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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 μ 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 *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 *E. z. hartmannae* Matschie, 1898
		Rhinoceros unicornis Linn., 1758	*E. burchelli* (Gray, 1824) *E. b. burchelli* (Gray, 1824)
		Rhinoceros sondaicus Desmarest, 1822	*E. b. antiquorum* H. Smith, 1841 *E. b. böhmi* Matschie, 1892
		Dicerorhinus sumatrensis (Fischer, 1814)	*E. b. crawshaii* de Winton, 1896 (= *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

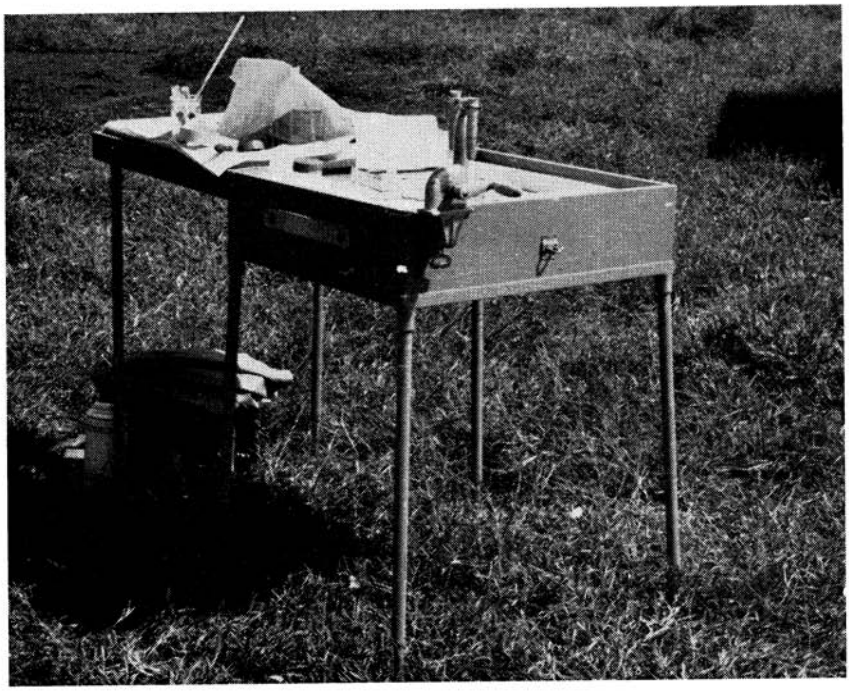

Plate 1. Portable work bench.

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