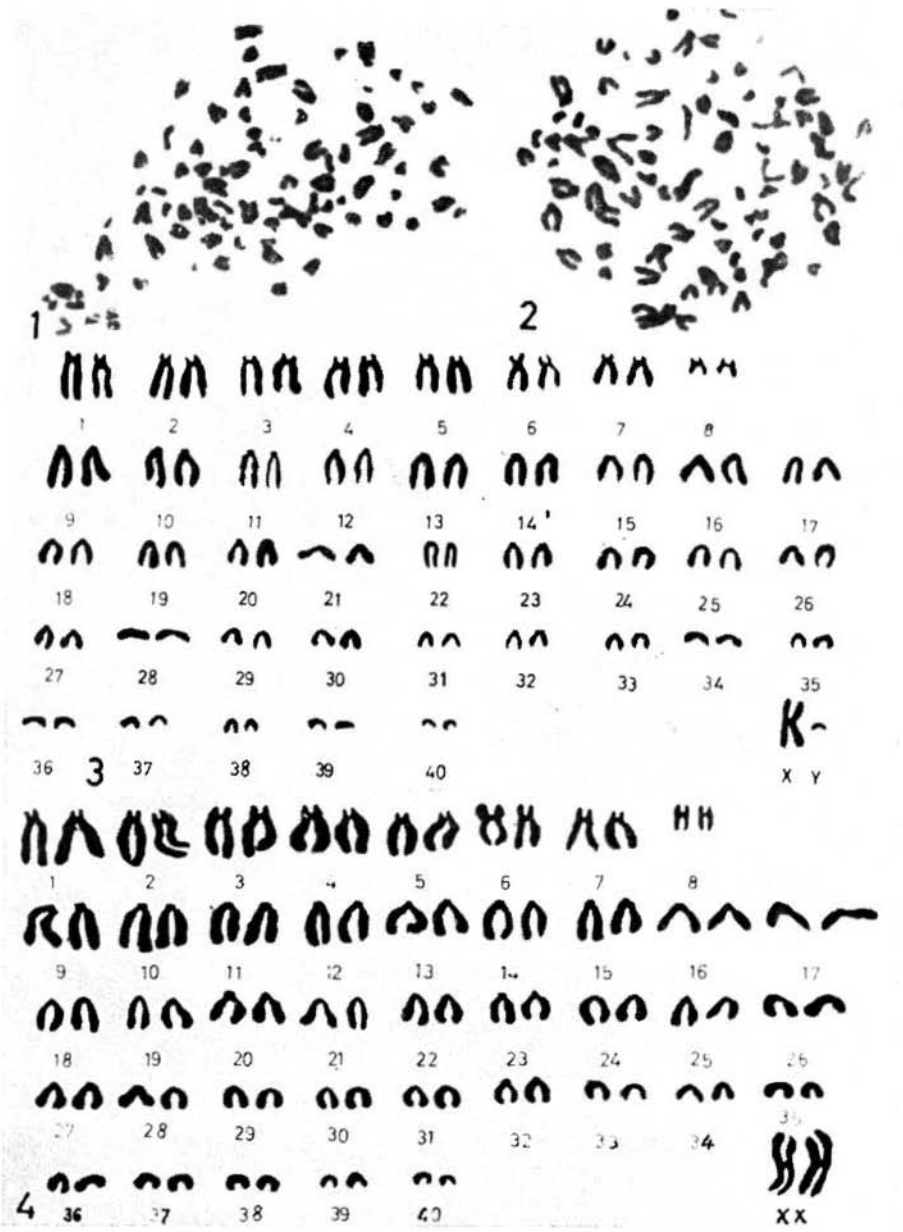



Plate 3. Mitotic chromosomes of *Ceratotherium simum simum*.
(1) Male (No. *C.s.s.* 3) and (2) female (No. *C.s.s.* 12) metaphase spread, ± × 1,200.
(3) Male karyogram, ± × 1,800 and (4) female karyogram, ± × 1,700

Table 3

Chromosome counts of *Ceratotherium simum simum.*

| *Animal number* | *Sex* | *Age* | *Time of collection* | *2n chromosome number* 79 | 80 | 81 | 82 | 83 | 84 | 85 | *Total No. of spreads counted* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *C.s.s.* 1 | ♂ | Adult | 8.30 a.m. | 0 | 0 | 1 | 3 | 2 | 0 | 0 | 6 |
| *C.s.s.* 3 | ♂ | Adult | 10.00 a.m. | 0 | 1 | 4 | 24 | 3 | 3 | 1 | 36 |
| *C.s.s.* 5 | ♂ | Juvenile | 8.45 a.m. | No mitoses found. | | | | | | | |
| *C.s.s.* 6 | ♂ | Adult | 12.40 p.m. | No mitoses found. | | | | | | | |
| *C.s.s.* 7 | ♂ | Adult | 9.20 a.m. | 0 | 1 | 0 | 3 | 0 | 0 | 0 | 4 |
| *C.s.s.* 2 | ♀ | Adult | 11.30 a.m. | 1 | 1 | 1 | 2 | 1 | 0 | 1 | 7 |
| *C.s.s.* 8 | ♀ | Adult | 9.45 a.m. | No mitoses found. | | | | | | | |
| *C.s.s.* 9 | ♀ | Juvenile | 11.00 a.m. | No mitoses found. | | | | | | | |
| *C.s.s.* 11 | ♀ | Adult | 11.30 a.m. | No mitoses found. | | | | | | | |
| *C.s.s.* 12 | ♀ | Adult | 9.45 a.m. | 1 | 1 | 4 | 36 | 5 | 2 | 1 | 50 |
| *C.s.s.* 13 | ♀ | Juvenile | 10.15 a.m. | No mitoses found. | | | | | | | |
| Total | | | | 2 | 4 | 10 | 68 | 11 | 5 | 3 | 103 |

Acrocentric; but numbers 9–15, 20 and 32 appear to be somewhat thickened at the one end, above the centromere.

The large X-chromosome is metacentric, while the Y is a small acrocentric chromosome. The sex-chromosomes resemble those of the Equidae very closely.

(*b*) *Polymorphic sexing*

Table 4

Polymorphic sexing of *Ceratotherium simum simum.*

| *Animal number* | *Sex* | *No. of cells without appendages* | *No. of appendages* *Type A* | *Type B* | *Type C* | *Type D* | *Total No. of cells counted* | $\frac{A + B^*}{C}$ |
|---|---|---|---|---|---|---|---|---|
| *C.s.s.* 1 | ♂ | 496 | 1 | 1 | 0 | 2 | 500 | ∞ |
| *C.s.s.* 5 | ♂ | 498 | 0 | 0 | 2 | 0 | 500 | 0.00 |
| *C.s.s.* 6 | ♂ | 499 | 0 | 0 | 1 | 0 | 500 | 0.00 |
| *C.s.s.* 7 | ♂ | 497 | 0 | 1 | 2 | 0 | 500 | 0.50 |
| *C.s.s.* 2 | ♀ | 474 | 17 | 3 | 5 | 1 | 500 | 4.00 |
| *C.s.s.* 4 | ♀ | 484 | 10 | 5 | 0 | 1 | 500 | ∞ |
| *C.s.s.* 8 | ♀ | 460 | 30 | 6 | 4 | 0 | 500 | 9.00 |
| *C.s.s.* 9 | ♀ | 461 | 25 | 8 | 4 | 2 | 500 | 8.25 |
| *C.s.s.* 11 | ♀ | 474 | 15 | 7 | 3 | 1 | 500 | 7.33 |
| *C.s.s.* 12 | ♀ | 479 | 13 | 4 | 1 | 3 | 500 | 17.00 |
| *C.s.s.* 13 | ♀ | 479 | 14 | 4 | 1 | 2 | 500 | 18.00 |

* = Formula according to Kosenow and Scupin (1956).

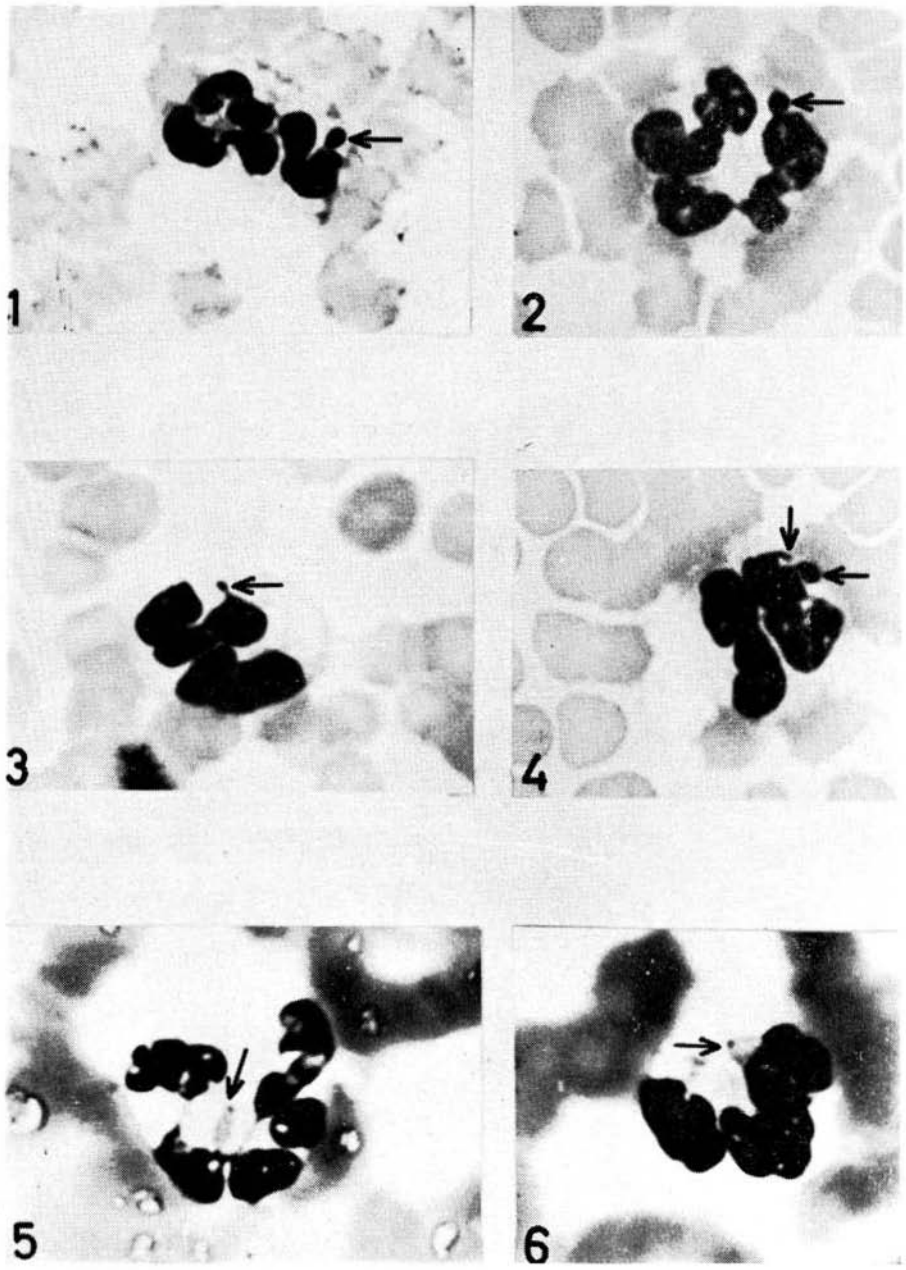



Plate 4. Appearance of drumsticks in Perissodactyla.
Zebra preparations. Arrow indicates the drumstick.
(1) No. *E.z.h.* 4 A-Type: a solid nodule, with filament.
(2) No. *E.b.a.* 6 B-Type: a sessile nodule, without filament.
(3) No. *E.b.b.* 2 C-Type: intermediate forms.
(4) No. *E.b.a.* 6 A- and C-Type: (5 and 6) No. *E.z.h.* 3 D-Type: (5) "rachet"-shaped form; (6) "ring"-shaped or sessile.

The results of the counts made of nuclear appendages occurring on neutrophils in blood smears are shown in Table 4. The various types of nuclear appendages are illustrated in Plate 4, which is a composite illustration, obtained from various zebra preparations, but is representative of all the animal species studied. No clear species differences were apparent as far as the morphology of the nuclear appendages is concerned.

D-forms found in these investigations resemble those illustrated by Gerneke (1965) for the hippopotamus. They had different sizes and were either sessile, Plate 4 (6), or, like the A-Type, connected to the nucleus with a thin filament, Plate 4 (5). Both drumsticks on these two figures have a black dot at the periphery.

Only one drumstick (nuclear appendage type A) was found among 500 neutrophils in the case of a male, whereas 10 to 30 were found in the females.

## B. *Diceros bicornis bicornis* (Linn., 1758).

Plate 2 (3 and 4).

