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 1½ 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:

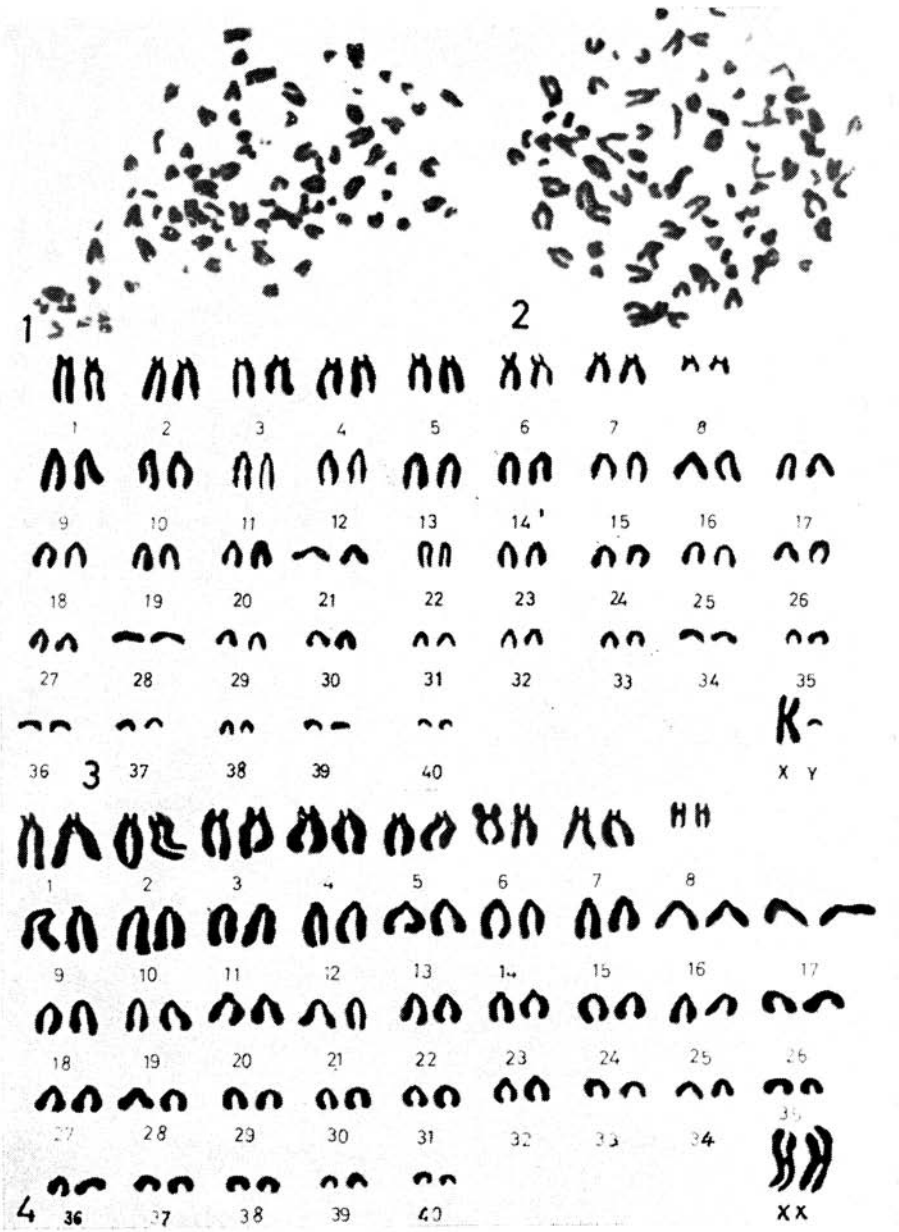

Plate 3. Mitotic chromosomes of *Ceratotherium simum simum*.
(1) Male (No. *C.s.s.* 3) and (2) female (No. *C.s.s.* 12) metaphase spread, ± × 1,200.
(3) Male karyogram, ± × 1,800 and (4) female karyogram, ± × 1,700

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).