### 1. *Material*

Blood smears were made and bone marrow collected from one adult female (No. *D.b.b.* 1) and one juvenile female (No. *D.b.b.* 2) during September 1968 in the Hluhluwe Game Reserve, Zululand, Natal. The two animals could only be immobilized at 1.30 p.m., so that the bone marrow biopsy on the adult and juvenile was taken at 2.15 p.m. and 3.15 p.m. respectively. These animals were cautiously stalked and the biopsy done immediately after immobilization. Unfortunately, no material from a male could be obtained.

### 2. *Results*

#### (*a*) *Chromosome number and karyotype*

No mitotic figures were evident in the adult female; in the juvenile several good chromosome spreads were found. Collection had been done on a cool, rainy day. The results of the counts are given in Table 5.

Table 5

Chromosome counts of *Diceros bicornis bicornis*.

| *Animal number* | *Sex* | *Age* | *Time of collection* | *2n chromosome number* 81 | 82 | 83 | 84 | 85 | 86 | 87 | *Total No. of spreads counted* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *D.b.b.* 1 | ♀ | Adult | 2.15 p.m. | No mitoses found. | | | | | | | |
| *D.b.b.* 2 | ♀ | Juvenile | 3.15 p.m. | 1 | 2 | 3 | 20 | 3 | 0 | 1 | 30 |
| Total | | | | 1 | 2 | 3 | 20 | 3 | 0 | 1 | 30 |

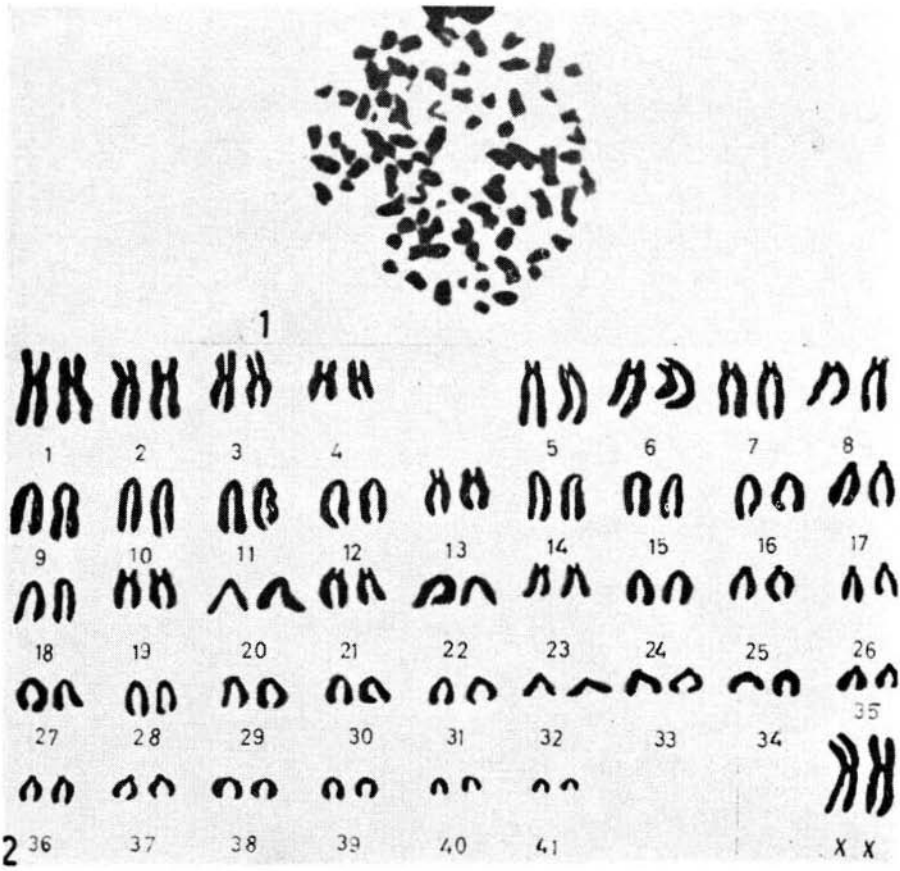



Plate 5. Mitotic chromosomes of *Diceros bicornis bicornis*.
(1) Female (No. *D.b.b.* 2) metaphase spread, ± × 1,200.
(2) Female karyogram, ± × 1,900.

A spread from the black rhinoceros female is illustrated in Plate 5 (1) and the karyogram in Plate 5 (2). From Table 5 it is seen that the black rhinoceros has a chromosome count of 2n = 84, the same as found recently in a female black rhinoceros investigated by Hungerford, Chandra and Snyder (1967).

As shown in the karyogram, Plate 5 (2), the autosomal chromosomes consist of one group being meta-submetacentric (1–4), and another group acro-subtelocentric (5–41).

Group A chromosomes 1–4: Meta-submetacentric; numbers 1 and 2 submetacentric and numbers 3 and 4 metacentric.

Group B chromosomes 5–41: Acro-subtelocentric; numbers 5 to 8, 13, 19, 21 and 22, subtelocentric and numbers 9 to 12, 14 to 18, 20 and 23

to 41 are all acrocentric with numbers 17, 25, 26, 33 and 35 to 37 appearing somewhat thickened at the one end, above the centromere.

Since no male material was obtained, the sex chromosomes could not definitely be identified. If, however, the chromosomes are compared with those from the white rhinoceros, it is seen that the one almost metacentric pair has the same size and morphology as the X-chromosomes of the white rhinoceros.

(*b*) *Polymorphic sexing*

The incidence of the nuclear appendages in neutrophils in blood smears of the females is shown in Table 6. Very likely the males will also have the same paucity of type A and type B appendages, as is the case in all the other species of this animal order.

Table 6

Polymorphic sexing of *Diceros bicornis bicornis*.

| *Animal number* | *Sex* | *No. of cells without appendages* | *No. of appendages* | | | | *Total No. of cells counted* | $\frac{A+B}{C}$ |
|---|---|---|---|---|---|---|---|---|
| | | | *Type A* | *Type B* | *Type C* | *Type D* | | |
| *D.b.b.* 1 | ♀ | 476 | 17 | 4 | 3 | 0 | 500 | 7.0 |
| *D.b.b.* 2 | ♀ | 482 | 13 | 3 | 2 | 0 | 500 | 8.0 |

C. *Equus zebra zebra* Linn., 1758.

Plate 6 (1, 2 and 3)

1. *Material*

During two expeditions to the Mountain Zebra National Park in March and April 1967, bone marrow was taken from three adult stallions (Nos. *E.z.z.* 1, 2 and 3) and one adult mare (No. *E.z.z.* 4).

The first expedition was an opportunistic attempt during the removal of a killer stallion. The animal (No. *E.z.z.* 1) used for specimen collection could only be obtained at 1.00 p.m. No blood smears were made. Consequently a second expedition had to be arranged specifically for the collection of material, which consisted of bone marrow and blood smears. Blood smears were also made from an adult mare (No. *E.z.z.* 5) from which no bone marrow could be obtained.

To avoid taxonomic confusion and possible misinterpretation, photographs were taken from each animal in frontal, lateral or latero-ventral and dorsal view. A typical example is shown in Plate 6 (1, 2 and 3). The only difference between the two subspecies of *E. zebra*, found during these studies, is that *E. z. hartmannae* Plate 6 (4, 5 and 6) is in general a little bigger than *E. z. zebra*.

2. *Results*

(*a*) *Chromosome number and karyotype*

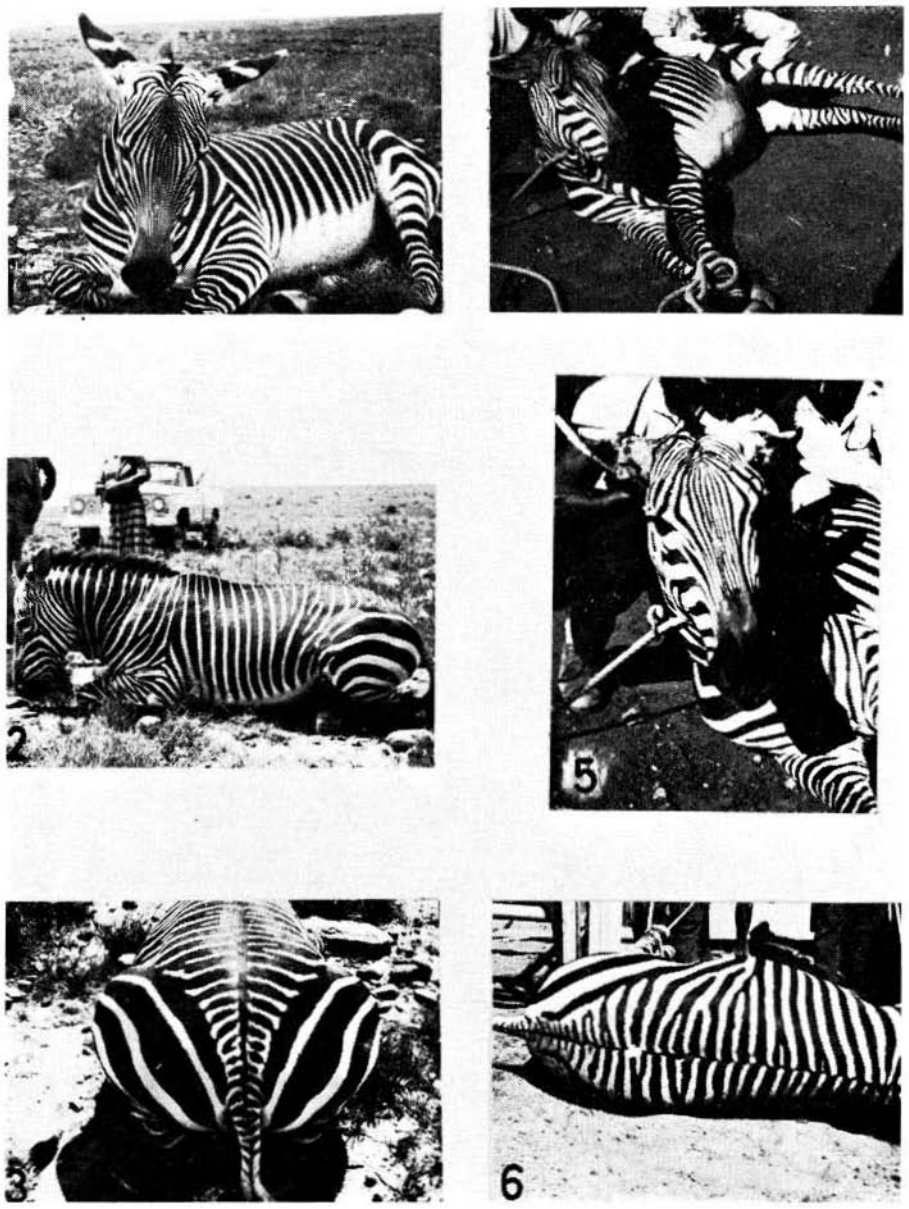



Plate 6. (1) Frontal and latero-ventral; (2) lateral and (3) dorsal views of *Equus zebra zebra*. (4) Frontal and latero-ventral; (5) frontal and (6) dorsal views of *Equus zebra hartmannae*.

Table 7

Chromosome counts of *Equus zebra zebra*.

| *Animal number* | *Sex* | *Age* | *Time of collection* | *2n chromosome number* 30 | 31 | 32 | 33 | 34 | *Total No. of spreads counted* |
|---|---|---|---|---|---|---|---|---|---|
| *E.z.z.* 1 | ♂ | *Adult* | 1.00 p.m. | No mitoses found. | | | | | |
| *E.z.z.* 2 | ♂ | *Adult* | 10.45 a.m. | 1 | 6 | 42 | 1 | 0 | 50 |
| *E.z.z.* 3 | ♂ | *Adult* | 8.30 a.m. | 1 | 5 | 46 | 0 | 0 | 52 |
| *E.z.z.* 4 | ♀ | *Adult* | 11.00 a.m. | 1 | 6 | 40 | 2 | 1 | 50 |
| Total | | | | 3 | 17 | 128 | 3 | 1 | 152 |

It will be noted from Table 7 that in the case of stallion No. *E.z.z.* 1 from which bone marrow was collected at 1.00 p.m., no mitoses could be found. A metaphase spread of cells from a male and a female *E.z. zebra* and a karyogram, produced by pairing off homologous chromosomes from these spreads, are shown in Plate 7 (1, 2, 3 and 4). *Equus zebra zebra* was found to have a diploid chromosome number of 32 (Table 7).

The chromosomes are divided into two groups:

Group A chromosomes 1–13: meta-submetacentric. (Nos. 1–3, 8, 11 and 13 are almost metacentric, while Nos. 4–7, 9, 10 and 12 are more submetacentric).

Group B chromosomes 14–15: acro-subtelocentric. No. 14 subtelocentric and No. 15 acrocentric.

The sex chromosomes comprise a large submetacentric X- and a small submetacentric Y-chromosome. The X-chromosome resembles that of the donkey, therefore differing slightly from that of the horse, which is more metacentric.

(*b*) *Polymorphic sexing*

The results of the counts of nuclear appendages are given in Table 8. Mare No. *E.z.z.* 5 had an exceptionally high drumstick count.

Table 8

Polymorphic sexing of *Equus zebra zebra*.

| *Animal number* | *Sex* | *No. of cells without appendages* | *No. of appendages* *Type A* | *Type B* | *Type C* | *Type D* | *Total No. of cells counted* | $\frac{A+B}{C}$ |
|---|---|---|---|---|---|---|---|---|
| *E.z.z.* 2 | ♂ | 500 | 0 | 0 | 0 | 0 | 500 | 0 |
| *E.z.z. 3* | ♂ | 498 | 1 | 1 | 0 | 0 | 500 | ∞ |
| *E.z.z.* 4 | ♀ | 473 | 23 | 3 | 0 | 1 | 500 | ∞ |
| *E.z.z.* 5 | ♀ | 444 | 36 | 13 | 5 | 0 | 500 | 9.8 |

D. *Equus zebra hartmannae* Matschie, 1898.

Plate 6 (4, 5 and 6).

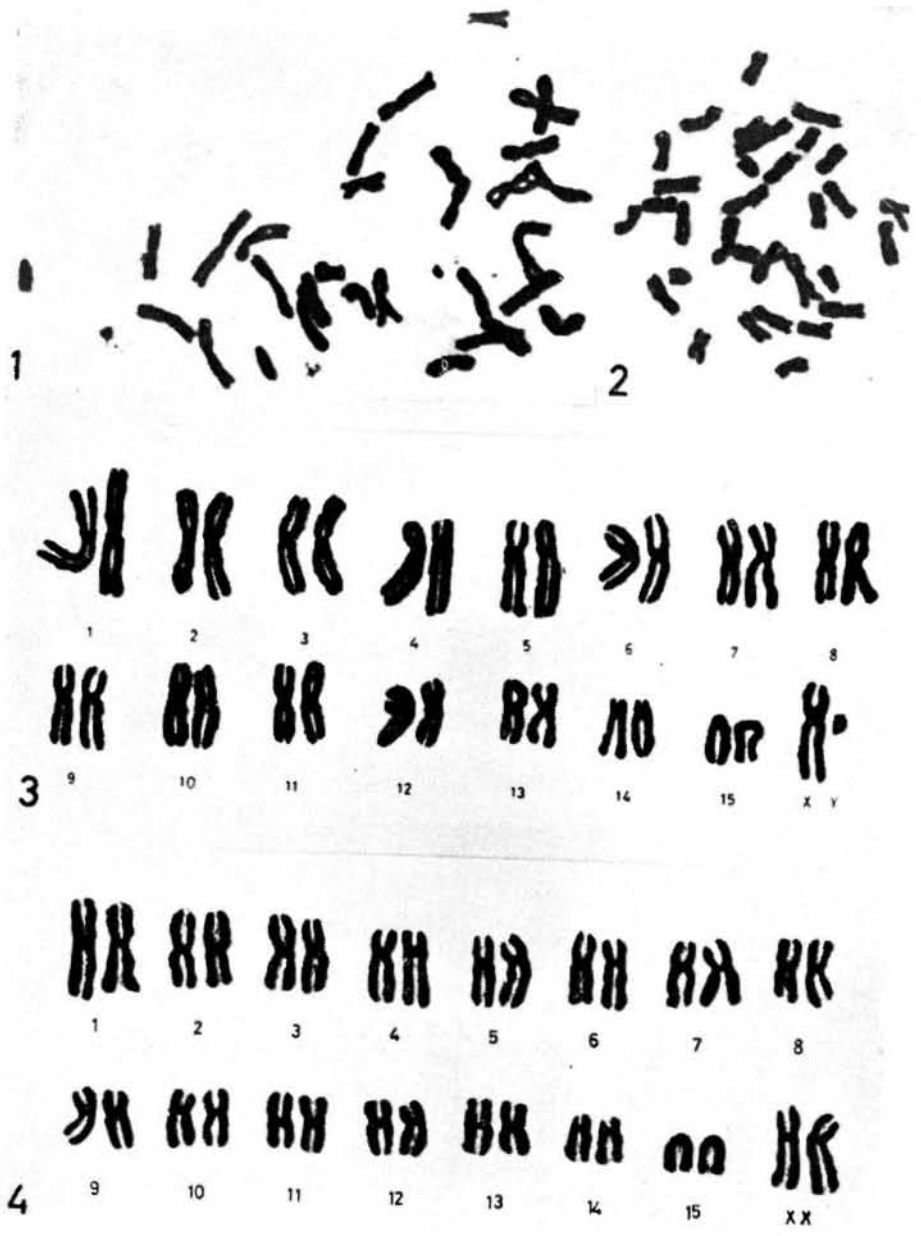



Plate 7. Mitotic chromosomes of *Equus zebra zebra*.

(1) Male (No. *E.z.z.* 2), and (2) female (No. *E.z.z.* 4), metaphase spread, ± × 1,200.
(3) Male karyogram, ± × 2,500 and (4) female karyogram ± × 2,200.


Plate 8. Mitotic chromosomes of *Equus zebra hartmannae*.

(1) Male (No. *E.z.h.* 2), and (2) female (No. *E.z.h.* 5), metaphase spread ± × 1,200.
(3) Male karyogram, ± × 2,200.
(4) Female karyogram, ± × 2,200.

1. *Material*

Bone marrow specimens and blood smears were obtained from a colt (No. *E.z.h.* 1) and a stallion (No. *E.z.h.* 2) and four mares (Nos. *E.z.h.* 3, 4, 5 and 6) on two neighbouring game farms in the vicinity of Windhoek, South West Africa.

2. *Results*

(*a*) *Chromosome number and karyotype*

The results of the chromosome counts are shown in Table 9, from which it is clear that the diploid number of chromosomes in the Hartmann zebra is 32.

Table 9

Chromosome counts of *Equus zebra hartmannae.*

| *Animal number* | *Sex* | *Age* | *Time of collection* | *2n chromosome number* | | | | | *Total No. of spreads counted* |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 30 | 31 | 32 | 33 | 34 | |
| *E.z.h.* 1 | ♂ | ± 1 year | 10.45 a.m. | 0 | 2 | 12 | 1 | 1 | 16 |
| *E.z.h.* 2 | ♂ | ± 2 years | 10.00 a.m. | 1 | 4 | 44 | 2 | 0 | 51 |
| *E.z.h.* 3 | ♀ | ± 5 years | 11.15 a.m. | 0 | 3 | 21 | 2 | 0 | 26 |
| *E.z.h.* 4 | ♀ | ± 3 years | 9.45 a.m. | 0 | 0 | 7 | 0 | 1 | 8 |
| *E.z.h.* 5 | ♀ | ± 7 years | 11.00 a.m. | 0 | 0 | 12 | 1 | 2 | 15 |
| *E.z.h.* 6 | ♀ | ± 1 year | 11.30 a.m. | All mitotic figures in anaphase stage. | | | | | |
| Total | | | | 1 | 9 | 96 | 4 | 4 | 116 |

On visual inspection the morphology of the chromosomes, Plate 8 (1 and 2), was found to be identical to that of *E.z.zebra*, Plate 7 (1 and 2) and thus the karyotypes, Plate 7 and 8 (3 and 4), are identical.

In a number of spreads from zebra No. *E.z.h.* 2, the mitotic figures were already in the anaphase stage, yet one chromosome was still clearly seen to be in the metaphase stage. This late-separating chromosome was thus assumed to be the X-chromosome (Plate 9). By pairing off the chromosomes during construction of the karyograms, the identity of this chromosome as the X-chromosome was confirmed.

(*b*) *Polymorphic sexing*

The results of the counts for nuclear appendages have been compiled in Table 10. Clear sex differences exist. The second youngest mare zebra (No. *E.z.h.* 4) had an exceptionally high drumstick count (Table 10), while a five year old mare (No. *E.z.h.* 3), with a colt of about one year, had a number of degenerating vacuoles adjacent to the neutrophil nuclei, of the D-type described previously.

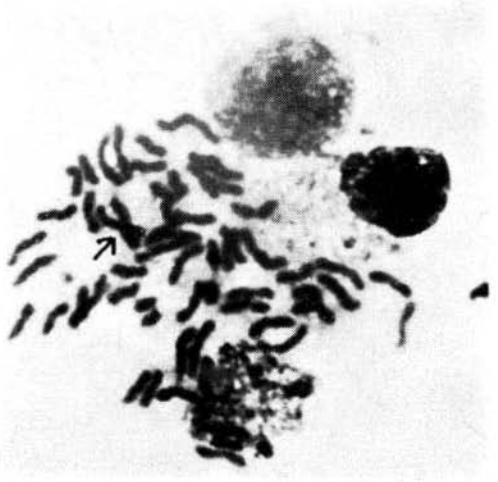


Plate 9. Mitotic early anaphase spread of *Equus zebra hartmannae*. Arrow indicates late-separating chromosome.

Table 10

Polymorphic sexing of *Equus zebra hartmannae*.

| *Animal number* | *Sex* | *No. of cells without appendages* | *No. of appendages* | | | | *Total No. of cells counted* | $\frac{A+B}{C}$ |
|---|---|---|---|---|---|---|---|---|
| | | | *Type A* | *Type B* | *Type C* | *Type D* | | |
| *E.z.h.* 1 | ♂ | 498 | 1 | 1 | 0 | 0 | 500 | $\infty$ |
| *E.z.h.* 2 | ♂ | 499 | 0 | 0 | 0 | 1 | 500 | 0 |
| *E.z.h.* 3 | ♀ | 480 | 13 | 3 | 1 | 3 | 500 | 16.0 |
| *E.z.h.* 4 | ♀ | 467 | 29 | 3 | 1 | 0 | 500 | 32.0 |
| *E.z.h.* 5 | ♀ | 484 | 13 | 2 | 1 | 0 | 500 | 15.0 |
| *E.z.h.* 6 | ♀ | 484 | 12 | 2 | 1 | 1 | 500 | 14.0 |

E. *Equus burchelli burchelli* (Gray, 1824).

Plate 10 (1, 2, 3 and 4).

Because of the uncertainty of the exact taxonomic status of subspecies of the plains zebra of South West Africa (see above) and in view of Benirschke's findings (see above), investigations on the karyotype of *E. burchelli* occurring in that region was undertaken (Heinichen, in press).

1. *Material*

Four zebras, two stallions (Nos. *E.b.b.* 1 and 2) and two mares (Nos. *E.b.b.* 3 and 4) were shot during a survey of diseases and parasitism of wild life in the Etosha Game Park in the northern part of S.W.A., towards the end of July, 1967. Bone marrow biopsies and blood smears were

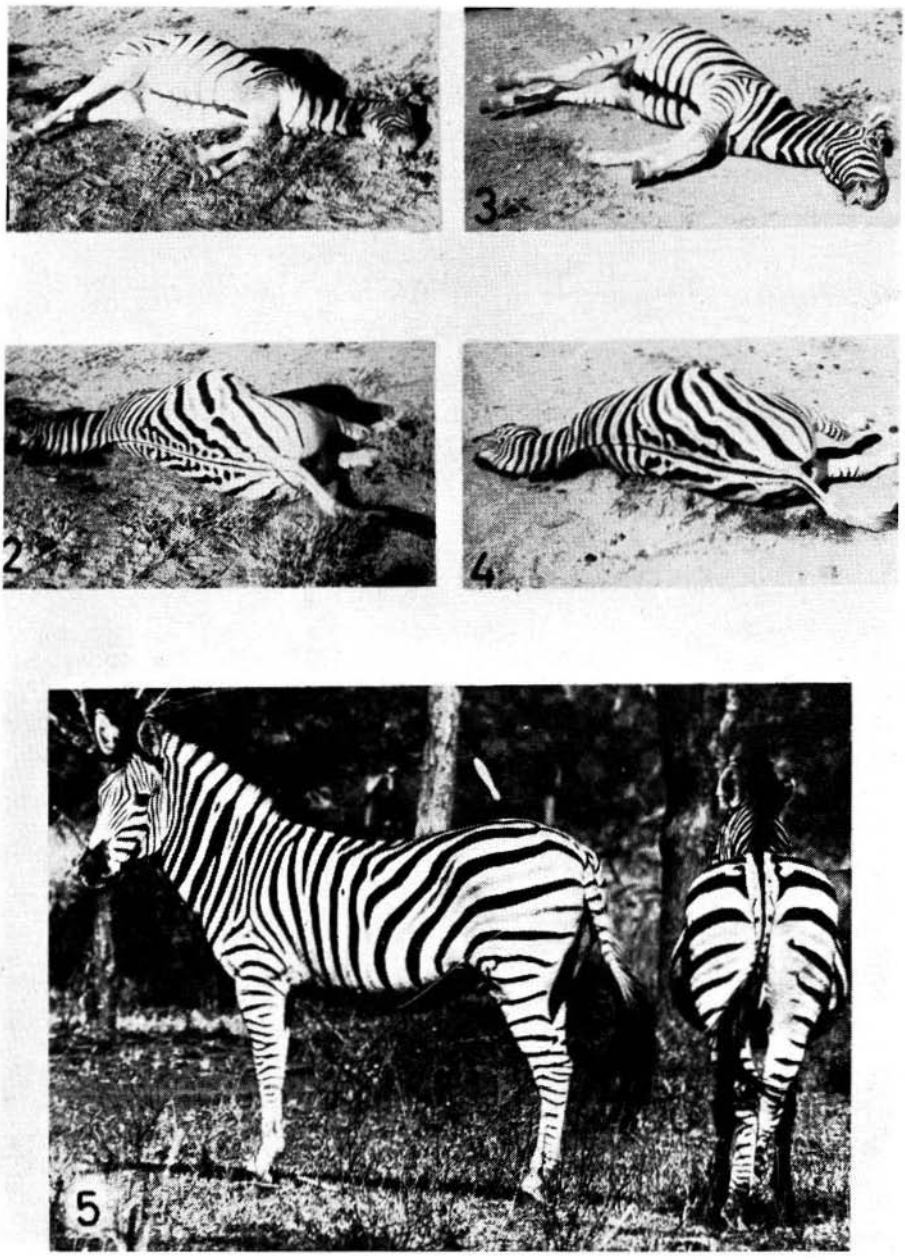



Plate 10. (1 and 2). Ventral and dorsal views of lightly striped specimen of *Equus burchelli burchelli* female.