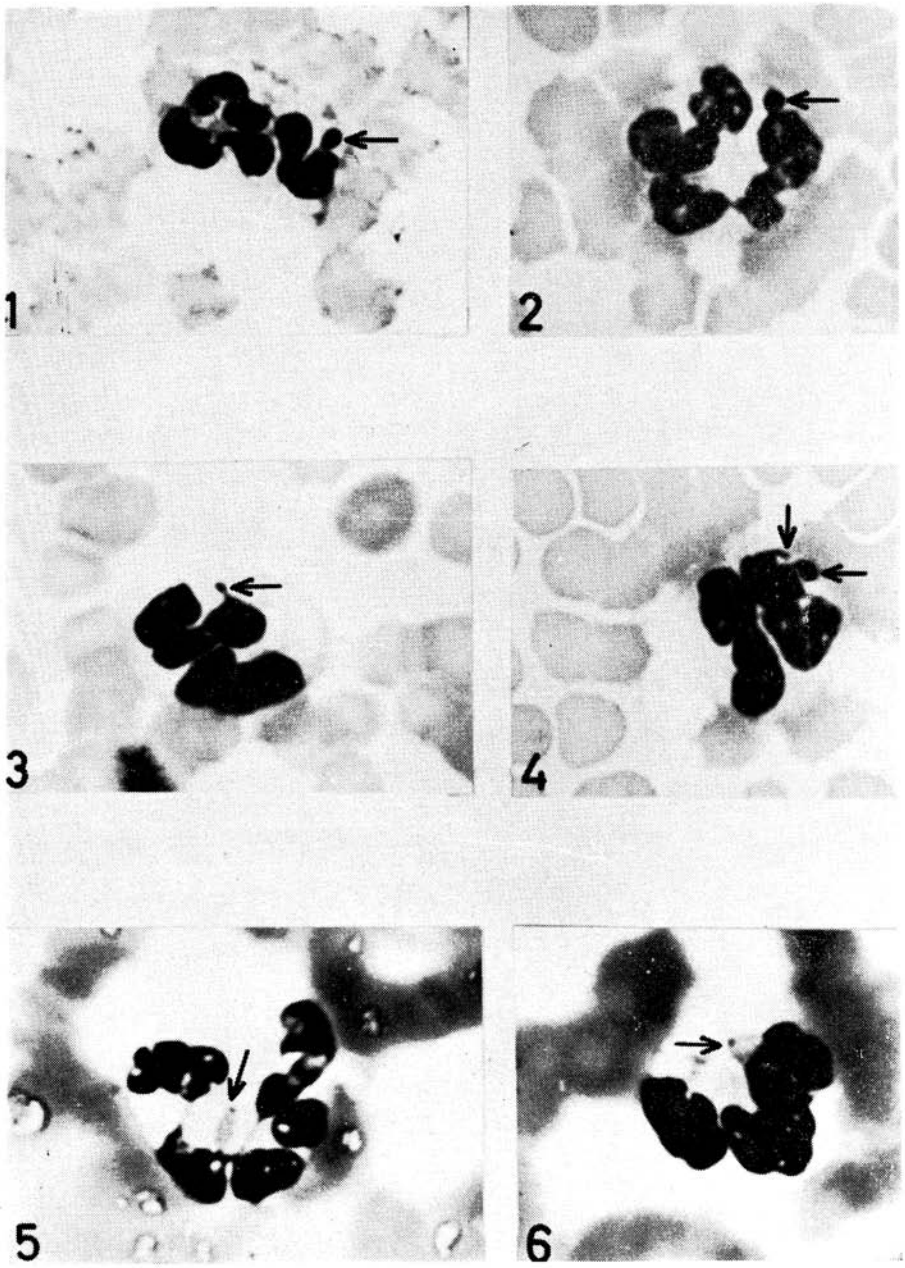

Plate 4. Appearance of drumsticks in Perissodactyla.
Zebra preparations. Arrow indicates the drumstick.
(1) No. *E.z.h.* 4 A-Type: a solid nodule, with filament.
(2) No. *E.b.a.* 6 B-Type: a sessile nodule, without filament.
(3) No. *E.b.b.* 2 C-Type: intermediate forms.
(4) No. *E.b.a.* 6 A- and C-Type: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