(3 and 4) Ventral and dorsal views of darkly striped specimen of *Equus burchelli burchelli* male.

(5) Lateral and caudal views of *Equus burchelli antiquorum*.

collected immediately after death. For reference purposes the two extremes of markings found in these four animals are illustrated on Plate 10 (1, 2, 3 and 4).

2. *Results*

(*a*) *Chromosome number and karyotype*

The results of the chromosome counts are listed in Table 11. The diploid chromosome number of 44 was found for this subspecies.

Table 11

Chromosome counts of *Equus burchelli burchelli.*

| *Animal number* | *Sex* | *Age* | *Time of collection* | *2n chromosome number* 42 | 43 | 44 | 45 | 46 | *Total No. of spreads counted* |
|---|---|---|---|---|---|---|---|---|---|
| *E.b.b.* 1 | ♂ | ± 5 years | 9.00 a.m. | 2 | 8 | 37 | 2 | 1 | 50 |
| *E.b.b.* 2 | ♂ | ± 9 years | 10.00 a.m. | 3 | 5 | 39 | 4 | 0 | 51 |
| *E.b.b.* 3 | ♀ | ± 8 years | 8.00 a.m. | No mitoses found. | | | | | |
| *E.b.b.* 4 | ♀ | ± 8 years | 9.15 a.m. | 2 | 5 | 22 | 3 | 1 | 33 |
| Total | | | | 7 | 18 | 98 | 9 | 2 | 134 |

As shown in the karyograms, Plate 11 (3 and 4), chromosomes can be classified into two groups:
Group A chromosomes 1–18: meta-submetacentric (Nos. 1–4, 7, 10, 11, 13, 14 and 17 have approximately submedian centromeres, whereas 5, 6, 8, 9, 12, 15, 16 and 18 have median ones).
Group B chromosomes 19–21: all, one large and two smaller pairs are acrocentric.

Both the X- and the small Y-chromosome are metacentric. The X-chromosome resembles that of the horse very closely.

(*b*) *Polymorphic sexing*

The results of the counts of nuclear appendages of the polymorphonuclear neutrophil leukocytes are given in Table 12. As before, the sex difference is distinct.

Table 12

Polymorphic sexing of *Equus burchelli burchelli.*

| *Animal number* | *Sex* | *No. of cells without appendages* | *No. of appendages* *Type A* | *Type B* | *Type C* | *Type D* | *Total No. of cells counted* | $\frac{A+B}{C}$ |
|---|---|---|---|---|---|---|---|---|
| *E.b.b.* 1 | ♂ | 500 | 0 | 0 | 0 | 0 | 500 | 0 |
| *E.b.b.* 2 | ♂ | 497 | 1 | 0 | 2 | 1 | 500 | 0.5 |
| *E.b.b.* 3 | ♀ | 480 | 16 | 3 | 1 | 0 | 500 | 19.0 |
| *E.b.b.* 4 | ♀ | 479 | 16 | 3 | 1 | 1 | 500 | 19.0 |

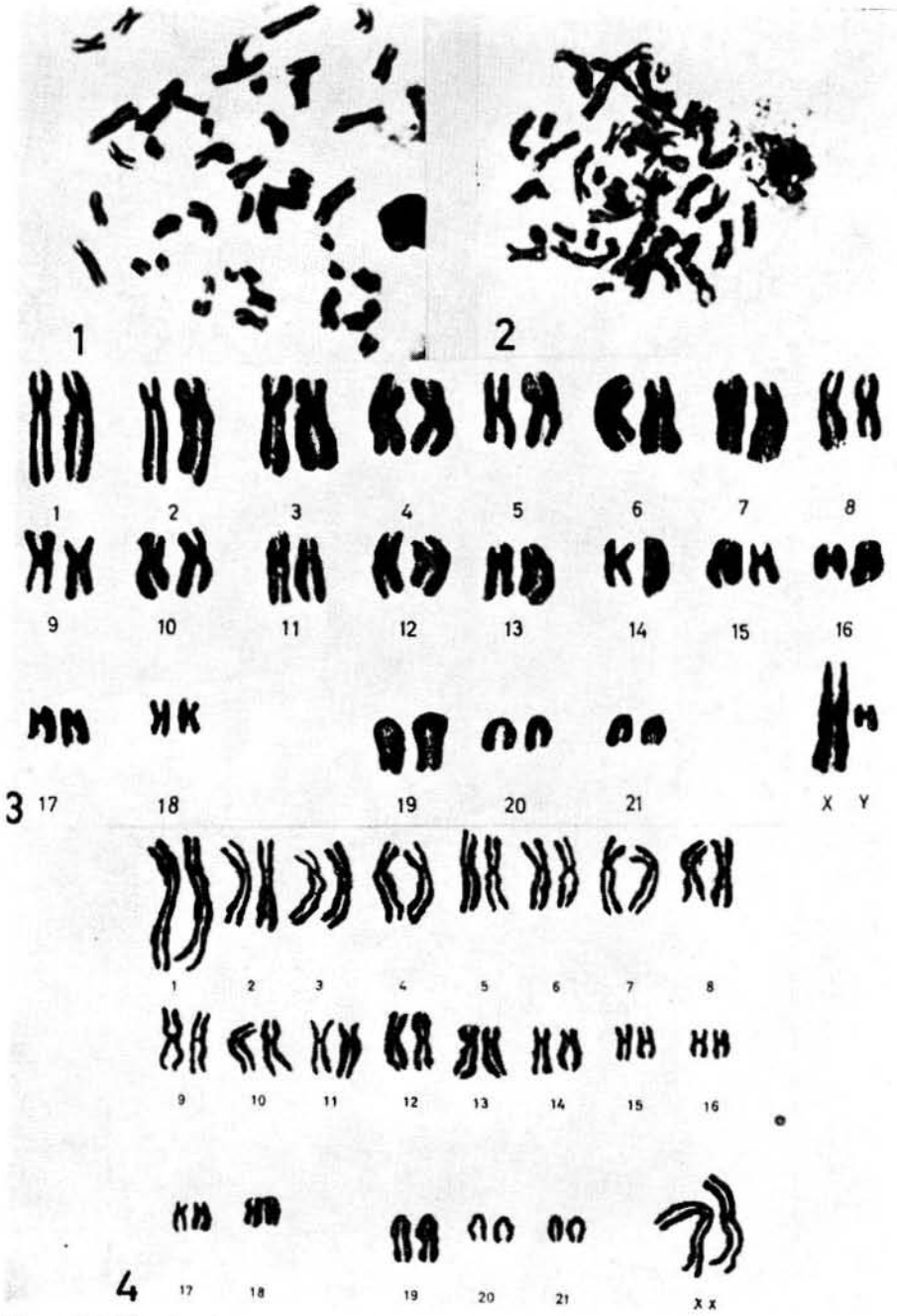



Plate 11. Mitotic chromosomes of *Equus burchelli burchelli*.

(1) Male (No. *E.b.b.* 1), and (2) female (No. *E.b.b.* 4), metaphase spread, ± × 1,200.

(3) Male karyogram, ± × 1,600 and

(4) female karyogram, ± × 1,600.

F. *Equus burchelli antiquorum* H. Smith, 1841.

Plate 10 (5).

1. *Material*

During an expedition to the Kruger National Park in April 1967, bone marrow biopsies were done on a colt and three stallions (Nos. *E.b.a.* 1, 2, 3 and 4, respectively) and two fillies and one mare (Nos. *E.b.a.* 5, 6 and 7, respectively). Blood smears were collected from Nos. *E.b.a.* 1, 3, 4, 6 and 7. As stated earlier, the three foals had been tied down, stallion No. *E.b.a.* 2 had been chemically immobilized, while stallions Nos. *E.b.a.* 3 and 4 and the mare No. *E.b.a.* 7, had been shot during operations for the artificial reduction of the overcrowded zebra population.

2. *Results*

(*a*) *Chromosome number and karyotype*

The chromosome number was found to be 2n = 44 (Table 13). It is noteworthy that the chromosomes from the three foals were smaller than those of the adult animals. The same also applies to the marrow cells in general.

Table 13

Chromosome number of *Equus burchelli antiquorum*.

| *Animal number* | *Sex* | *Age* | *Time of collection* | *2n chromosome number* 42 | 43 | 44 | 45 | 46 | *Total No. of spreads counted* |
|---|---|---|---|---|---|---|---|---|---|
| *E.b.a.* 1 | ♂ | Foal | 9.45 a.m. | 1 | 1 | 42 | 4 | 2 | 50 |
| *E.b.a.* 2 | ♂ | ± 2 years | 11.00 a.m. | 1 | 6 | 39 | 4 | 1 | 51 |
| *E.b.a.* 3 | ♂ | ± 5 years | 6.00 a.m. | 1 | 5 | 57 | 4 | 1 | 68 |
| *E.b.a.* 4 | ♂ | ± 4 years | 6.30 a.m. | 3 | 6 | 32 | 1 | 0 | 45 |
| *E.b.a.* 5 | ♀ | Foal | 10.45 a.m. | 2 | 2 | 41 | 5 | 0 | 50 |
| *E.b.a.* 6 | ♀ | Foal | 11.15 a.m. | 2 | 5 | 38 | 3 | 0 | 48 |
| *E.b.a.* 7 | ♀ | ± 2 years | 10.30 a.m. | 2 | 6 | 42 | 2 | 1 | 53 |
| Total | | | | 12 | 31 | 291 | 23 | 5 | 365 |

These chromosomes were found to be morphologically indistinguishable from those of the other subspecies, *Equus burchelli burchelli*, as will be noted on comparing the microphotographs of the two spreads and the two karyograms illustrated in Plate 11 (1, 2, 3 and 4) with those of Plate 12 (1, 2, 3 and 4).

(*b*) *Polymorphic sexing*

The results of sex determination, according to counts of nuclear appendages, are shown in Table 14. As before, there was a distinct sex difference.

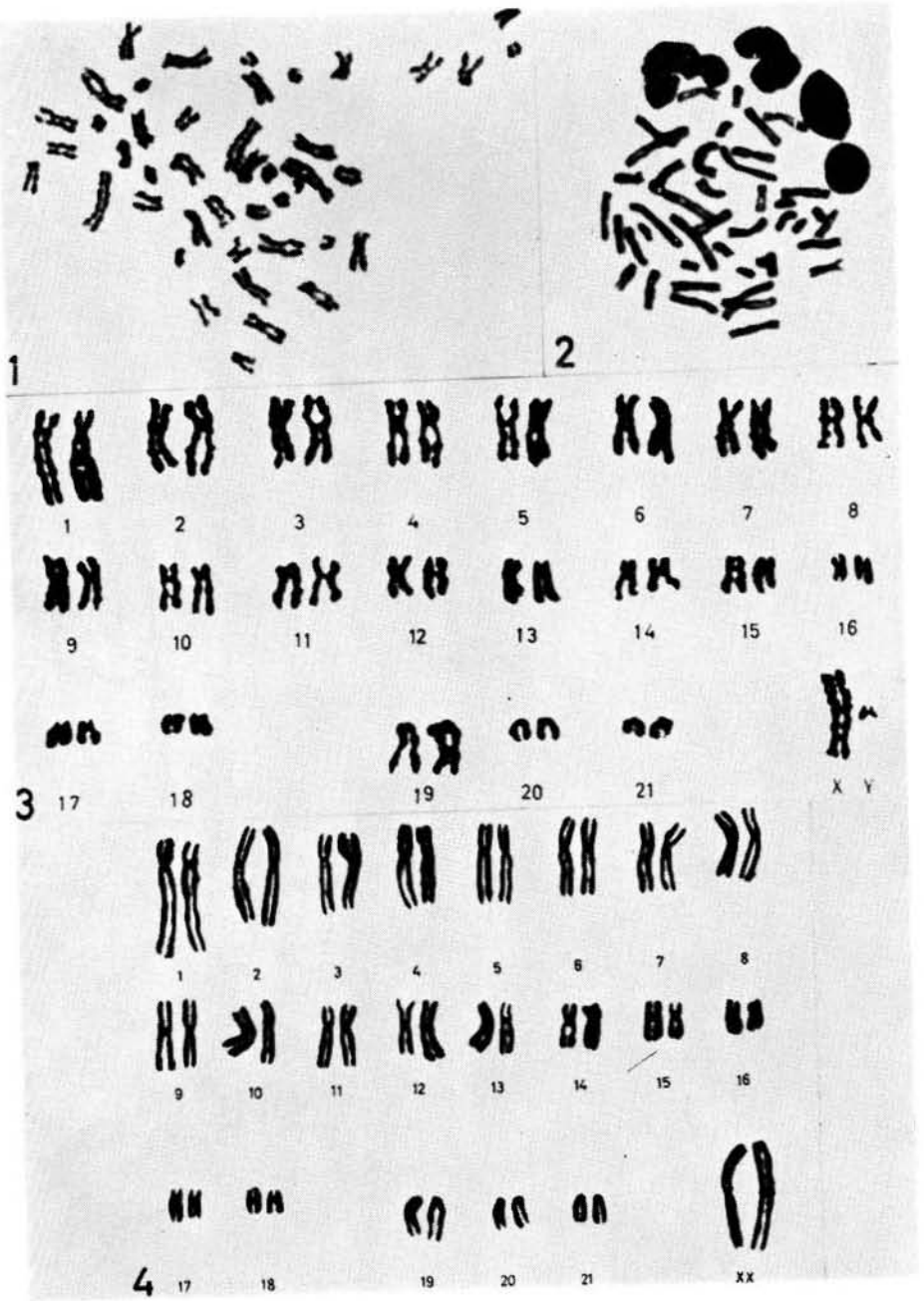



Plate 12. Mitotic chromosomes of *Equus burchelli antiquorum*.

(1) Male (No. *E.b.a.* 2), and (2) female (No. *E.b.a.* 7), metaphase spread, ± × 1,200.

(3) Male karyogram, ± × 2,000, and (4) female karyogram, ± × 2,000.

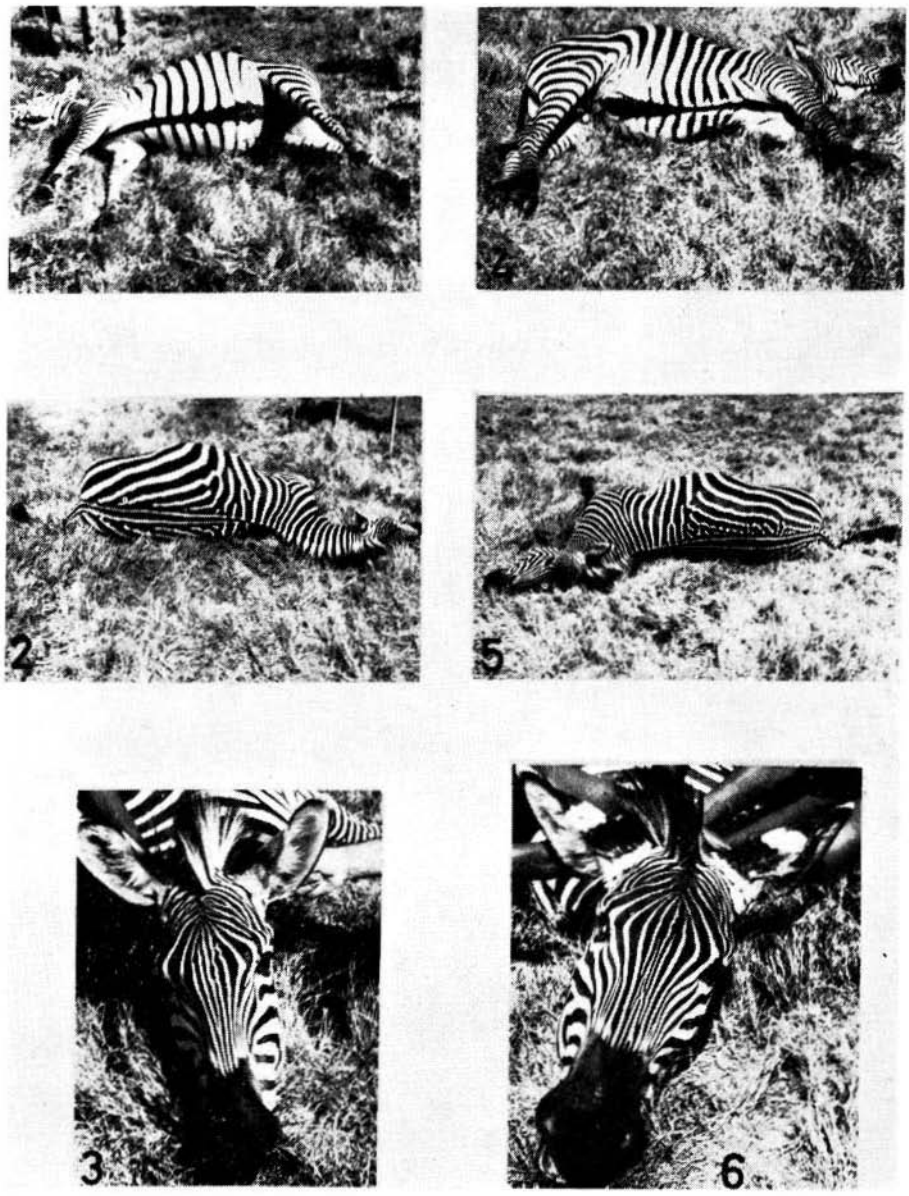


Plate 13. (1, 2 and 3).Ventral, dorsal and frontal views of *Equus burchelli crawshaii* female from Gorongosa.
(4, 5 and 6). Ventral, dorsal and frontal views of *Equus burchelli crawshaii* male from Gorongosa.

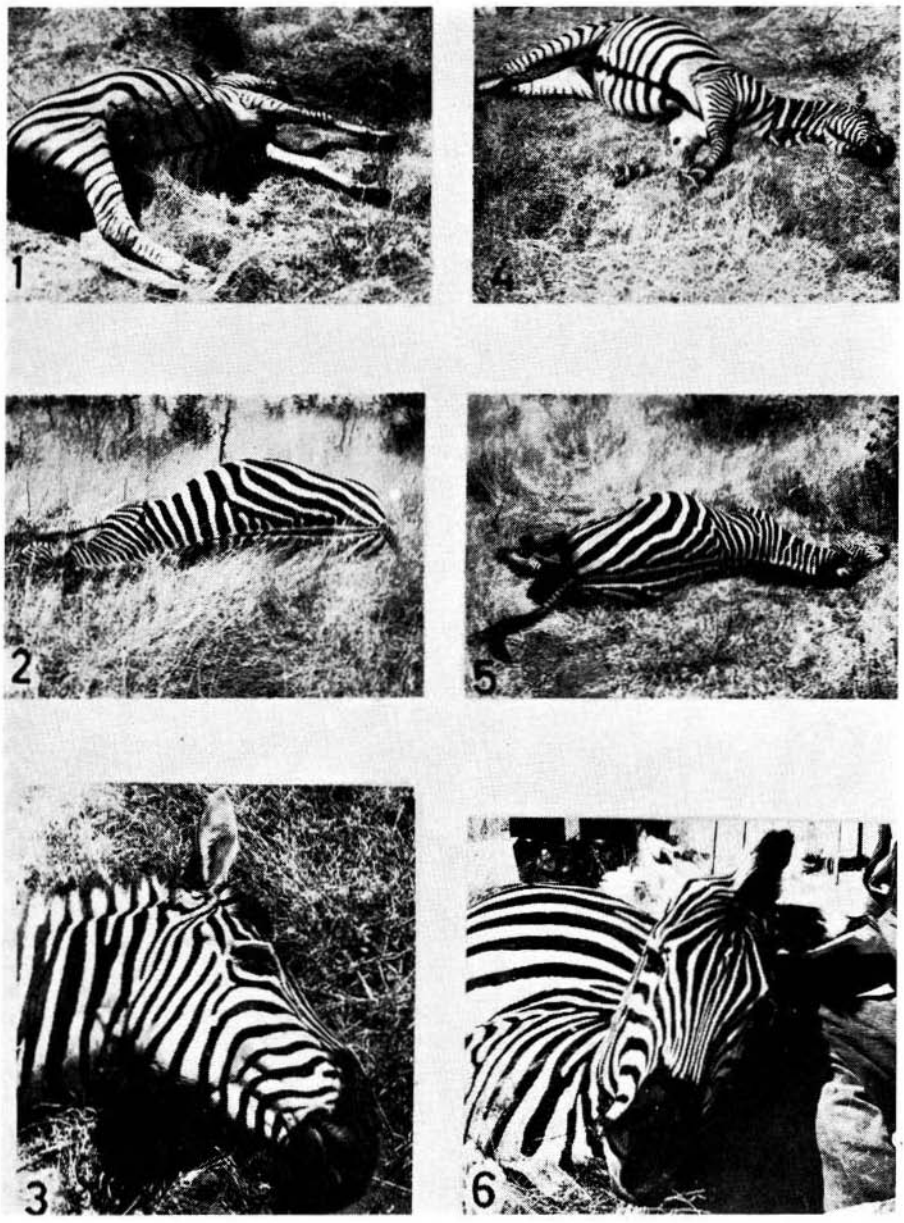



Plate 14. (1, 2 and 3). Ventral, dorsal and lateral facial views of *Equus burchelli* female from the Wankie area.
(4, 5 and 6). Ventral, dorsal and frontal views of *Equus burchelli* male from the Wankie area.

Table 14

Polymorphic sexing of *Equus burchelli antiquorum*.

| *Animal number* | *Sex* | *No. of cells without appendages* | *No. of appendages* | | | | *Total No. of cells counted* | $\frac{A+B}{C}$ |
|---|---|---|---|---|---|---|---|---|
| | | | *Type A* | *Type B* | *Type C* | *Type D* | | |
| *E.b.a.* 1 | ♂ | 500 | 0 | 0 | 0 | 0 | 500 | 0 |
| *E.b.a.* 3 | ♂ | 500 | 0 | 0 | 0 | 0 | 500 | 0 |
| *E.b.a.* 4 | ♂ | 498 | 2 | 0 | 0 | 0 | 500 | ∞ |
| *E.b.a.* 6 | ♀ | 459 | 29 | 9 | 2 | 1 | 500 | 19.0 |
| *E.b.a.* 7 | ♀ | 481 | 14 | 4 | 1 | 0 | 500 | 18.0 |

G. *Equus burchelli crawshaii* de Winton, 1896.
( = *selousi* Pocock, 1897).

Plates 13 and 14 (1, 2, 3, 4, 5 and 6).

Since there is still some uncertainty about the westward limits of *E.b. crawshaii* in Rhodesia (see above), it was decided to obtain material from zebras in two different areas.

1. *Material*

Three animals (Nos. *E.b.c.* 1, 2 and 4) of the Wankie Game Reserve in Rhodesia and two zebras (Nos. *E.b.c.* 3 and 5) of the Gorongosa National Park were investigated in July 1968. Bone marrow biopsies and blood smears were collected immediately after the animals had been shot.

The striping of the zebras from Gorongosa (see Plate 13) was found to be quite different to that from the Wankie area (see Plate 14). The animals from Wankie resemble *E. burchelli antiquorum* and *E. burchelli burchelli*, while the Gorongosa type has no shadow stripes, is more black with thin white stripes and has the reddish brown colour on the face like the mountain zebras, but restricted to the lateral aspect. It is likely that the Wankie zebras represent an intermediate type between *E. burchelli antiquorum* and *E. burchelli crawshaii* (Ansell, personal communication 1968). They have been grouped here under *E. b. crawshaii* purely as a matter of convenience.

2. *Results*

(*a*) *Chromosome number and karyotype*

The results of the chromosome counts are shown in Table 15. The diploid chromosome number was also found to be 44.

Table 15

Chromosome counts of *Equus burchelli crawshaii*.