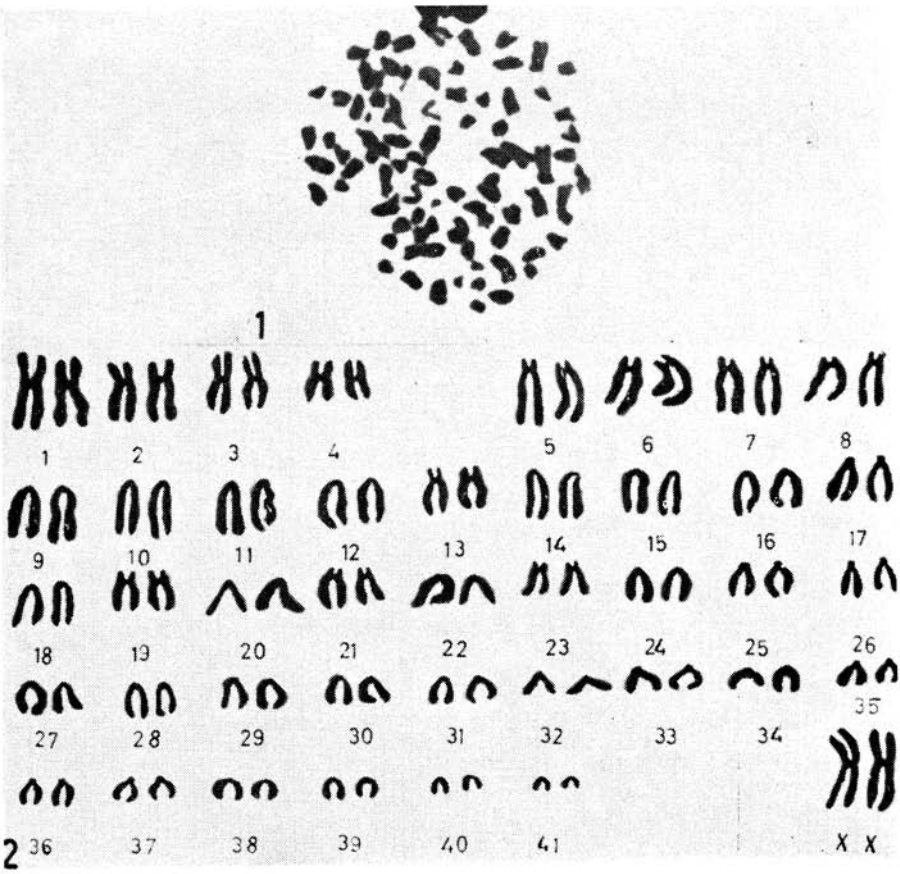

Plate 5. Mitotic chromosomes of *Diceros bicornis bicornis*.
(1) Female (No. *D.b.b.* 2) metaphase spread, ± × 1,200.
(2) Female karyogram, ± × 1,900.

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*

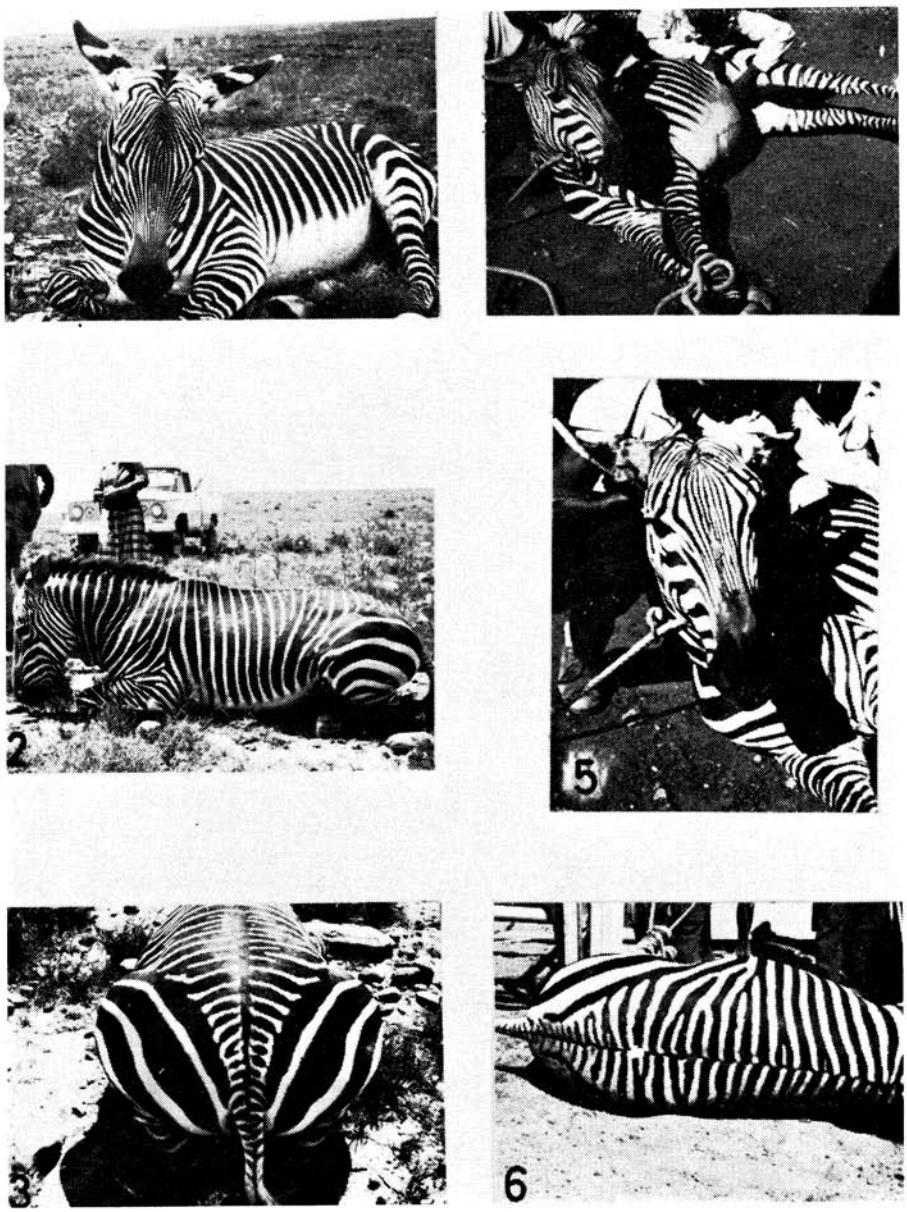

Plate 6. (1) Frontal and latero-ventral; (2) lateral and (3) dorsal views of *Equus zebra zebra*. (4) Frontal and latero-ventral; (5) frontal and (6) dorsal views of *Equus zebra hartmannae*.

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* 30	31	32	33	34	*Total No. of spreads counted*
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	∞
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

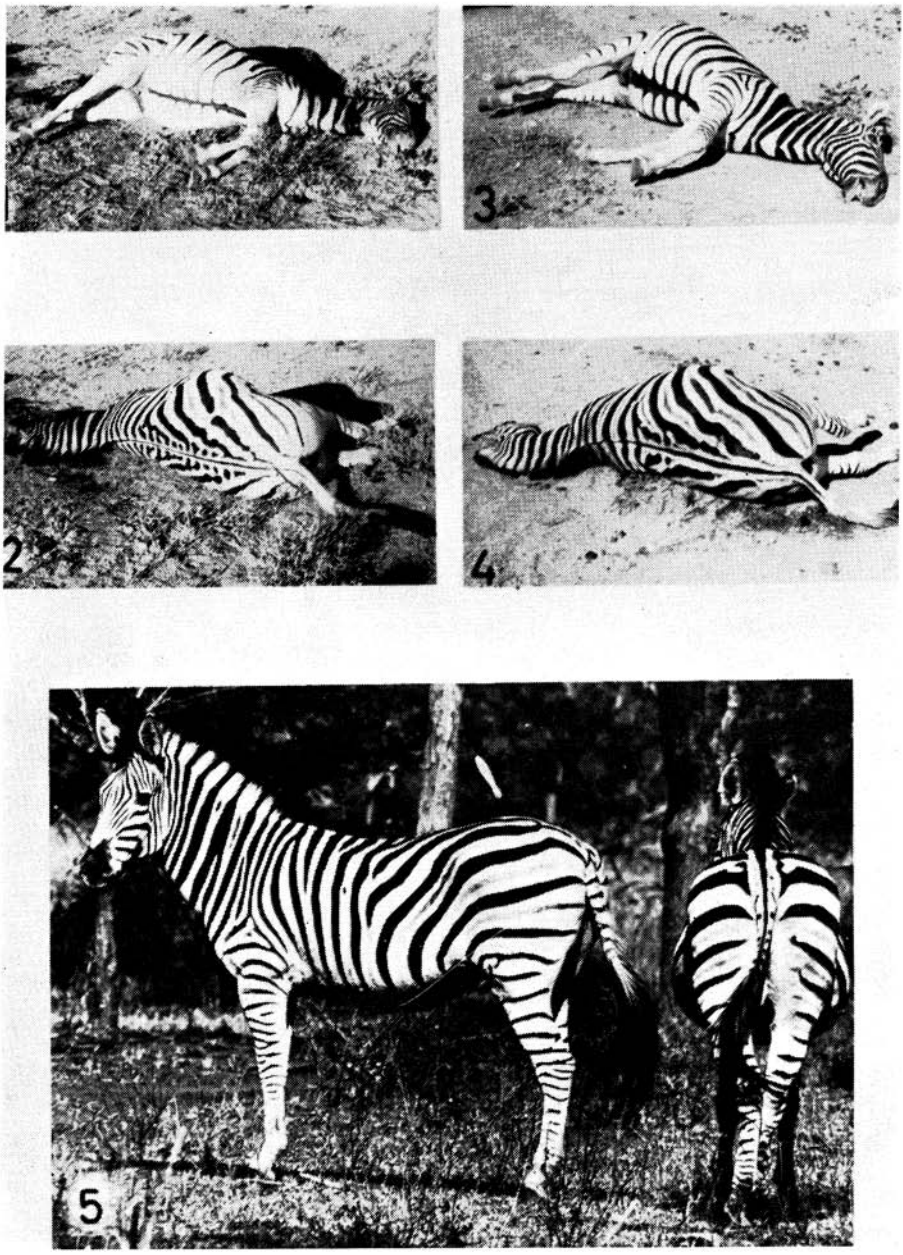

Plate 10. (1 and 2). Ventral and dorsal views of lightly striped specimen of *Equus burchelli burchelli* female.

(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*.