(*a*) *Counts on dividing bone marrow cells*

| *Animal number* | *Sex* | *Age* | *Time of collection* | *2n chromosome number* 42 | 43 | 44 | 45 | 46 | *Total No. of spreads counted* |
|---|---|---|---|---|---|---|---|---|---|
| *E.b.c.* 1* | ♂ | ± 8 years | 12.00 p.m. | 2 | 1 | 16 | 2 | 0 | 21 |
| *E.b.c.* 2* | ♂ | Foal | 12.30 p.m. | 1 | 1 | 4 | 1 | 0 | 7 |
| *E.b.c.* 3 | ♂ | Adult | 11.30 a.m. | 0 | 1 | 8 | 1 | 1 | 11 |
| *E.b.c.* 4* | ♀ | Adult | 12.15 p.m. | 1 | 5 | 35 | 1 | 1 | 43 |
| *E.b.c.* 5 | ♀ | ± 6 years | 9.45 a.m. | 4 | 4 | 33 | 3 | 0 | 44 |
| Total | | | | 8 | 12 | 96 | 8 | 2 | 126 |

(*b*) *Counts on meiotic spermatocytes*

| *Animal number* | *Sex* | *Age* | *Time of collection* | *n chromosome number* 20 | 21 | 22 | 23 | 24 | *Total No. of spreads counted* |
|---|---|---|---|---|---|---|---|---|---|
| *E.b.c.* 1* | ♂ | ± 8 years | 12.00 p.m. | 1 | 2 | 44 | 3 | 2 | 52 |
| *E.b.c.* 3 | ♂ | Adult | 11.30 a.m. | 1 | 2 | 41 | 4 | 2 | 50 |
| Total | | | | 2 | 4 | 85 | 7 | 4 | 102 |

These chromosomes were found to be morphologically indistinguishable from those of the other subspecies, *Equus burchelli burchelli* and *Equus burchelli antiquorum*, as shown in the spreads and karyograms illustrated in Plate 15 (1, 2, 3 and 4), nor was there any difference in this respect between the zebras from the Wankie area and Gorongosa.

*Likely to be intermediate types, resembling *E. burchelli antiquorum*.

(*b*) *Polymorphic sexing*

The sex difference was distinct as before and the results of counts of nuclear appendages are shown in Table 16.

Table 16

Polymorphic sexing of *Equus burchelli crawshaii*.

| *Animal number* | *Sex* | *No. of cells without appendages* | *No. of appendages* *Type A* | *Type B* | *Type C* | *Type D* | *Total No. of cells counted* | $\frac{A+B}{C}$ |
|---|---|---|---|---|---|---|---|---|
| *E.b.c.* 1* | ♂ | 496 | 0 | 0 | 2 | 2 | 500 | 0·0 |
| *E.b.c.* 2* | ♂ | 498 | 1 | 0 | 1 | 0 | 500 | 1·0 |
| *E.b.c.* 3 | ♂ | 498 | 0 | 0 | 2 | 0 | 500 | 0·0 |
| *E.b.c.* 4* | ♀ | 485 | 10 | 3 | 0 | 2 | 500 | ∞ |
| *E.b.c.* 5 | ♀ | 485 | 11 | 2 | 1 | 1 | 500 | 13·0 |

H. *The effect of excitement and physical stress.*

To test the effect of excitement and physical stress on the number of chromosome spreads obtained by bone marrow biopsy, the following


Plate 15. Mitotic chromosomes of *Equus burchelli crawshaii*.

(1) Male (No. *E.b.c.* 1*), and (2) female (No. *E.b.c.* 4*), metaphase spread, ± × 1,200.
(3) Male karyogram, ± × 2,200 and (4) female karyogram, ± × 1,600.

pilot test was undertaken. An aged stallion that had to be destroyed for other purposes, was quietly handled and placed under Fluothane inhalation anaesthesia and a sternal bone marrow biopsy performed. Three days later the animal was driven hard for 40 minutes and slaughtered immediately afterwards.

Without delay a second biopsy was performed. Treatment of the two specimens was identical. The incidence of mitotic figures obtained from the two biopsy specimens is compared in Table 17.

Table 17

| *Preparation number* | *Total number of mitoses* | |
|---|---|---|
| | *Biopsy* 1 *No physical stress* | *Biopsy* 2 *After physical stress* |
| 1 | 45 | 12 |
| 2 | 55 | 13 |
| 3 | 40 | 9 |
| 4 | 52 | 7 |
| 5 | 35 | 3 |
| Total | 227 | 44 |
| *Average* | 45.4 | 8.8 |

Although this was only a preliminary trial on one animal, the results suggest that excitement and physical stress have a depressing effect on the number of mitotic figures observed in bone marrow biopsy specimens, although, remarkably enough, more cells are noted in the preparations.

On the strength of these preliminary results, rhinoceroses Nos. *C.s.s.* 12 and 13 were only chased a short distance before being darted and rhinoceroses Nos. *D.b.b.* 1 and 2 were cautiously stalked and not chased at all.

*Discussion and Conclusions*

The ensuing discussion and conclusions refer to mammals only, although some statements may be applicable to other classes or phyla.

A. *Mitosis and the mitotic cycle*

The existence of a diurnal mitotic cycle is generally accepted (Bullough, 1963). Gerneke (1967 and personal communication, 1966) incidentally found some evidence for such a cycle in the bone marrow of mammals, both domestic and wild.

In the case of the white rhinoceros the small number of spreads found in Nos. *C.s.s.* 1 and 2 (Table 3) are directly referable to technical difficulties experienced in the early stages of the work. In the case of No. *C.s.s.* 3 (Table 3) bone marrow was collected at 10.00 o'clock in the morning and yielded sufficient spreads.

It is considered that other factors, which will be discussed subsequently, contributed to the poor results obtained in the case of white rhinoceroses Nos. *C.s.s.* 5 to 9 and 11. Bone marrow biopsy was performed on black rhinoceroses No. *D.b.b.* 1 at 2.15 p.m. and no mitoses were found, whereas the material from No. *D.b.b.* 2, collected at 3.15 p.m., yielded a reasonable number of spreads, suggesting the occurrence of a second mitotic wave in the afternoon (perhaps occurring only in juveniles).

No mitoses could be found in the case of No. *E.z.z.* 1 (Table 7) from which the collection was done at 1.00 p.m., whereas sufficient metaphase spreads were obtained from the other mountain zebras from which material was collected between 8.30 a.m. and 11.00 a.m. In the case of *Equus burchelli* a good yield of mitotic spreads was obtained between 9.00 a.m. and 11.15 a.m. Although in some instances reasonable preparations were obtained as late as 12.00 p.m. and 12.15 p.m. (Nos. *E.b.c.* 1 and 4), there was a tendency for more anaphase stages to appear in material taken later in the morning. Interesting enough, No. *E.b.b.* 3 yielded no mitoses at 8.00 a.m., whereas Nos. *E.b.a.* 3 and 4, collected at 6.00 a.m. and 6.30 a.m. respectively, yielded an adequate number of mitotic figures for chromosome counting, but the cycle was still at the early metaphase stage. It may be noted that the material from No. *E.b.b.* 3 was collected during winter at a longitude further west.

The impression is also gained that young animals, approximately one year of age or less, have a mitotic cycle more sharply circumscribed in terms of time than older animals.

Because he found fewer lysosomes in mitotic cells than in cells in the interphase stage, Allison (1967) believed mitotic inhibition to be released by enzymes freed by breakdown of lysosome membranes, such enzymes then acting as mitotic stimulants.

Bullough (1963) tried to find an explanation more directly linked to externally visible factors. He postulated that adrenalin, probably in combination with chalone, a tissue specific substance, acts as a mitotic inhibitor. In this way mitotic rhythm and the rhythm of the animal's activity are inversely linked.

The number of observations made and the nature of the investigation do not allow one to come to any definite conclusions, but nevertheless point to a number of external factors as having some influence on the duration of mitotic cycle and number of dividing cells formed in this cycle. Possible influences that suggest themselves are: 1. temperature, 2. daylight, 3. time of year (seasons), 4. longitude and latitude, 5. height above sea-level and 6. age of the animal. These may influence the activity of the animal, either directly or indirectly, and it would appear as if activity were the main factor. These suggestions need further experimental study, which should include observations on a number of diurnal and noctural "cycles" and the occurrence of such "cycles" in nocturnal animals.

As already indicated above there is suggestive evidence that excitement

and physical stress as well as delayed collection after immobilization, had a depressing effect on the number of mitotic figures obtained by bone marrow biopsy. It is difficult to believe that such an effect could be due to unusually rapid completion of the mitoses; it is more likely that circulatory changes in the bone marrow would affect sampling.

It was not the purpose of this work to determine which factors influence the number of mitotic figures obtained, but the possibility of there being a specific, chronological cycle, as well as stress effects, is mentioned for the benefit of others.

On death of the animal, all dividing cells complete their mitosis. Due to lack of oxygen, no new mitoses are initiated and "cell death" gradually takes place. It is therefore essential to collect bone marrow within half an hour after death, otherwise mitotic figures may be too scarce.

### B. *Chromosome numbers*

#### 1. *Rhinoceros*

In a previous article (Heinichen, 1967) *Ceratotherium simum simum* (2n = 82) was described incidentally as possessing the highest chromosome number in mammals. Since then, however, Hungerford, Chandra and Snyder (1967) reported a female *Diceros bicornis* to have 84 chromosomes. The chromosome count of the one black rhinoceros female investigated here was also found to be 2n = 84. In contrast to the findings of the authors mentioned, four instead of three meta-submetacentric chromosome pairs were observed. This difference may merely represent a difference in interpretation but indicates that further observations should be made. The Indian rhinoceros, *Rhinoceros unicornis* has been described by Benirschke (personal communication, 1967) as also possessing 82 chromosomes as the diploid number.

Two rodents, *Dipodomys merriami merriami* with a "2n = 86±" and *Geomys breviceps breviceps* with "2n = 84±", (Cross, 1931) are also amongst those with high numbers. Cross (1931), however, was uncertain and listed them as "approximately" 84 and 86. The squash techniques used in earlier days were not accurate in determining such high numbers. If these two rodents are omitted, as is the case in "An Atlas of Mammalian Chromosomes" (Hsu and Benirschke, 1967), then *Diceros bicornis* (2n = 84) has the highest known chromosome number in mammals, *C. simum simum* and *R. unicornis* (2n = 82) the second highest, and *Tarsius bancanus* (2n = 80) (Klinger, 1963) and the dog (2n = 78) third and fourth respectively.

#### 2. *Zebra*

As a result of this study it can be definitely concluded that both *Equus zebra zebra* and *Equus zebra hartmannae* have a chromosome number 2n = 32. This confirms the figure given by Benirschke and Malouf (1967) for *E. z. hartmannae* and that of Hamerton (personal communication to Benirschke, 1966). Of the two explanations given for a diploid chromo-

some number of 48 possessed by a hybrid between an *E. asinus* stallion and a mare that was possibly *E. z. hartmannae* (see above), it is now clear that the first alternative is probably the more correct one, namely that the donkey stallion had an aberrant chromosome number.

In contrast to Benirschke's finding of 42 chromosomes for the "Damara zebra", this study has revealed that the plains zebra from South West Africa, to which Benirschke probably referred, has 44 chromosomes.

As no differences were found between *E. z. zebra* and *E. z. hartmannae* nor between *E. burchelli burchelli*, *E. burchelli antiquorum* and *E. burchelli crawshaii*, it is concluded that, at the subspecies level, chromosome numbers do not offer a means of distinction. In the case of *E. b. burchelli* and *E. b. antiquorum* it has to be remembered that the subspecies status of the South West African plains zebra is by no means resolved and the same applies to the zebras in the north-western (Wankie) region of Rhodesia (see above).

The three species of zebra namely *E. grevyi*, *E. burchelli* and *E. zebra* thus have diploid chromosome numbers of 46, 44 and 32 respectively (see Table 18).

### 3. *Chromosome number as a characteristic of species*

As shown in Table 18 a division at species level according to the chromosome number is possible amongst the Equidae, but not amongst the Rhinocerotidae. In the latter instance *Rhinoceros unicornis* and *Ceratotherium simum simum* both have 82 chromosomes, but the chromosomes of the one species are morphologically distinct from those of the other: the white rhinoceros possesses only eight subtelocentric autosomal chromosome pairs, whereas the Indian rhinoceros has 10 such pairs.

In view of this, one could postulate in a general way that if two animals have a different chromosome number they should be regarded as specifically distinct. An exception to this rule occurs in cases of chromosome polymorphism, which has been described in several animal species: spiny mouse (Wahrman and Zahavi, 1955); common shrew (Ford, Hamerton and Sharman, 1957); impala (Wallace and Fairrall, 1967) and silver fox (Gustavsson and Sundt, 1967). The latter authors found 35, 36 and 37 chromosomes in a study on four silver foxes. Wallace and Fairrall (1967) counted 60 chromosomes in 17 impalas, 59 in 14 individuals and 58 in three. No externally visible morphological distinction could be made between these three groups.

The converse of the above rule, namely, that if two animals have the same chromosome number, they should belong to the same species, does not hold at all. It has already been pointed out that the Indian and the white rhinoceros have the same chromosome number. On the other hand their karyotypes are morphologically distinct. Similar examples are quoted in Table 19:

Table 18

*Presently known chromosome numbers of the Perissodactyla*

| *Family* | *Species* | *Subspecies* | *2n chromosomes* | *Metacentric (=meta-sub-meta-centric) chromosome pairs* | *Acrocentric (=acro-sub-telo-centric) chromosome pairs* | *References* |
|---|---|---|---|---|---|---|
| **Equidae** | *E. przewalskii* (Przewalski's horse) | | 66 | 13 | 19 | Benirschke, Malouf and Low (1965) |
| | *E. caballus* (Domestic horse) | | 64 | 13 | 18 | Benirschke, Brownhill and Beath (1962); Trujillo, Stenius, Christian and Ohno (1962) |
| | *E. asinus* (Donkey) | | 62 | 19 | 11 | Trujillo *et al.* (1962); Benirschke, *et al.* (1962) |
| | *E. hemionus* (Onager) | | 56 | 23 | 4 | Benirschke and Malouf (1967) |
| | *E. grevyi* (Grevy's zebra) | | 46 | 16 | 6 | Mutton, King and Hamerton (1964) |
| | *E. burchelli* (Burchell's zebra) | *E. b. burchelli* | 44 | 18 | 3 | Heinichen (in press) |
| | | *E. b. antiquorum* | 44 | 18 | 3 | Eloff (1966); Benirschke and Malouf (1967); Heinichen (in press) |
| | | *E. b. böhmi* | 44 | 18 | 3 | Benirschke, Brownhill and McFeely (1963) |
| | | *E. b. crawshaii* | 44 | 18 | 3 | Present investigation |
| | *E. zebra* (Mountain zebra) | *E. z. zebra* | 32 | 13 | 2 | Heinichen (1967) |
| | | *E. z. hartmannae* | 32 | 13 | 2 | Benirschke and Malouf (1967); Heinichen (in press); Hamerton (according to Benirschke, 1966) |
| **Rhinocerotidae** | *D. bicornis* (Black rhinoceros) | *D. b. bicornis* | 84 | 4 | 37 | Present investigation; *Hungerford, Chandra and Snyder (1967) |
| | *R. unicornis* (Indian rhinoceros) | | 82 | 0 | 40 | Benirschke (personal communication, 1967) |
| | *C. simum* (White rhinoceros) | *C. s. simum* | 82 | 0 | 40 | Heinichen (1967) |

*See "Discussion and Conclusions"

Table 19

*Examples of distantly related species having the same chromosome number but different karyotypes.*

| *Order* | *Family* | *Genus and species* | *Chromosome number* |
|---|---|---|---|
| Rodentia | Sciuridae | *Tamiasciurus hudsonicus streatori* (Red squirrel) | 46 |
| Rodentia | Heteromyidae | *Perognathus intermedius* (Rock pocket mouse) | 46 |
| Rodentia | Cricetidae | *Microtus pennsylvanicus pennsylvanicus* (Meadow vole) | 46 |
| Rodentia | Cricetidae | *Baiomys taylori subater* (Northern pygmy mouse) | 48 |
| Rodentia | Cricetidae | *Onychomys leucogaster* (Northern grasshopper mouse) | 48 |
| Carnivora | Procyonidae | *Bassariscus astutus* (Ringtailed cat) | 38 |
| Carnivora | Felidae | *Felis catus* (Domestic cat) | 38 |
| Primates | Callithricidae | *Callithrix jacchus* (Common marmoset) | 46 |
| Primates | Hominidae | *Homo sapiens* (Man) | 46 |

It is even possible to have distinct species with the same chromosome number and apparently morphologically identical karyotypes (Table 16).

Table 20

*Examples of closely related species having apparently morphologically identical karyotypes.*

| *Order* | *Family* | *Genus and species* | *Chromosome number* |
|---|---|---|---|
| Lagomorpha | Leporidae | *Lepus alleni alleni* (Antelope jack rabbit) | 48 |
| Lagomorpha | Leporidae | *Lepus californicus eremicus* (Black-tailed jack rabbit) | 48 |
| Carnivora | Canidae | *Canis familiaris* (Dog) | 78 |
| Carnivora | Canidae | *Canis latrans* (Coyote) | 78 |
| Carnivora | Ursidae | *Selenarctos thibetanus* (Asiatic black bear) | 74 |
| Carnivora | Ursidae | *Ursus americanus* (American black bear) | 74 |
| Artiodactyla | Cervidae | *Odocoileus hemionus* (Mule deer) | 70 |
| Artiodactyla | Cervidae | *Odocoileus virginianus* (White-tailed deer) | 70 |

On comparing the data in Tables 19 and 20, obtained from Hsu and Benirschke (1967), it will be noted that generally species with the same chromosome number, but belonging to different families, have a different karyotype, whereas species with the same family, as a rule have apparently morphologically similar karyotypes (the word "apparently" has been used, as no detailed measurements have been made on the chromosomes). Exceptions, however, do occur, as in the case of the meadow vole and pygmy mouse (Table 19).

In the final analysis it must be concluded that an identical chromosome number and an identical karyotype may not be advanced uncritically for the identity of species, neither may differences in chromosome number be accepted as proof of difference in species, unless one excludes chromosome polymorphism.

4. *Hybrids*

For successful hybridization there should be: 1. mating compatibility (although this may be overcome theoretically by artificial insemination); 2. compatibility of the gametes; 3. genetic compatibility and 4. acceptability of the hybrid conceptus to the maternal uterus.

Under natural conditions the behaviour of the population is such that mating between animals from different species is not likely to occur. Hybridization is the result of artificial circumstances, as a rule man-made. Incompatibility of gametes has been postulated where an attempt at hybridization has not resulted in the development of a fertilized ovum. Attempts at crossing goats with sheep have resulted in non-viable embryos and the fourth reason quoted above has then been advanced (Berry, 1938).

Interspecific hybrids in the Equidae are well known. In this regard it is of interest to note that this occurs despite considerable difference in chromosome number in many instances. In all of these cases the diploid number of the hybrid was equal to the sum of the haploid number of both parents (Benirschke, 1966), with the exception of the donkey x *E. z. hartmannae* cross referred to previously. Even in this case it seems likely that the donkey stallion had an aberrant number of chromosomes. Clearly, genetical compatibility is not a simple matter of chromosome number or karyotype and the mechanism of the first and subsequent divisions of the zygote are not upset by the disparity between chromosomes contributed by the male and female cells. In amphimixis, the two sets of chromosomes come together and the mitotic spindle seems well able to deal with the unexpected disparity.

In meiosis, however, the disparity between maternal and paternal chromosomes causes the mechanism to break down and, as a rule, no viable gametes are formed, with the result that the hybrid is sterile. Benirschke (1966) is very sceptical about the supposed fertility of the few cases of mare mules described in the literature. King *et al.* (1966) mentions "block" in the meiotic division of the primary spermatocyte at late pachytene to diplotene stage as the cause of failure to produce gametes

in a male *E. burchelli böhmi* x female *E. asinus* hybrid, and possible physiological disturbance before the meiotic prophase in male *E. grevyi* x female *E. caballus* hybrids.

The fertility of the hybrid between *E. przewalskii* and *E. caballus* announced by Koulischer and Frechkop (1966) forms an exception to the rule. This horse, as mentioned in the article, "may not be a pure Prjewalski's horse". It may also have a closer relationship to *E. caballus* than exists between *E. caballus* and *E. asinus*.

On account of the relatively common fertility of the hybrids between *Vulpes fulvus* and *Vulpes vulpes*, Gustavsson and Sundt (1967) do not consider them as distinct species, but rather as subspecies.

Where polymorphism occurs, the so-called "hybrids" between males having a specific chromosome number and females of the same species having a different chromosome number seem to be fertile. In other words, it is not a simple matter of difference in chromosome numbers which must be held responsible for meiotic failure.

## C. *Karyotypes*

Levan *et al.* (1964) stated that it is important to identify chromosomes and to try to recognize homologies between those from one species and another. In this way it may be possible to find a "standard" karyotype for a particular taxon. When considering the various karyotypes as constructed for the Perissodactyla, it does not seem possible to postulate a hypothetical standard. The morphological relationship between the autosomes seem to have a certain evolutionary significance, which is discussed under the appropriate heading below. On the other hand there is a similarity in appearance of the sex-chromosomes throughout the order.

### 1. *Sex-chromosomes*

#### (*a*) *Sex chromosomes of the Rhinocerotidae* (*see also Fig.* 1)

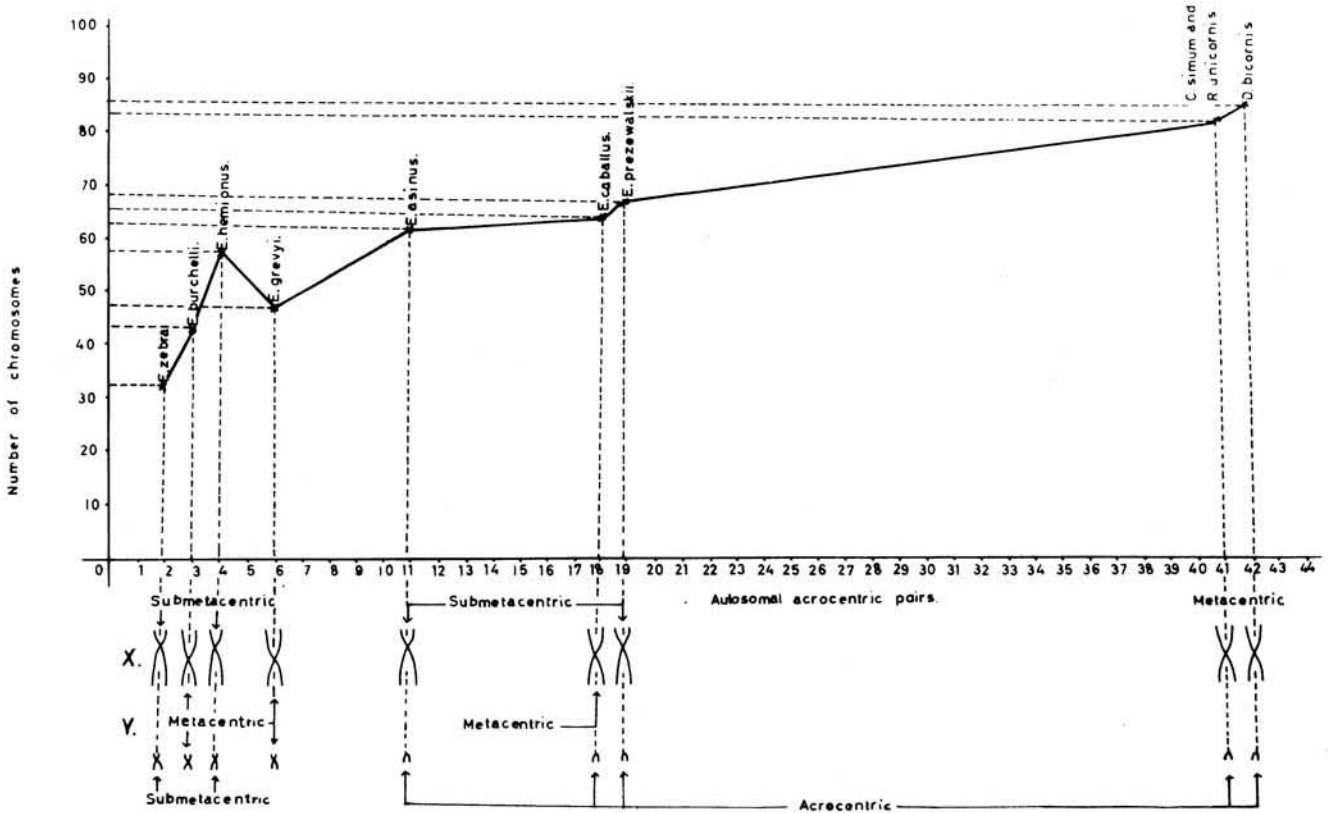
The relationship between Rhinocerotidae and the Equidae is revealed in their sex chromosomes, since the rhinoceroses have sex chromosomes very much similar to those of the horse (see next subsection).

It would be most interesting to compare the karyotype of the Tapiridae with those of the above families.

#### (*b*) *Sex chromosomes of the Equidae* (*see also Fig.* 1)

Previous studies (Mutton *et al.*, 1964; Benirschke *et al.*, 1963; Benirschke and Malouf, 1967; Heinichen, 1967 and Heinichen, in press) and the present results have revealed that the three zebra species: *E. grevyi*, *E. zebra* and *E. burchelli* have X-chromosomes ranging from meta- to submetacentric. According to Mutton *et al.* (1964), Trujillo *et al.* (1962) and Benirschke and Malouf (1967), the X-chromosome of the donkey differs in structure from that of the other Equidae in being the most submetacentric, in fact almost subtelocentric. In *E. przewalskii*, *E. hemionus* and *E. zebra* the position of the centromere of the X-chromosome resembles more closely that of the donkey, whereas in *E. grevyi* and *E. burchelli* it is

Fig.1. Graphic representation of decreasing acrocentric chromosome pairs accompanied by a decrease in chromosome number.



situated in a more median position, similar to the X-chromosome of the horse. Bourdelle (1941) describes *E. zebra* as having more asinine features (especially the ears and tail), while the other zebras are more horse-like. At this stage one can do no more than point out that a relationship exists between the external features and the position of the centromere on the X-chromosomes amongst some members of this order, a relationship which probably is purely coincidental.

Differences in centrometric position in Y-chromosomes also exist. As revealed in Fig. 1, three species have an acrocentric Y-chromosome (Hsu and Benirschke, 1967). Although Mutton *et al.* (1964) described *E. grevyi* as having a metacentric Y-chromosome and *E. burchelli* an acrocentric Y-chromosome, Benirschke *et al.* (1963) list the Y-chromosome in both these species as a small metacentric element. In these studies, the Y-chromosome of *E. zebra* was classified as submetacetric, whereas all members of *E. burchelli* had a metacentric Y-chromosome.

2. *Karyotype evolution in the Perissodactyla*

Of special interest is the fact that the known diploid chromosome numbers for the Perissodactyla range from 32 to 84. Decrease of chromosome number often appears to be accompanied by a decrease in number of acro-subtelocentrics and an increase in number of meta-submetacentric chromosomes, as illustrated in Table 18 and Fig. 1.

It has been postulated that a decrease in acro-subtelocentrics could be due to the so-called Robertsonian fusion or centric fusion as well as tandem fusion (Bender and Chu, 1963; Gustavsson and Sundt, 1967; Chiarelli, 1968). A study of the karyotype of *E. przewalskii* and *E. zebra* (both with 26 meta-submetacentric chromosomes) proves that the decrease of acro-subtelocentrics is not necessarily accompanied by an equivalent increase of meta-submetacentrics. Some of the smaller chromosomes could possibly 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 meta-submetacentric chromosomes: such chromosomes may revert to acro-subtelocentric ones especially as a result of pericentric inversion. The black and white rhinoceroses could well serve as an example of such reversion: the black rhinoceros has 84 chromosomes and the white rhinoceros 82, yet the black rhinoceros has four pairs of meta-submetacentric chromosomes and the white rhinoceros none.

The graph in Fig. 1 follows a direction parallel to the evolution of the Perissodactyla, from rhinoceroses (and tapirs?), being the most primitive, with the highest chromosome number and acro-subtelocentric chromosomes, to the Equidae, with the lowest chromosome number and acro-subtelocentric chromosome pairs (Table 18). The zebras, with the lowest

chromosome count and number of acro-subtelocentric chromosomes, are the most specialized as Thenius (1966), regards them.

The suggestion, that the more highly specialized animals have a smaller chromosome count and few acro-subtelocentric chromosomes, has also been made for a number of animal groups among the primates (Bender and Chu, 1963).

Specialization of animals does not necessarily mean that they are more intelligent. In fact a horse seems more intelligent to us than a zebra. The horse with a higher chromosome number, may have more chromosome material (and possibly more genes) with a greater chance of variation. Even if the zebras have the same number of genes, they will be present in fewer linkage groups, that is, a greater number of them will be linked, with the result that a smaller chance exists for recombination by independent assortment and therefore also a decrease in variation of genetic characteristics could be expected. During diplotene of meiosis, chiasmata (the cytological expression of genetical crossing over) are formed (Swanson, 1960). Length of the chromosome determines to a certain extent the number of crossovers that can occur. It is a well-known fact that one crossover inhibits the occurrence of another in its immediate vicinity. This phenomenon is known as chiasma-interference. In *Drosophila* this interference is complete for 10 map units (Swanson, 1960). It is clear then, that if the distance between two genes increases, the chances of crossing over occurring increases. One would expect that if zebras have the same number of genes on fewer chromosomes than horses and donkeys, the crossover percentage of zebra chromosomes will be lower than that of horses and donkeys.

The zebras, with a smaller chromosome count, inhabit only small parts of the African continent. Today, mainly through the action of man, their distribution area is still decreasing. This applies especially to the mountain zebras inhabiting only a very small part of South Africa and here being restricted to the mountainous regions (Bigalke, 1952). The chance of their becoming extinct through just a slight change in their "evolutionary niche" is certainly much greater than for horses and donkeys. The latter two species, however, owe their present wide distribution to a great extent to the fact that they have become domesticated. In fact, the horse has been re-introduced to the American continent where it previously had become extinct. Here it has also been able to propagate itself in the feral state.

The Rhinocerotidae, with a *high* chromosome count are also threatened with extinction. Cooke (1950) regards them as very primitive and not subject to rapid changes. Their chromosome complement, most autosomal chromosomes being acro-subtelocentric, also bears this out. They have undergone little adaptive change, in fact these animals do not seem to have changed at all since their origin in the Eocene (Thenius, 1966). Reasons for their threatened extinction in modern times must rather be sought in the fact that they have a long generation time and in man's

superstitious belief in the medicinal value of rhino horn.

The argument, that decrease in chromosome number leads to a lessening of the scope for genetic variation, would not seem to hold in the case of the rhinoceros. It is therefore clear that, despite the parallelism between karyotype evolution and animal evolution, oversimplified conclusions are not justified.

Polyploidy plays an important rôle in plant evolution, but among animals there is little evidence for its existence (White, 1945). In the family Cercopithecidae, chromosome numbers of 48, 54, 60, 66 and 72 (all multiples of 6) were found and polyploidy was suggested as an explanation for this phenomenon (White, 1945). White, however, raised several objections to this as an explanation: 1. It is difficult to understand how the sex determining mechanism of a polyploid primate would function. 2. Each species of the Cercopithecidae has one chromosome pair with a secondary constriction. If some of the species developed by polyploidy one would find several numbers of these constrictions. 3. The DNA values were measured and were found the same among all these species. If polyploidy was the reason for the differences in chromosome number, then the DNA value should also be different.

Polyploidy could never be used as an explanation for the wide range of chromosome numbers found in the Perissodactyla, as it presupposes doubling of a certain basic chromosome number with increase in evolutionary status. In this order there is a progressive decrease in number which does not occur in relative submultiples.

A better concept of karyotype evolution can only be gained once the karyotype of a much greater number of existing mammals becomes known.

### D. *Taxonomy and cytogenetics*

The Cradock mountain zebra and the mountain zebra of S.W.A. seem to be conspecific: their karyotypes are morphologically and numerically identical (see Plate 7 and 8) (no relative arm lengths could be determined, due to insufficiency of spreads available for this particular purpose, nor were DNA determinations possible). Therefore the practice of regarding the one as *E. zebra zebra* and the other as *E. zebra hartmannae* is purely a subspecific (not specific) taxonomic differentiation and one on which most taxonomists agree (Ansell, 1967).

Similarly the plains zebras of southern Africa, as well as Grant's zebra (occurring north of the middle Zambezi, Luangwa and Rovuma rivers according to Ansell, 1967) appear to be conspecific judging by their karyotypes (see Plates 11, 12 and 15; Benirschke *et al.*, 1963).

In this respect the karyotypes do not assist in clarifying the taxonomic problem at subspecies level. At best, it has confirmed the existing classification at the species level.

Differences among animals with the same chromosome number are usually morphological, resulting in two subspecies or rather races, as

is seen in the zebras, where distinctions between the subspecies are based mainly on the stripe pattern of the animals (stripe pattern and colour could be the same as differences in hair- or eyecolour in man or distinctions as described by Cabrera (1936) caused by the animals' geographic distribution).

These studies can only give some support for the taxonomists and not solve their problems. All the subspecies of a particular species of zebra have an identical chromosome number, which is typical for the species to which they belong. Taxonomists can and do still place them in various subspecies mainly according to the basic pattern formed by the stripes.

As only four to seven representatives of each species were examined (examination of more animals was practically impossible) variations of the typical numbers may perhaps still be encountered, but then as examples of polymorphism only, and this at low frequency. Nevertheless, this work forms a basis from which further expansions can be made.

### E. *Polymorphic sexing*

In all the animal species studied, distinct polymorphic sexing was possible, the relative frequency of drumsticks serving as the most useful guide. The highest number of this type of nuclear appendage found in the male was two and the lowest number in females was ten per 500 polymorphonuclear leukocytes. Kosenow and Scupins' formula was not useful, because of the frequent absence of nuclear appendages of the C-type, giving a value of $\infty$ for both males and females in these cases. The polymorphic sexing in the southern African forms of the Perissodactyla is thus simplified: only the drumsticks need to be counted.

The vesicular D-type appendages shown in Plate 4 (5 and 6) differ in some detail from those described by Kosenow and Scupin (1956) and have to my knowledge been described only by Gerneke (1965) in the hippopotamus. The suggestion was made there that they resemble the "paranuclear vacuoles" appearing with "cell death", studied by Bessis (1964). Possibly these structures could be similar to the degenerating vacuoles occurring near the junction of the nucleus and the cytoplasm described by Dustin (*cit.* Bessis, 1961).

*Summary*

A cytogenetic survey was undertaken on the southern African species and subspecies of the order Perissodactyla, taking advantage of the relatively recent improvements whereby chromosome number and karyotypes could be established with greater accuracy.

Material was obtained from male and female animals either chemically immobilized, caught or shot in various game parks or game farms in South Africa, South West Africa, Rhodesia and Mozambique. The bone marrow biopsy technique based on that of Sandberg, Crosswhite and Gordy (1960) with some adaptations (Gerneke, 1967) was employed.

Several, up to about 50, good chromosome spreads were counted and karyograms were constructed. Simultaneously blood smears were made and the nuclear appendages on 500 neutrophil polymorphonuclear leukocytes counted to determine the feasibility of polymorphic sexing.

The following results were obtained:

| *Species* | *2n Chromosomes* | *Metacentric (= meta-submetacentric) chromosome pairs* | *Acrocentric (= acro-subtelocentric) chromosome pairs* | *Number of animals* |
|---|---|---|---|---|
| *Ceratotherium simum* | 82 | 0 | 40 | 5 |
| *Diceros bicornis* | 84 | 4 | 37 | 1 |
| *Equus burchelli* | 44 | 18 | 3 | 15 |
| *Equus zebra* | 32 | 13 | 2 | 8 |

The subspecies of *Equus burchelli*, namely *E. b. burchelli*, *E. b. antiquorum* and *E. b. crawshaii* (= *selousi*) and intermediate types between the latter two all have the same chromosome number, namely 2n = 44, and morphologically apparently identical karyograms. The same applies to the subspecies of *Equus zebra*, *E. z. zebra* and *E. z. hartmannae*, with a diploid chromosome number of 32.

The karyotypes of the different species of the Perissodactyla were compared with each other. There is a great variation in number and morphology of the karyotypes, so that no morphological relationship between the autosomes was found, although a similarity was recognized in the sex chromosomes throughout the order. The sex chromosomes of the rhinoceroses resemble those of the horse.

Karyotype evolution among the Perissodactyla was difficult to explain. Robertsonian fusion, whereby a decrease of chromosome number is accompanied by a decrease in number of acro-subtelocentric chromosomes could not be the only reason for the existence of such a wide range from 32 to 84 chromosomes in this order. Robertsonian fusion must have been accompanied by other phenomena, such as tandem fusion, translocations with subsequent loss of heterochromatic centromeres, translocations reverting to acro-subtelocentric chromosomes as a result of pericentric inversion or possibly the loss of very small chromosomes. Polyploidy could not be offered as an explanation here.

These cytogenetic studies could not assist in clarifying the taxonomic problems among the zebras at subspecies level. Nevertheless, it has confirmed the existing classification at species level, with every species of this order having its specific diploid chromosome count.

It was concluded that an identical chromosome number and an identical karyotype may not be advanced uncritically for the identity of species, neither may differences in chromosome number be accepted as proof of difference in species, unless one excludes chromosome polymorphism.

Although no chromosomes of hybrids were studied, the findings of other authors on hybrids were discussed, showing that in nearly all known hybrids of the Equidae, the diploid number of the hybrid was equal to the sum of the haploid number of both parents and that all Equidae hybrids, excluding a few exceptional mules, were sterile.

Suggestive evidence was found for the existence of a mitotic cycle with peak activity during about 9 to 11 a.m. Although not specifically investigated, indications were found that activity of the animal (and thus external factors influencing that activity) may play a rôle, yet that physical stress, excitement, and delay in collection of bone marrow after immobilization may possibly depress the number of mitotic figures obtained, presumably due to circulatory changes in the bone marrow.

Clearcut sex differences exist in all the species and subspecies examined; the female sex can be determined by counting typical "drumsticks" only.

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